Original Article

Bronchodilatory and B-adrenergic effects of methanolic and aqueous extracts of *Althaea* root on isolated tracheobronchial smooth rat muscle

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Abstract

Background: The smooth muscle contractions of the tracheobronchial airways are mediated through the balance of adrenergic, cholinergic and peptidergic nervous mechanisms. This research was designed to determine the bronchodilatory and B-adrenergic effects of methanolic and aqueous extracts of root *Althaea* on the isolated tracheobronchial smooth muscle of the rat.

Materials and Methods: In this experimental study, 116 tracheobronchial sections (5 mm) from 58 healthy male Sprague-Dawley rats were dissected and divided into 23 groups. The effect of methanolic and aqueous extracts of the root *Althaea* was assayed at different concentrations (0.2, 0.6, 2.6, 6.6, 14.6 μg/ml) and epinephrine (5 μm) in the presence and absence of propranolol (1 μM) under one g tension based on the isometric method. This assay was recorded in an organ bath containing Krebs-Henseleit solution for tracheobronchial smooth muscle contractions using potassium chloride (KCl) (60 mM) induction.

Results: Epinephrine (5 μm) alone and root methanolic and aqueous extract concentrations (0.6-14.6 μg/ml) reduced tracheobronchial smooth muscle contractions induced using KCl (60 mM) in a dose dependent manner. Propranolol inhibited the antispasmodic effect of epinephrine on tracheobronchial smooth muscle contractions, but could not reduce the antispasmodic effect of the root extract concentrations.

Conclusion: The methanolic and aqueous extracts of *Althaea* root inhibited the tracheobronchial smooth muscle contractions of rats in a dose dependent manner, but B-adrenergic receptors do not appear to engage in this process. Understanding the mechanism of this process can be useful in the treatment of pulmonary obstructive diseases like asthma.

Key Words: Adrenergic, *Althaea* root, bronchodilator, methanolic and aqueous extracts, tracheobronchial muscle

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INTRODUCTION

Asthma is a chronic inflammatory disease of the airways that is identified by increased respiratory tract responsiveness to a variety of endogenous and exogenous stimuli. Its physiological manifestations are in the form of reversible obstructions of the small respiratory tracts/alveoli that are clinically detected

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by shortness of breath (dyspnea), coughing and wheezing.[1,2]

Smooth muscle cells form much of the airways, blood vessels, gastrointestinal tract and other systems. Neuronal receptors in airway smooth muscle cells regulate the neuronal and chemical action of mediators and cause constriction or dilatation of the airways.[3,4] The most important receptors in smooth muscle airway linings are beta-adrenergic and cholinergic receptors. As opposed to beta-adrenergic receptors that cause increased dilatation of the respiratory tract, cholinergic receptors cause contraction, narrowing and stimulation of bronchial glands.[5]

Modern principles of the treatment of asthma are based on short-term or long-term approaches.[6] Short-term treatments increase respiratory tract dilatation (broncho-dilatation) and provide rapid resolution of symptoms; long-term treatments suppress inflammation of the respiratory tract and prevent the incidence of the disease.[6,7] Chemical agonists of beta-adrenergic receptors cause increased broncho-dilatation and are considered the most effective bronchodilators to manage acute asthma symptoms.[8] Nonetheless, these chemical compositions have many side-effects, including skeletal muscle tremor, tachycardia, tolerance and activation of other receptors by the excessive consumption of high doses.[9]

Hollyhock is a perennial plant that reaches a height of 150 cm. This plant is from the family *Malvacaea* and is native to parts of Europe and Asia, especially tropical regions. There are two species of hollyhock, *Alcea* and *Althaea*, that have historically been linked to countless medicinal attributes.[10] The use of the hollyhock species *Althaea officinalis* L, stems from its antibiotic and anti-inflammatory properties; it has long been used in traditional medicine for the treatment of oral discomfort, respiratory tract diseases, chest diseases and conjunctivitis.[11] *Althaea* root extract has antitussive and pain relief properties and has been traditionally used to reduce inflammation of the lungs, severe coughing, rhinopharyngitis, angina and bronchitis.[12]

The chemical composition of *Althaea* root is in mucilage form with polysaccharides, flavonoids, tannins, lecithin, pectin and astriola.[13-16] Studies have shown that *Althaea* root mucilage helps relieve inflammation caused by bronchitis and asthma and improve dryness, tenderness and irritation of the chest and throat normally caused by cold and chronic coughs.[14-17] Studies on the polysaccharide components extracted from *Althaea* root mucilage have shown that these compounds revive the trachea mucous layer, reduce irritation of the respiratory tract and inhibit coughs in cats.[18] The rhamnogalacturonan in *Althaea* root reduces citric acid-induced coughs in guinea pigs and potassium canal inhibitors do not alter this effect.[19] There have also been reports of antioxidant and nitric oxide/cyclic guanosine monophosphate cGMP-relaxation properties in vascular endothelial cells by the flavonoids in *Althaea* root.[20]

Traditional medicine plays a significant role in health-care in developing countries. The varied climates of Iran are conducive to the growth of species of plants with medicinal properties that are catalogued in reference books on traditional medicine. Given the unfavorable side-effects of manufactured drugs, it is advisable to conduct a comprehensive scientific study of the pharmacological properties of traditional plants in Iran. An extensive literature review has shown that no study has been conducted on the pharmacological effects of *Althaea* root extracts on the smooth tracheobronchial muscles. The present study investigated the bronchodilatory effects of both aqueous and methanolic *Althaea* root extracts on stimulated smooth trachea muscle in rats and their agonistic properties on beta adrenergic receptors.

MATERIALS AND METHODS

Preparation of aqueous and methanolic Althaea root extract

Althaea roots were dried in the shade and then ground to a powder. Extracts were prepared by dissolving the powder in distilled water and then in methanol for 9 h in a Soxhlet extractor. To separate the extract from the solvent, the solution was placed in a rotary evaporator for 1 h. Each extract was poured into an hour-glass and placed in a kiln for 48 h to fully evaporate the remaining solvent. Each extract powder was maintained at 4°C until use.[17]

Laboratory animals and preparation of samples

In this experiment, 58 male Sprague-Dawley rats, weighing 220 ± 5 g were procured from Pasteur Institute in Tehran in compliance with regulations for animal maintenance and research. Four rats were placed in each polycarbonate cage and maintained in the Kashan University of Medical Sciences animal house. After administering 50 mg/kg ketamine to the rats, they were dissected from the chest to under the jaw and about 1 cm of trachea was removed. The removed trachea segment was immediately placed in a cold oxygenated solution of Krebs-Henseleit buffer with a pH of 7.4 (118 mM sodium chloride, 4.7 mM potassium chloride (KCl), 2.25 mM calcium chloride, 2.52 mM magnesium sulfate, 1.64 mM potassium dihydrogen phosphate, 1.18 mm sodium hydrogen carbonate and 5.5 mM glucose).

Experiment method

To adjust the environment outside the body, the trachea segment was initially refrigerated at 4°C and then transferred to a tissue bath (American Biosystems) containing 50 ml solution of Krebs-Henseleit buffer at 37°C. The trachea was connected to two horizontal steel bars, one submerged in the solution bath and the other connected by a thread to an isometric transducer (F-60, American Biosystems) while maintaining a constant flow of oxygen bubbles from the bottom of the bath (Longfei oxygen generating device, China). Tracheal contractions were recorded using a 4-channel physio-graph (American Biosystems) on paper at a speed of 0.1 mm/s. The initial tension was 1.5 g for a 60 min adaptation period. The bath solution was replaced every 15 min.[21]

Dissolution in Krebs-Henseleit buffer produced aqueous and methanolic extract concentrations of 14.6, 6.6, 2.6, 0.6 and 0.2 μg. After an adaptation period, the trachea segment was contracted using 60 mM KCl to reach a plateau, then 0.1 ml of aqueous extract concentration was added to the isolated tissue environment in an organ bath and the level of relaxation was measured each time. After each test, the bath solution was replaced 3 times at 10 min intervals. When the contraction returned to a normal level, the test was repeated 3 times and the mean value was used as the sample response for each test. The experiment was performed under the same conditions for different concentrations of *Althaea* methanolic extract.

To examine the role of adrenergic receptors of smooth trachea muscle in the performance of aqueous and methanolic extracts, 1 μM propranolol (as a beta-adrenergic receptor blocker) was used synergistically with each hollyhock root extract. The results from the muscle contraction behaviors for 5 μM epinephrine and 1 μM propranolol synergy were compared.

Statistical analysis

The trachea smooth muscle contraction from the 60 μM KCl was considered to be 100% and the reduction in the percentage of contractile change induced by each aqueous and methanolic extract and drugs for the KCl contractions were calculated. SPSS-16 software (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412. Patent No. 7,023,453) was used for comparison of all mean values obtained. One-way analysis of variance and Tukey *post hoc* tests were used to compare the means of control and the experimental groups and $P < 0.05$ was considered to be significant.

RESULTS

The results of the effect of aqueous and methanolic hollyhock root extract concentrations on trachea smooth muscle contractions induced by 60 μM KCl indicated that the 0.2 μg/ml concentration of both extracts had no significant relaxation effect. Methanolic extract concentrations of 0.6-14.6 μg/ml significantly reduced rat trachea smooth muscle spasmodic power induced by 60 μM KCl ($P < 0.001$). This significant reduction was also observed in aqueous extract concentrations of 0.6-14.6 μg/ml ($P < 0.001$).

There was a significant dose dependent reduction in trachea smooth muscle contraction from both extracts; however, no significant difference was observed between the results of the effects of the individual extracts. To examine the agonistic effects of *Althea* root extracts, 0.6-14.6 μg/ml concentrations of aqueous and methanolic extracts were added with 1 μM propranolol to the medium. The results indicated that both extracts in synergy with propranolol still showed relaxation effects and the propranolol caused no significant change in antispasmodic effects of either extract [Figure 1].

The relaxation effect of epinephrine synergy with propranolol on reducing trachea smooth muscle spasm was compared. 5 μM epinephrine (as a beta-adrenergic receptor agonist) significantly reduced trachea smooth muscle spasmodic power induced by 60 mM KCl ($P < 0.001$). The presence of propranolol in the medium significantly inhibited the action of the 5 μM epinephrine and showed that propranolol, as an adrenergic receptor antagonist, inhibited epinephrine action and its relaxation effect [Figure 2].

The antispasmodic effects of 0.6-2.6 μg/ml concentrations of both aqueous and methanolic extracts with 5 μM epinephrine were investigated. A comparison of the epinephrine group and extract group showed that 5 μM epinephrine antispasmodic effects for 0.6 and 2.6 μg/ml concentrations of aqueous and methanolic extracts was significantly different for both groups ($P < 0.001$) [Figure 3].

DISCUSSION

Today, the side-effects of manufactured medicines have made herbal medicines particularly attractive because of their homologous medicinal compounds, low side-effects and higher compatibility with the body. Since asthma is a relatively common disease,

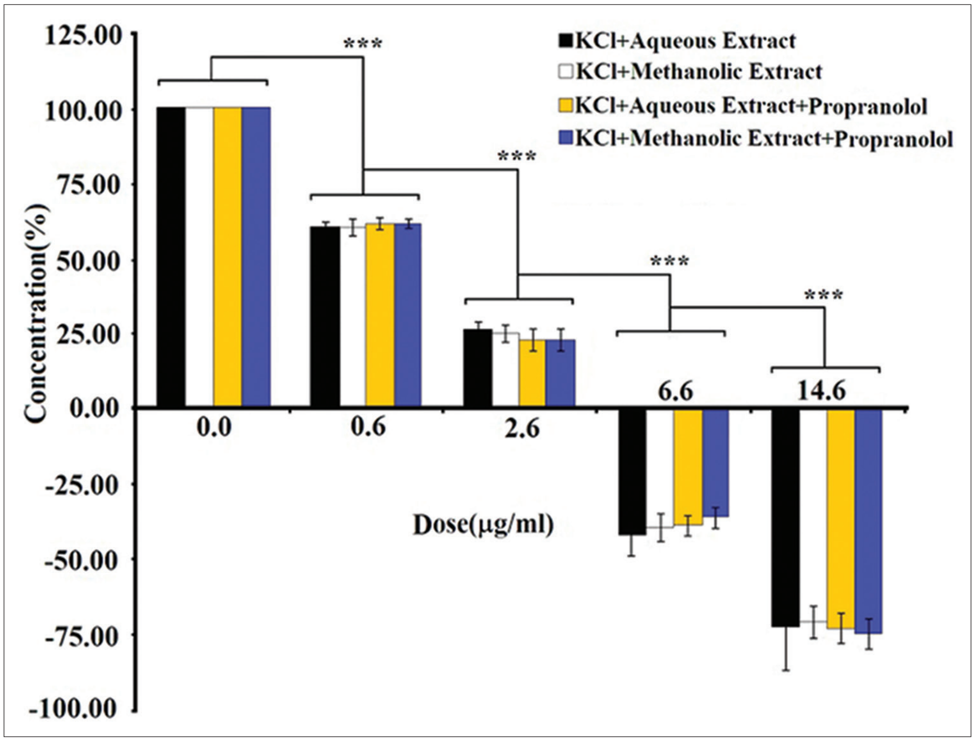

Figure 1: Comparison of the cumulative effect of Althaearoot aqueous and methanolic extracts at concentrations of 14.6, 6.6, 2.6, and 0.6 μg/ml with and without 1 μM propranolol on the contraction power of trachea muscle induced by potassium chloride (60 mM). The statistical differences in the data in the experimental groups were found using one-way analysis of variance and Tukey tests with SPSS-16 software; *P*<0.001 was considered as significant for all concentration

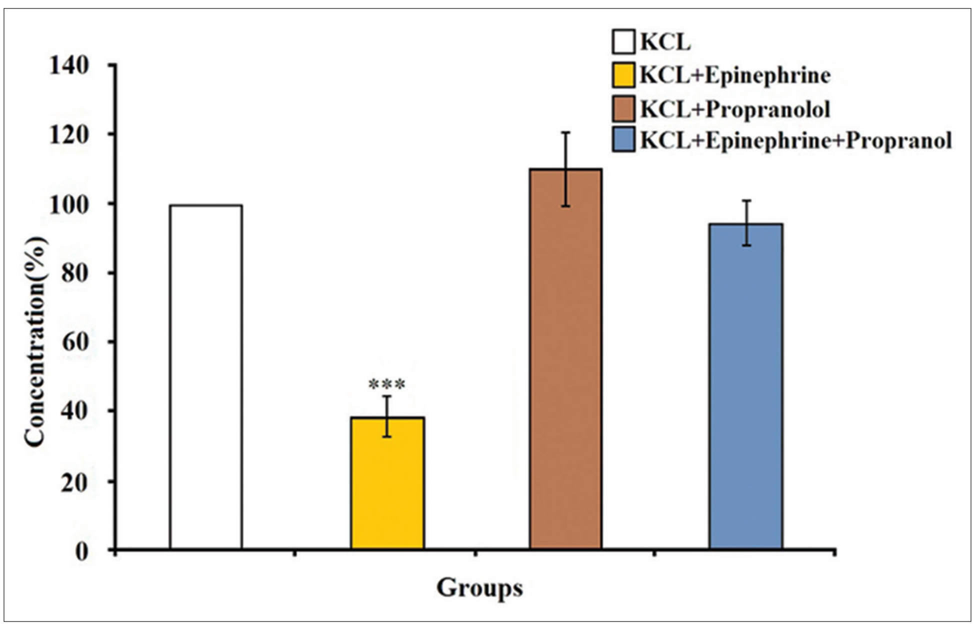

Figure 2: Effect of epinephrine on rat trachea smooth muscle contractions induced using potassium chloride (60 mM) with and without 1 μm propranolol. The statistical differences in the data in the experimental groups were found using one-way analysis of variance and Tukey tests with SPSS-16 software; *P*<0.001was considered as significant for all concentrations

the proper identification of herbal drug characteristics can open a way for producing drugs of natural origin as alternatives to manufactured drugs for long-term use and chronic diseases in the future.[22,23]

In the present study, trachea smooth muscle contraction induced using a 60 mM KCl model was used to investigate the bronchodilatory properties of aqueous and methanolic root extracts of the hollyhock

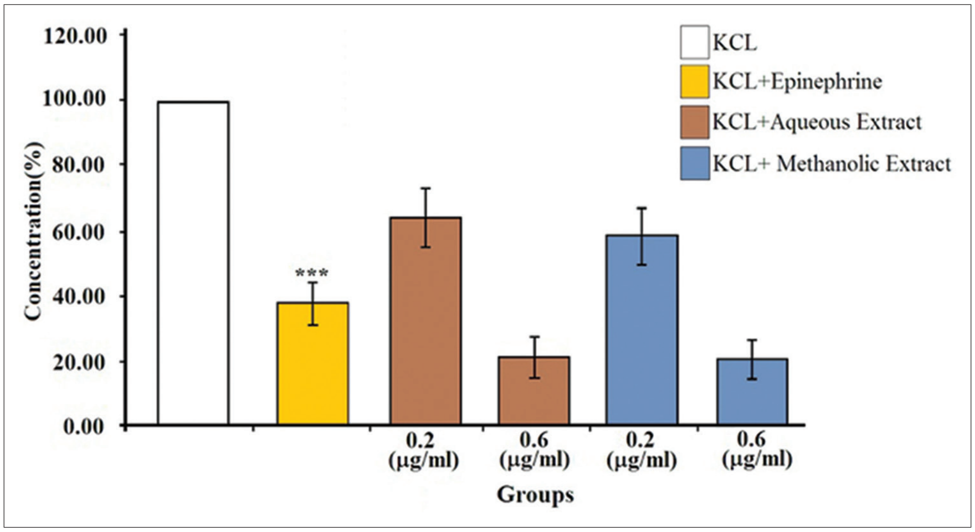

Figure 3: Comparison of the inhibitory effects of 5 μM epinephrine with 0.2 and 0.6 μg/ml concentrations of aqueous and methanolic extracts on contractions induced using potassium chloride (60 mM). The statistical differences in the data in the experiment groups were found using one-way analysis of variance and Tukey tests with SPSS-16 software. Between the potassium and epinephrine groups, the difference was significant at *P*<0.001

plant.[24] In a study conducted at science academy hospital in Russia on children aged 5-12 years with bronchial asthma, it was shown that a brewed combination of herbal medicines that included the hollyhock flower had more positive antispasmodic effects on the smooth muscles of the airways compared to common drugs. This was a result of the spasmolytic and expectorant properties of the brew; however, it was not clarified, which plant extract or mechanism was effective.[25]

In the present experiment, it was observed that the doses of 14.6, 6.6, 2.6 and 0.6 μg/ml of aqueous and methanolic *Althaea* root extract significantly attenuated contractions induced by 60 mM KCl and that this reduction in spasmodic power of smooth muscle increased as the dosage of the extracts increased. To assess the agonistic properties of extracts, propranolol with 1 μM concentration was used as a non-elective antagonist of beta adrenergic receptors. Studies have shown that antagonists of beta adrenergic receptors reduce smooth muscle cyclic adenosine monophosphate cells through inhibition of G protein-dependent cellular adenylyl cyclase pathways.[21] The results showed that propranolol did not have a significant effect on the relaxation effect of aqueous nor methanolic extracts, but significantly reduced the antispasmodic effect of 5 μM epinephrine as an agonist of adrenergic receptors. The absence of an effect for propranolol on relaxation of the extracts for KCl induced contractions and its effect on epinephrine function indicate the lack of compounds with agonistic effects on trachea smooth muscle beta adrenergic receptors in aqueous and methanolic root extracts of hollyhock. The same rationale is applicable to the lack of an effect of propranolol on the inhibitory action of ivy leaf extract on uterine smooth muscle contractions.[26]

In this study, both aqueous and methanolic *Althaea* root extracts showed similar results for the reduction in rat trachea smooth muscle contractions and demonstrated a dose-dependent dilatory effect on smooth muscle contractions. No significant difference was observed for the same concentrations of extracts individually or with 1 μM propranolol. It appears that antispasmodic elements are equally present in both extracts. Both compositions in water and in fat can be extracted using alcoholic extraction, but the aqueous extraction method only extracts water-based compositions. Given the similar antispasmodic properties and lack of statistical difference between the two extracts, the most effective elements to reduce the spasmodic power of rat trachea in *Althaea* root are aqueous.

Studies have shown that anticholinergic compounds, activators of non-adrenergic non-cholinergic receptor inhibitory pathways, calcium channel antagonists, potassium channel activators and adrenergic receptor agonists play a significant role in regulating the tone of the respiratory tract.[27-30] Few studies were found on the antispasmodic effects of hollyhock plant extracts prepared from different regions and their mechanisms. Sutovská *et al*. investigated the effect of rhamnogalacturonan extracted from *Althaea* root on smooth muscle of the respiratory airways of guinea pigs and showed that the effect of this polymeric compound on cough suppression was caused by the 5-hydroxytryptamine 2 serotonergic receptors.[31] Kobayashi *et al*. also showed that hollyhock flower extract can reduce the activity of melanocyte induced endothelin-1 by preventing intracellular calcium movement.[20] In general, from the failure of agonistic effects for aqueous and methanolic extracts on beta adrenergic receptors in this study, it can be suggested that effective elements for the antispasmodic effect of the extracts act through other receptors. Clarifying the exact bronchodilatory mechanism of these two extracts requires extraction of the effective ingredients and examining their influence on all neuronal and non-neuronal receptors. This can provide the means to investigate the relaxation mechanism of influential elements of hollyhock root and their potential for asthma treatment drug production.

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