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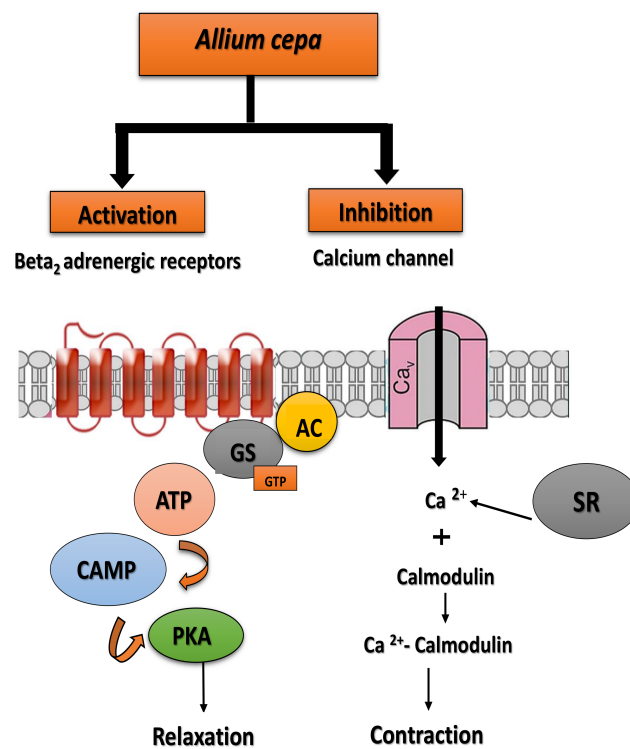
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The contribution of beta-2 adrenergic, muscarinic and histamine (H₁) receptors, calcium and potassium channels and cyclooxygenase pathway in the relaxant effect of *Allium cepa* L. on the tracheal smooth muscle

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Running title: Relaxant effect of *Allium cepa* extract

ABSTRACT

Ethnopharmacological relevance: There are report regarding therapeutic effects for *Allium cepa* L. (*A. cepa*) in Iranian traditional medicine and the plant has showed anti-inflammatory, anti-allergic, anti-hyperglycemic, antioxidant, anti-cancer, anti-hypertension, anti-hypercholesterolemia and anti-asthmatic activities in previous studies.

Aim of the study: In this study, the contribution of β_2 adrenergic, muscarinic and histamine (H1) receptors, calcium and potassium channels, and cyclooxygenase pathway in the relaxant effect of *A. cepa* extract on tracheal smooth muscle (TSM) was assessed.

Materials and Methods: TSM was contracted by KCl (60 mM) or methacholine (10 μ M) for 5 min and cumulative concentrations of *A. cepa* extract (2, 4, 8, 16, 32 and 64 mg/ml) were added to organ bath every 5 minutes. Theophylline (0.2, 0.4, 0.6 and 0.8 mM) as positive control, and saline (1 ml) as negative control were also examined in non-incubated tissues. The relaxant effect of *A. cepa* extract was examined on non-incubated and incubated TSM with propranolol, chlorpheniramine, diltiazem, atropine, glibenclamide and indomethacin.

Results: *A. cepa* showed concentration-dependent relaxant effects on non-incubated TSM contracted by KCl (60 mM) or methacholine (10 μ M), ($P < 0.01$ to $p < 0.001$). There was no significant difference in the relaxant effects of *A. cepa* between non-incubated and incubated tissues with glibenclamide, atropine, chlorpheniramine and indomethacin. The plant extract showed significant lower relaxant effects in incubated TSM with propranolol and diltiazem compared to non-incubated tissues. EC₅₀ values of *A. cepa* extract in incubated TSM with propranolol and diltiazem were significantly lower than non-incubated tissues ($p < 0.001$ and $p < 0.05$, respectively). The relaxant effects of different concentrations of the extract of *A. cepa* were not significantly different with those of theophylline. The concentrations of *A. cepa* extract and theophylline were significant correlated with their relaxant effects ($p < 0.05$ to $p < 0.001$). In incubated TSM with propranolol and diltiazem, concentration ratio minus one (CR-1) values was positive (2.65 ± 0.63 and 1.28 ± 0.43 respectively).

Conclusion: The *A. cepa* extract showed relatively potent relaxant effect on TSM which was comparable to the effect of theophylline. The results showed that β_2 -adrenergic stimulatory and/or calcium channel blockade are the possible mechanisms for the relaxant effects of the plant.

Keywords: *Allium cepa*; smooth muscle; trachea; relaxant effect; β_2 -adrenergic receptor; calcium channel.

1. Introduction

Asthma is a common chronic inflammation disease which leads to considerable morbidity worldwide every year. Airway hyperresponsiveness, increased mucosa secretion, smooth muscle hyperplasia, infiltration of inflammatory cells, are the main characteristics of asthma (Barnes, 2003; Nauta et al., 2008). Airway inflammation (Pniewska and Pawliczak, 2013) and airway constriction (Nauta et al., 2008; Zar and Levin, 2012) are the main characteristic features of asthma. Therefore, treatment of asthma such included education to self-care of patients, management of asthma symptoms and drug therapy is a long and difficult processes in medical field (Zar and Levin, 2012). Treatment of asthma focused on reduction of lung inflammation and prevention of airway smooth muscle constriction (Masoli et al., 2004). Therefore, two types of drug are used for treatment of asthma including preventive drug to reduce lung inflammation and relieving drug to reduce airway constriction (Rabe and Schmidt, 2001). With regard to adverse effects of currently used asthma drugs and also the absence of their full therapeutic efficacy, new anti-asthmatic drugs need to be developed (Mullane, 2011).

Allium cepa L. (*A. cepa*) belong to the Liliaceae family which grows all over the world. Onion mostly cultivated in China, India, United States, Russia, Turkey, and Iran (Slimestad et al., 2007). *A. cepa* showed various pharmacological activities such as antioxidant, antimicrobial, anti-hyperglycemic, antidiabetic, anti-inflammatory (M.Manasa, 2014) and immunomodulatory (Marefati et al., 2018) effects. Previous studies also showed preventive effects on the tracheal responsiveness and lung inflammation (Ghorani et al., 2018) for *A. cepa* extract in sensitized rats.

The constituents of *A. cepa* include flavonoids, organosulfur, and phenol showed anti-allergic (Roldán et al., 2008), anti-inflammatory, antioxidant activities (Lee and Jung, 2016) and smooth muscle relaxant effects (Capasso et al., 2009; Joskova et al., 2011). Anti-asthmatic effects of *A. cepa* extract and its constituent, quercetin, were evaluated in a murine model of asthma (Oliveira et al., 2015). *A. cepa* has many traditional and long history of medical use (Kumar et al., 2010). The effect of *A. cepa* on respiratory diseases were indicated in ancient Iranian traditional medical books (Aghili Shirazi, 1992; Ansarishirazi, 1993; Emami et al., 2010). The effects of *A. cepa* and its constituents on respiratory and allergic diseases such as inhibitory effect on bronchoconstriction were shown (Bornet et al., 2002). World Health Organization (WHO) recommends the use of *A. cepa* extract for the treatment of diseases such as: common colds, coughs, asthma, bronchitis and allergic disease (Kumar et al., 2010).

Therefore, in the present study the relaxant effect of *A. cepa* extract on tracheal smooth muscle and its possible mechanisms were examined.

2. Methods and materials

2.1. Preparation of the extract

A. cepa was bought from a market in Mashhad, Iran in June 2018 and identified by Mr Joharchi, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran and a specimen was deposited in the herbarium of Faculty of Agriculture (Vucher sp. No. E1028-

FUMH). *A. cepa* was peeled and its juice was prepared using a Pars Khazar (Iran) fruit juice producer. The extract was condensed by evaporation using a rotary evaporator at 60–70°C.

2.2. Characterization of the extract of *A. cepa*

HPLC fingerprint of the extract of *A. cepa* was performed using a Shimadzu (LC-2030). Detection wavelengths was set exactly at 254 nm. Lab solution software was utilized for instrument control, data collection and data processing. The column used was an Technochroma, BRISA LC C18 (0.46 × 25, 5 µm). A gradient elution (methanol and water) with a flow rate of 1 ml/min was applied for this analysis. Injection volume for sample and standard solutions was 20 µl (Fig. 1).

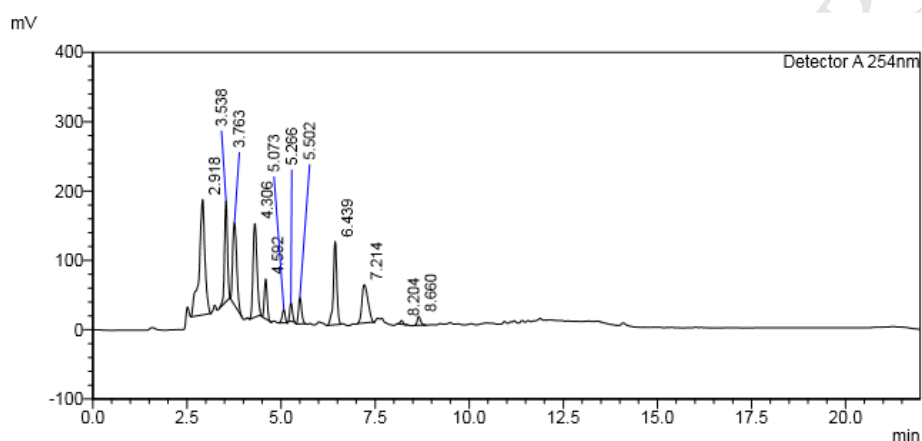


Fig. 1. HPLC finger print of the extract of *A. cepa* at 254 nm (40 mg/ml)

2.1 Animals and experimental groups

Forty seven male Wistar rats (weight, 200-250) were in standard condition including; 22±2 °C temperature, 12h light/dark cycles and free access to standard diet and tap water. They were kept in the Animal House, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. approved by the Ethics Committee of Mashhad University of Medical Sciences (#961800). All experiments on animals were done according to National Laws regarding the use care of laboratory animals. The study was performed in nine groups of rat (Table 1).

Table 1. Different experimental groups of the present study

	Condition	Incubating agent	Mechanisms	n
KCl (60 mM)	Non-incubated tissues			n = 5
		Atropine (1 µM)	Muscarinic receptor inhibition	n = 6
	Incubated tissues	Indomethacin (1 µM)	Cyclooxygenase inhibition	n = 5
		Chlorphenamine (1 µM)	Histamine(H1) receptor inhibition	n = 5
Methacholine (10 µM)	Non-incubated tissues			n = 7
		Diltiazem (5 µM)	Calcium channel blocking	n = 6
	Incubated tissues	Glibenclamide (1 µM)	Potassium channel opening	n = 5

Propranolol (1 μ M) B_2 -adrenoceptor stimulation

n = 8

2.2 Tissue preparation

The chest rats were opened after their scarification and tracheal ring containing 2-3 cartilage was prepared (Boskabady et al., 2010) and mounted in a (10 ml) organ bath containing Krebs-Henseliet (NaCl 120, KCl 4.72, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCl_2 \cdot 2H_2O$ 2.5, $NaHCO_3$ 25 and Dextrose 11 in mM unit). The tissues were aerated with 95% O_2 + 5% CO_2 and kept in 37°C⁰ under isometric tension of 1g for 45 min for tissue equilibration while washed every per 15 min (Boskabady et al., 2010). An isometric transducer (MLT0202, AD Instruments, Australia) connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia) was used for recording of responses.

2.3 Evaluation of smooth muscle relaxant effect

Tracheal smooth muscle (TSM) was contracted by KCl (60 mM), Sigma Chemical Ltd, UK) or methacholine (10 μ M), Sigma Chemical Ltd, UK) for 5 min (Emami et al., 2017). Then, cumulative concentrations of *A. cepa* extract, (2, 4, 8, 16, 32 and 64 mg/ml), theophylline (0.2, 0.4, 0.6 and 0.8 mM) as positive control, or saline (1 ml) as negative control were added to organ bath every 5 min (Emami et al., 2017; Naghdi et al., 2018).

The percentage of relaxation due to each concentration of *A. cepa* extract or theophylline in appropriate to the maximum contraction due to KCl or methacholine was plotted against *A. cepa* extract or theophylline concentrations to generate a concentration response curve in each experiment.

The effective concentration of *A. cepa* extract causing 50% of maximum relaxation effect (EC_{50}) was calculated from concentration response curve as described previously (Emami et al., 2017; Naghdi et al., 2018). In incubated groups, different antagonists or channel blocker were added to organ bath first followed by contraction of TSM by methacholine or KCl after 10 min. Then, the relaxant effect of *A. cepa* extract was examined as described for non-incubated groups. In incubated tissue which caused rightward shift in concentration-response curve of the relaxant effect of the plant, the concentration ratio minus one (CR-1) was also calculated as (EC_{50} in the incubated tissues/ EC_{50} in non-incubated tissues)-1. A schematic diagram for examining the relaxant effect of *A. cepa* extract was shown in Fig. 2.

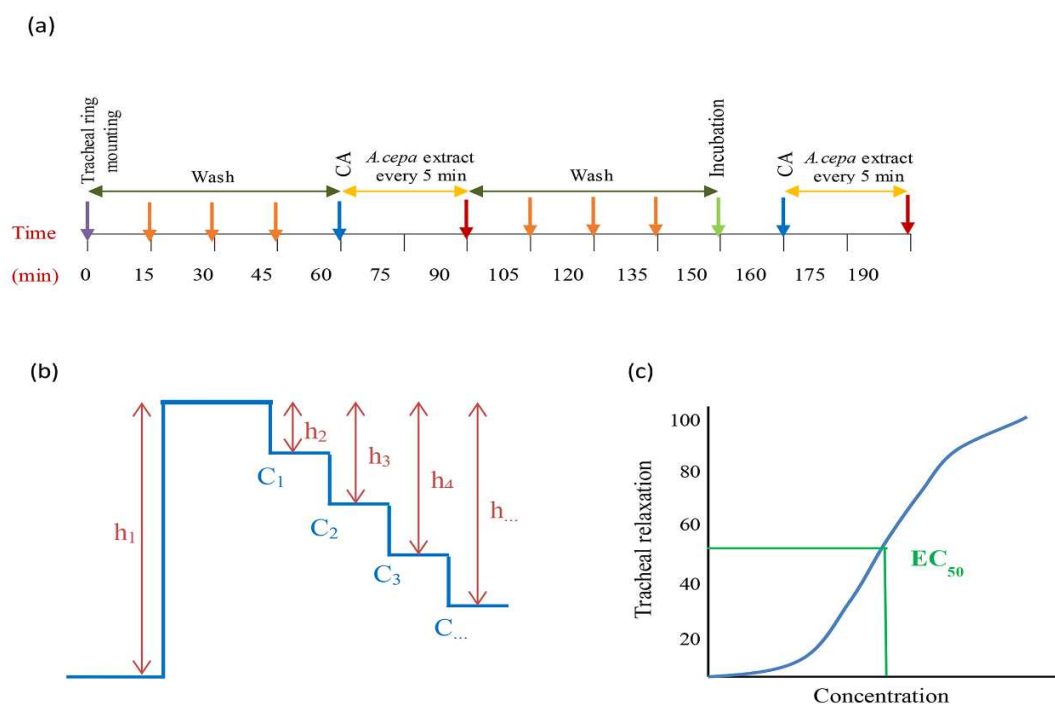


Fig. 2. (a), Protocol (time course) of the examining of the relaxant effect, (b), maximum contraction due to contractile agent (i.e. 10 μ M methacholine or 60 mM KCl, h_1) and concentration-response relaxant effect due to cumulative concentration (c) of *A. cepa* extract, (h_2, h_3, h_4 and $h_{\dots}$), and (c), concentration-response curve of the relaxation effect of *A. cepa* and the method of measurement of EC_{50} . Each tissue was used for one or two experiments and it was equilibrated for at least 1 hr, while it was washed with Krebs-Henseleit solution every 15 min before examining the relaxant effect. Relaxation response of different concentrations of the extract or theophylline was calculated as follows; First concentration = $h_2 / h_1 \times 100$, Second concentrations = $h_3 / h_1 \times 100$, Third concentrations = $h_4 / h_1 \times 100$ and ... concentrations = $h_{\dots} / h_1 \times 100$.

2.4 Statistical analysis

Data were expressed as mean $\pm$ SEM. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. InStat (GraphPad Software, Inc, La Jolla, USA) software was used for statistical analysis. Statistical significance was considered as $P < 0.05$.

3 Results

3.1 The relaxant effect of *A. cepa* extract on TSM contracted by methacholine

3.1.1 Non-incubated tissues

In methacholine (10 μ M)- induced contraction of TSM in non-incubated condition, all concentration of theophylline and the extract caused concentration-dependent and significant relaxant effect ($p < 0.01$ for 2 mg/ml of the extract and $p < 0.001$ for other cases). The relaxant effects of four concentrations of theophylline with those of four higher concentrations of the extract were not significantly different (Fig. 3a).

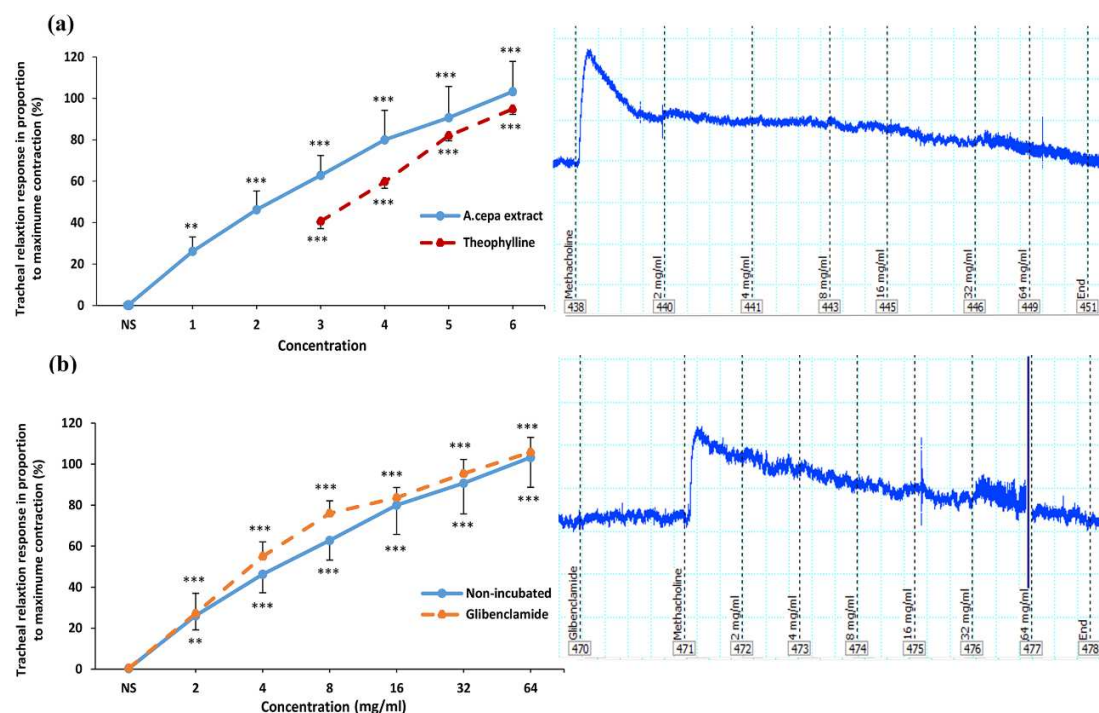


Fig. 3. Concentration-response relaxant effects (mean $\pm$ SEM) of (a) the extract of *A. cepa* in non-incubated TSM contracted by 10 μ M methacholine ($n = 7$). 1, 2, 3, 4, 5 and 6 in X-axis represent six concentration of the extract (2, 4, 8 and 16, 32 and 64 mg/ml) and 3, 4, 5 and 6 represent theophylline concentrations (0.2, 0.4, 0.6 and 0.8 mM) and (b) incubated tissues with glibenclamide (1 μ M, $n = 5$). *** $p < 0.001$ compared to the effect of saline (NS), + $p < 0.05$ compared to the effect of theophylline. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.1.2 Glibenclamide-incubated TSM

In incubated TSM with glibenclamide (1 μ M), the relaxant effect of all concentrations of the extract was significantly higher than saline ($p < 0.001$ for all cases). In addition, there was no significant difference between the relaxant effects of various concentrations of the extract between incubated and non-incubated tissues (Fig. 3b). EC_{50} values of the extract were not significantly different in between incubated tissues with glibenclamide, non-incubated TSM (Fig. 4a).

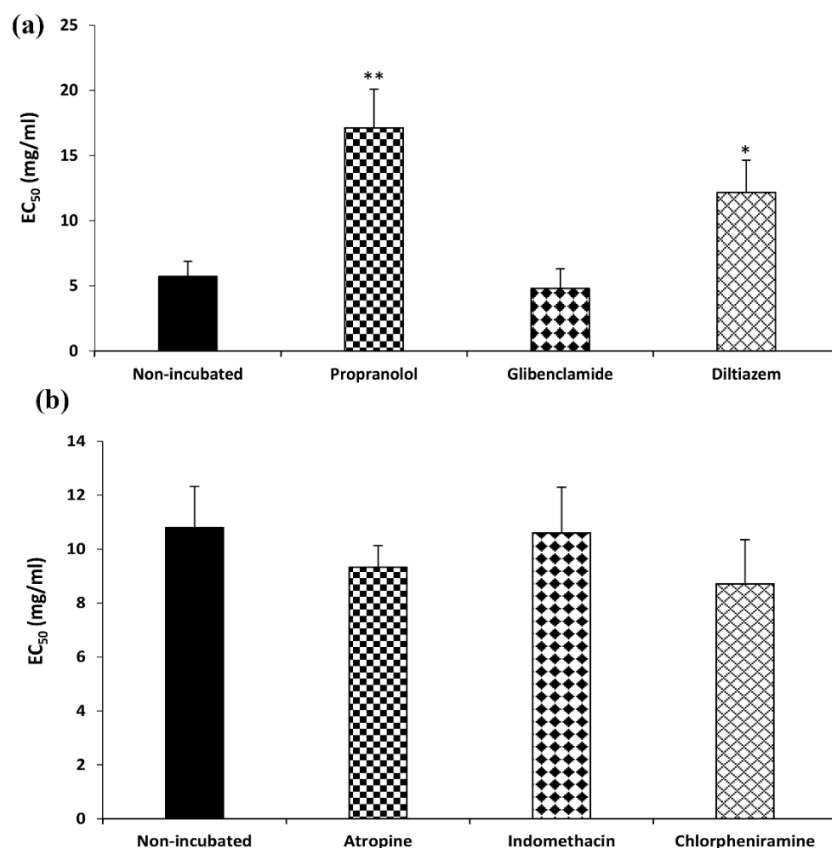


Fig. 4. EC₅₀ values of *A. cepa* extract induced TSM relaxation in non-incubated and incubated TSM with various agents and contracted with methacholine (a) or KCl (b). *, P < 0.05, **, P < 0.01 compared to non-incubated tissues. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.1.3 Propranolol-incubated TSM

In incubated TSM with propranolol (1 μ M), all concentrations of the extract caused significant relaxant effect ($p < 0.001$ for all concentrations). However, the effect of 2, 4 and 16 mg/ml concentrations of the extract in incubated tissues with propranolol were significantly lower than those of non-incubated tissues ($p < 0.05$ for all cases, Fig. 5a). The EC₅₀ value of *A. cepa* in tissue incubated with propranolol was significantly lower than non-incubated TSM contracted by methacholine ($p < 0.001$, Fig. 5a).

A rightward shift in concentration-response relaxation curve of the extract was observed in propranolol-incubated TSM compared to non-incubated tissues and the maximum response was achieved. The (CR-1) value of the extract in incubated TSM with propranolol was 2.65 ± 0.63 .

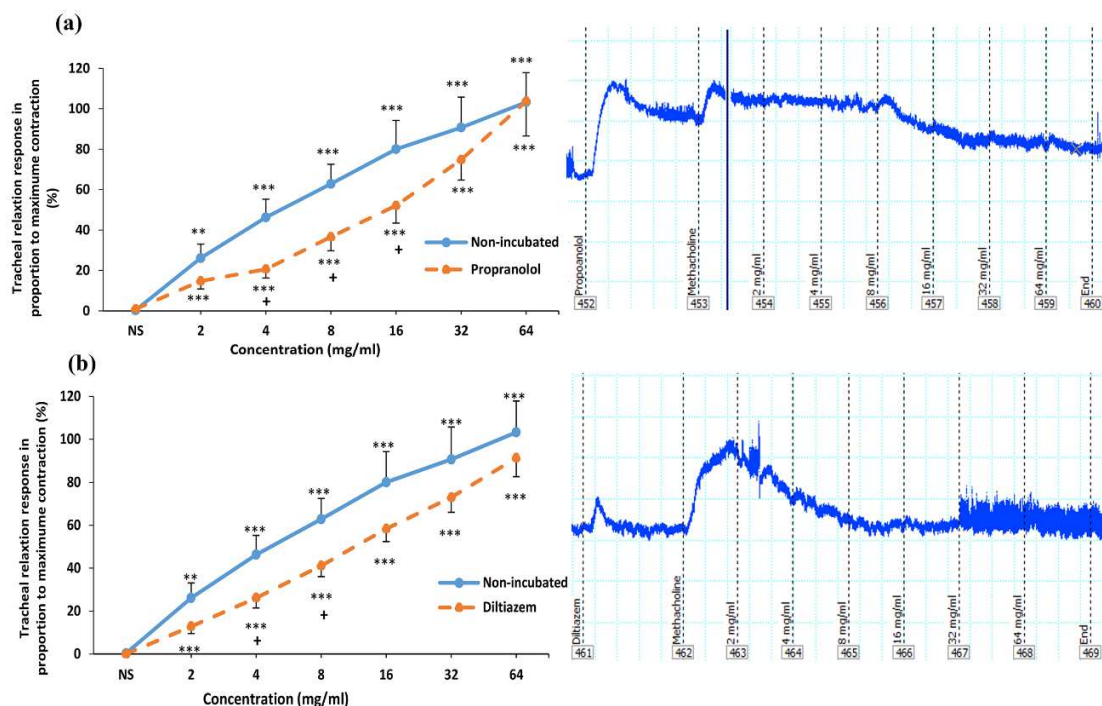


Fig. 5. Concentration-response relaxant effects (mean $\pm$ SEM) of the extract of *A. cepa* on 10 μ M methacholine-induced contraction of TSM in non-incubated ($n = 7$) and incubated tissues with propranolol (1 μ M, $n = 8$), (a) and diltiazem (1 μ M, $n = 6$), (b). ** $P < 0.01$, *** $p < 0.001$, compared to the effect of saline (NS), +: $p < 0.05$, compared to the effect of the extract on non-incubated tissues. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.1.4 Diltiazem-incubated TSM

Various concentrations of the extract showed concentration dependent and significant relaxant effects on tissues incubated with diltiazem ($p < 0.001$ for all cases), However, in incubated TSM with diltiazem (1 μ M), two concentrations of the extract (4 and 8 mg/ml) showed significantly lower relaxant effect compare to non-incubated tissues ($p < 0.05$ for both cases, Fig. 5b). The EC_{50} value of *A. cepa* in tissue incubated with diltiazem was significantly lower than non-incubated TSM contracted by methacholine ($p < 0.05$, Fig. 4a).

A rightward shift in concentration-response relaxation curve of the extract was observed in diltiazem-incubated TSM compared to non-incubated tissues and the maximum response was achieved. In diltiazem incubated TSM, also concentration-response curve of the extract shifted to right, maximum response was achieving and (CR-1) value of the extract was 1.28 ± 0.43 .

3.2 The relaxant effect of *A. cepa* extract on TSM contracted by KCl

3.2.1 Non-incubated tissues

The relaxant effects of all concentrations of theophylline and *A. cepa* extract in non-incubated TSM and contracted by KCl (60 mM) were significantly higher than the effect of saline ($P < 0.01$ for 2 and 4 mg/ml of the extract and $P < 0.001$ for other cases, Fig. 5a). The relaxant effects four higher concentrations of the extract were not significantly different with the effects of four concentrations of theophylline (Fig. 6a).

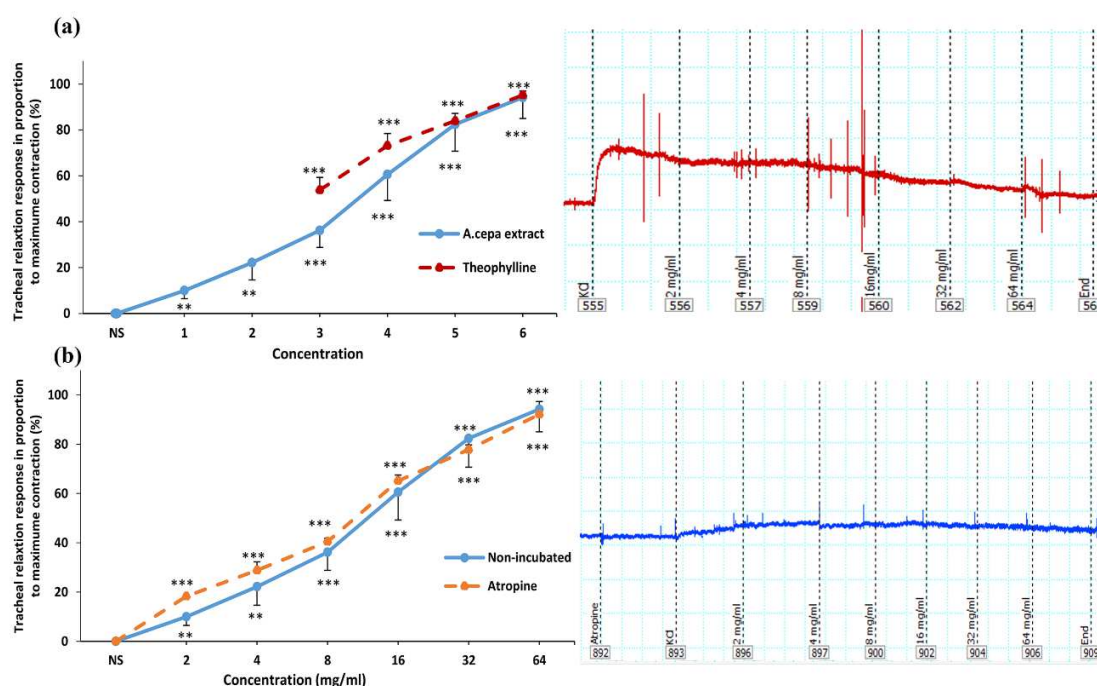


Fig. 6. Concentration-response relaxant effect (mean $\pm$ SEM) of (a) the extract of *A. cepa* in non-incubated TSM contracted by 60 mM KCl ($n = 5$). 1, 2, 3, 4, 5 and 6 in X-axis represent six concentration of the extract (2, 4, 8, 16, 32 and 64 mg/ml) and 3, 4, 5 and 6 represent theophylline concentrations (0.2, 0.4, 0.6 and 0.8 mM) and (b) incubated tissues with atropine (1 μ M, $n = 6$). **: $P < 0.01$ ***: $p < 0.001$, compared to the effect of saline (NS), +: $p < 0.05$ compared to the effect of the extract on non-incubated tissues. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.2.2 Atropine, chlorpheniramine and indomethacin-incubated TSM

All concentrations of *A. cepa* extract in incubated TSM with atropine, chlorpheniramine and indomethacin showed concentration dependent relaxant effects ($p < 0.05$ to $p < 0.001$). The relaxant effects of different concentrations of the extract between incubated tissues with these three drugs and non-incubated TSM were not significantly different (Figs. 6b and 7). EC_{50} values of the extract were not significantly different between incubated tissues with atropine, chlorpheniramine, indomethacin with that of non-incubated condition (Fig. 4b).

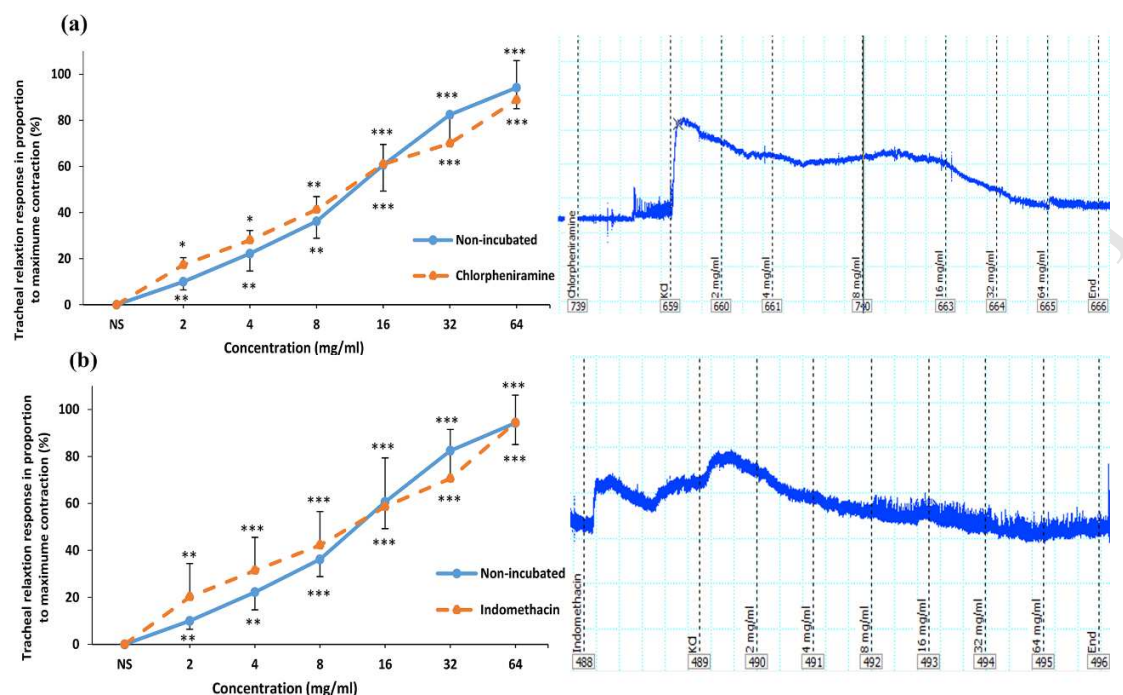


Fig. 7. Concentration-response relaxant effects (mean $\pm$ SEM) of the extract of *A. cepa* on 60 mM KCl-induced contraction of TSM in non-incubated ($n = 5$) and incubated tissues with chlorpheniramine (1 μ M, $n=5$) (a) and incubated tissues with indomethacin (1 μ M, $n = 5$) (b) **: $P < 0.01$ ***: $p < 0.001$, compared to the effect of saline (NS), +: $p < 0.05$ compared to the effect of the extract on non-incubated tissues. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.3 The relaxant effect of *A. cepa* extract in non-incubated TSM contracted by methacholine compared to tissues contracted by KCl

The relaxant effects of different concentrations of the extract of *A. cepa* in non-incubated TSM contracted by methacholine (10 μ M) and KCl (60 mM) were not significantly different (Fig. 8).

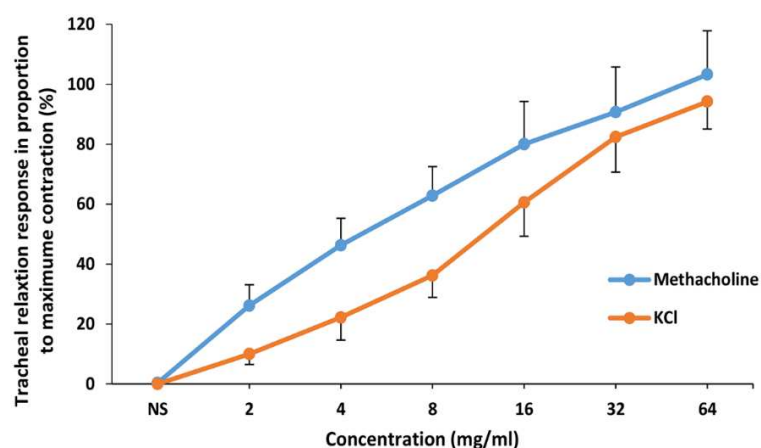


Fig. 8. Concentration-response relaxant effects of the extract of *A. cepa* in non-incubated tissues, contracted with 10 μ M methacholine (n = 7) and contracted with 60 mM KCl (n = 5). There was no significant difference between the relaxant effect of the plant on methacholine and KCl induced TSM contraction. ANOVA with Tukey Kramer post-test was used for statistical comparison.

3.4 Correlations of the relaxant effect of *A. cepa* and theophylline with their concentrations

The relaxant effect of theophylline and *A. cepa* in non-incubated TSM contracted by methacholine (10 μ M) and KCl (60 mM) were significantly correlate with their concentrations ($P < 0.001$ and $P < 0.05$ for methacholine and KCl induced contraction, respectively for theophylline $P < 0.001$ for both cases of *A. cepa*), (Table 2). There were significant correlations between the relaxant effect of *A. cepa* and its concentration in incubated TSM by all agents and contracted by either methacholine or KCl ($P < 0.001$ for all cases, Table 2).

Table 2. Correlations between the relaxant effect of *A. cepa* and theophylline with their concentrations

Contractile agents	Studied agents	Conditions	R	P Value
KCl	<i>A. cepa</i>	Non-Inc	0.784	$P < 0.001$
		Atropine-Inc	0.872	$P < 0.001$
		Indomethacin-Inc	0.867	$P < 0.001$
		Chlorpheniramine-Inc	0.667	$P < 0.001$
	Theophylline	Non-Inc	0.817	$P < 0.001$
		Non-Inc	0.573	$P < 0.001$
		Diltiazem-Inc	0.814	$P < 0.001$
		Glibenclamide-Inc	0.706	$P < 0.001$
Methacholine	<i>A. cepa</i>	Propranolol-Inc	0.738	$P < 0.001$
		Non-Inc	0.320	$P < 0.05$

Data were presented as mean $\pm$ SEM

4 Discussion

The results showed potent and concentration dependent relaxant effect of *A. cepa* extract in non-incubated TSM contracted by KCl and methacholine comparable to the effect of theophylline. There was no significant difference between the relaxant effect of *A. cepa* extract on non-incubated TSM contracted by methacholine and KCl. There were also significant correlations between *A. cepa* extract concentrations and their relaxant effects. The relaxant effect of *A. cepa* extract on rat ileum (Naseri et al., 2008b) and its vascular smooth muscle and hypotensive properties (Naseri et al., 2008a)

as well as the effect of the plant on smooth muscle contraction in a murine model of asthma (Oliveira et al., 2015) were previously demonstrated. The relaxant effect of quercetin a major constituent of *A. cepa* on rat tracheal smooth muscle (Capasso et al., 2009) and its bronchodilatory effect in experimental allergic asthma (Joskova et al., 2011) were also reported. The relaxant effect of *A. cepa* on methacholine-induced TSM contraction in non-incubated tissues may indicate the effect of the plant on calcium channels and β_2 adrenoceptors. On KCl-induced contraction of non-incubated tissues also, the relaxant effect of the plant may show the effect of *A. cepa* extract on muscarinic receptors, cyclooxygenase pathway or histamine receptors. However, these mechanisms are non-specific and should be evaluated more precisely using specific receptor antagonists and channel blockers.

Therefore, in order to evaluate the contribution of β_2 -receptor stimulating effect on the relaxant effect of *A. cepa* extract, the effect of the extract on incubated TSM with propranolol and contracted by methacholine was studied (Linden et al., 1993). The results of this group showed lower relaxant effect of the plant compared to non-incubated tissues. The EC_{50} value of the extract obtained in incubated tissues with propranolol was also significantly lower than non-incubated tissues. These results indicated a stimulatory effect of the extract of *A. cepa* on β_2 -receptor which could contribute on the relaxant effect of the plant on TSM.

Rightward shift of concentration-response relaxation curve of the extract, achieving the maximum relaxation response and positive (CR-1) in incubated TSM with propranolol as another pharmacological principle evidence for the stimulatory effect of the extract on β_2 adrenoceptors.

To study the effect of *A. cepa* on calcium channels and the role of this mechanism in the relaxant effect of the plant, its relaxant effect was studied on TSM incubated with diltiazem (Miyahara et al., 1993). The results showed significantly lower relaxant effects of two concentrations of *A. cepa* (4 and 8 mg/ml) in incubated tissues with diltiazem compared to non-incubated TSM. The EC_{50} values of *A. cepa* in incubated tissues with diltiazem were also significantly lower than non-incubated TSM with diltiazem. Lower relaxant effect of the plant and its EC_{50} indicated a calcium channel blocking effect for the plant which perhaps contribute to its relaxant effect on TSM.

Incubation of TSM with diltiazem also caused rightward shift in concentration-response relaxation curve of the extract and achievement of the maximum relaxation response and positive

(CR-1) value which are further pharmacological evidences for the inhibitory effect of the extract on calcium channels.

In previous studies, the inhibitory effect of *A. cepa* on calcium channels of ileum (Naseri et al., 2008b) and vascular (Naseri et al., 2008a) smooth muscle contractions were demonstrated which support the results of the present study.

To assess the effect of *A. cepa* on muscarinic (Lronards et al., 1992) and histamine (H_1), (Popa, 1977) receptors, ATP-sensitive potassium channels (Miura et al., 1992) and arachidonic acid metabolism (Slattery et al., 2001) pathways and the role of this mechanism, the relaxant effect of the plant was examined on tracheal smooth muscle incubated with atropine, chlorpheniramine, glibenclamide and indomethacin. There was no any significant difference in the relaxant effects of the plant between non-incubated and incubated tissues with these drugs. Therefore, the plant was not affected the above receptors, channels or pathways and these mechanisms were not contributing in the relaxant effect of the plant.

In the present study, the relatively potent relaxant effect of *A. cepa* was shown for first time. In addition, the possible mechanisms responsible for the relaxant effect was also examined. The results showed that the one mechanism responsible for the relaxant effect of the plant is to β_2 -adrenergic receptors stimulatory effect which is the most effective bronchodilator drug (Arunlakshana and Schild, 1959). In addition, the results also showed the calcium channel blocking effect of the plant is contributed in its relaxant effect.

Based on pharmacological principles, a parallel shift to the right of the concentration-response curve to an agent in the presence of a competitive antagonist compared to the absence of antagonist, indicates the effect of the studied agent on this receptor (Arunlakshana and Schild, 1959). In addition, several studies on various extract of medicinal plants and their constituents have already been published using the same method, including the relaxant effect of hydro-ethanolic extract of *Lavandula angustifolia* flowers on TSM (Barnes, 1998). It is impossible to evaluate all factors in a single study. Therefore, siRNA targeting on specific receptor for addressing of the role of receptors-related signaling involved in the extract is a limitation of the present study which should be addressed in further studies.

The other possible mechanisms responsible for the relaxant effect of the plant including the activation of rectifying K^+ channels, Ca^{2+} -and voltage-activated K^+ channels and voltage-gated channels, glucocorticoid receptor activation, involvement of nitric oxide synthase, stimulation/potencialization of soluble guanylyl cyclase, phosphodiesterase inhibition (Menezes et al., 2018), and inhibition of non-adrenergic non-cholinergic (NANC) system should be also evaluated in further studies.

Generally, the results of this study showed a potent relaxant effect of *A. cepa* on TSM which may indicate a bronchodilator effect for the plant on obstructive pulmonary diseases. The results also suggested that the relaxant effect of the plant is perhaps due to β_2 -adrenergic receptors stimulatory and/or calcium channel blockade.

Although the relaxant effect of quercetin on rat tracheal smooth muscle was shown previously (Emami et al., 2017), *A. cepa* contain rich in flavonols including quercetin,

kaempferol, tannins and organosulfur compounds like allyl propyl disulfide and diallyl disulfide (Miean and Mohamed, 2001). Therefore, the relaxant effects of this plant may not be due to quercetin alone and other flavonols may play a role in the smooth muscle relaxation of the plant. Moreover, as described in the methods and materials section, in this study, *A. cepa* was peeled and its juice was prepared freshly which its quercetin content is 48-fold less than in the onion peel as described previously (Goshev, 2008).

Previous studies also showed anti-oxidant, immunomodulatory (Marefati et al., 2018), and anti-inflammatory effects on tracheal responsiveness and lung inflammation (Ghorani et al., 2018) for *A. cepa* extract and its constituent quercetin on cytokines, inflammatory cells, and smooth muscle contraction in animal models of asthma (Oliveira et al., 2015). Therefore, the results of the present and previous studies may suggest the therapeutic effect of the plant on asthma as both relieving (bronchodilator) and preventive (anti-inflammatory) mechanisms. The bronchodilatory effects of *A. cepa* in obstructive pulmonary diseases also should be examined in clinical studies for evaluation of the possible clinical use of the plant.

5 Conclusion

The Results showed concentration dependent and potent relaxant effect for *A. cepa* extract on TSM comparable to the effect of theophylline. Therefore, the plant may be used as bronchodilatory drug in obstructive pulmonary diseases. The results also suggest that the relaxant effect of the plant is mediated by β_2 -adrenergic receptors stimulatory and calcium channels inhibitory effects.

6 Conflict of interest

The authors declare that there are no conflicts of interest.

7 Acknowledgment

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8 Authors Contribution

MHB conceived the presented idea, encouraged AM to investigate the relaxant effect of *A. cepa* extract on rat tracheal smooth muscle, and supervised the findings of this work. AM, FA and SS carried out the experiment. MJ and ZG helped contributed to the interpretation of the results. AM wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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