The Bronchodilator Potential of Astragalus sarcocolla: An in vitro Experiment

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ABSTRACT

Objective: To evaluate the bronchodilatory mechanism of Astragalus sarcocolla (ASE) extract on tracheal smooth muscles of rabbits.

Study Design: In-vitro experimental study.

Place and Duration of the Study: The animal house of CMH Lahore Medical College, Lahore, and Institute of Dentistry, NUMS, from October 2022 to May 2023.

Methodology: Six rabbits were randomly divided into four groups. After euthanising the rabbit, the trachea was carefully dissected out and stabilised in Kreb’s Henseleit solution for 30 minutes and then, stimulated by acetylcholine (Ach) 1µm, under mimicked physiological conditions. Group I served as the control group with tracheal smooth muscles stabilised with 1g tension. In Group II (positive control), tracheal smooth muscles were stimulated by potassium chloride (KCl) (80 mM and 25 mM, respectively) to get maximum tracheal smooth muscle contractions. Later, the tissue was exposed to theophylline with three molar concentrations 0.2, 0.4, 0.6, and 0.8 mM, and cumulative dose response curves were formed. In Group III (ASE group), tracheal smooth muscles were stimulated by KCl (80 mM and 25 mM) and was exposed to increasing concentration of ASE. In group IV, tissue was stimulated by KCl (25 mM) and glibenclamide (3 µM), later exposed to increasing concentration of ASE to confirm the bronchodilatory mechanism. The change in isometric contraction of the tissue was recorded using the force displacement transducer connected to a PowerLab data acquisition system. Concentration response curves were drawn, and median effective concentrations (EC50 values) and percentage inhibition were calculated. Non-linear regression was applied for the analysis of the concentration-response curves.

Results: ASE inhibited the KCl-induced low potassium (25 mM) contractions (EC50 = 0.38 mg/ml, 95% CI: 0.04 - 0.38, n = 6). It only partially inhibited the high potassium-induced contractions in tracheal smooth muscles. Pretreatment with glibenclamide showed a rightward shift of the dose-response curve. Theophylline and ASE significantly reduced the low K+ induced smooth muscle contractions in comparison to the control group (p <0.001, each).

Conclusion: Astragalus sarcocolla extract produced bronchodilator effects through the activation of ATP sensitive potassium channels in isolated rabbit trachea.

Key Words: Astragalus sarcocolla, Bronchodilators, ATP-sensitive potassium channels, Effective concentration 50, Concentration response curves.


INTRODUCTION

Asthma is characterised by recurrent episodic wheezing, breathlessness, and bouts of cough often leading to severe symptoms like tachypnoea cyanosis and respiratory failure. The complexity of the development of asthma is due to multiple factors including genetics, environment, and immunological. Viral infections, allergens, air pollution, and stress have been documented to trigger asthma followed by inflammation and constriction of the airways. Asthma is characterised by persistent inflammation in the airways, which causes chemotaxis of inflammatory cells and goblet cells hyperplasia leading to excessive mucus production. Blockage of small airways with thick mucus, collagen deposition underneath the alveolar basement membrane, hypertrophy of smooth muscles of the bronchioles, oedema in the airways, release of histamine and other inflammatory mediators by basophils, and the mast cells, all contribute to the pathology of asthma.
**Astragalus sarcocolla (ASE)** is a traditional herb used for the treatment of multiple ailments. It is commonly known as milkvetch in English and **Anzarut** in Urdu language. The plant grows abundantly in Iran, Pakistan, India, and China. **Astragalus** species has a high content of antioxidant flavonoids and polysaccharides. Traditionally, it has been used for treatment of flu, common cold, intestinal colic, visual impairment, acne vulgaris, urolithiasis, immune-related disorders like rheumatoid arthritis, digestive problems, and respiratory ailments. Previously, antispasmodic effect of this herb had been reported to mediate through opening the potassium channels (KCO) in ileal smooth muscle in rabbits.

Herbal medicine as anti-asthmatic agents in respiratory diseases has gained increasing interest due to its potential benefits and minimal side-effects as compared to synthetic agents. However, their use in smooth muscles of lung has not been explored. With this rationale, this study was designed to investigate the in-vitro bronchodilatory effect of **Astragalus sarcocolla** on isolated rabbit trachea smooth muscle strips.

**METHODOLOGY**

This experimental study was carried out in CMH Lahore Medical College and Institute of Dentistry, from October 2022 to May 2023. An ethical approval was obtained from the Ethics Review Committee of CMH Lahore Medical College and Institute of Dentistry, Lahore, before the commencement of the study (ERC number: 708/ERC/CMH/LMC).

Locally purchased adult rabbits, of both genders, weighing between 1-1.5 kg were used as the sample. The animals were placed in allocated rabbit cages in the animal housing facility of CMH Lahore Medical College and Institute of Dentistry, Lahore. The housing conditions were maintained at a range of 23-25°C, with humidity levels of 60 ± 4%. The rabbits had 12-hour light-dark cycle maintained. They had access to water and were provided with diet and libitum. Rabbits were allocated to different groups by simple random sampling, through the lottery method. Sample size was calculated with the standard WHO formula, using mean difference values from the reference study. It was calculated to be six animals per group.

The plant resin of **Astragalus sarcocolla** was acquired from a local herbarium market of Lahore, Pakistan, further identified by a Pharmacognosy expert of the Punjab University and was marked as voucher specimen no. 421 for further reference. The resin was then poured into a powdered form. The extraction process was performed using the cold extraction (maceration) method. For this purpose, 1000 g of resin was placed in absolute ethanol and allowed to soak for the duration of 72 hours at room temperature. Afterwards, filtration was carried out on the macerated plant, and the resulting liquid was filtered additionally with the help of a Buchner funnel vacuum filtration, using Whatman filter paper no.1. The filtrate obtained was evaporated to remove the solvent using a rotary evaporator model N-1000 under reduced pressure. The evaporation process was carried out at 104°F temperature. Subsequently, the residual material was dried in an oven until it reached a paste-like consistency. Finally, the resulting paste was refrigerated at 12 degrees in an airtight container until needed. The extract was stored in a cool dry place.

After euthanising the rabbit through lethal injection of ≥100 mg/kg of Pentobarbital, the trachea was carefully dissected out. A tracheal strip, consisting of a central part of smooth muscle between 2-3 cartilage rings was obtained. This tracheal strip was then suspended in a tissue bath (Radnoti 159920-X1/C; Radnoti Llc, Covina, CA) constituting Kreb’s Henseleit mixture. The solution was carbogenated, with a combination of O2 and CO2, at a ratio of 19:1. The tissue bath was maintained at a temperature of 37°C to maintain the physiological conditions. The tracheal tissue was left to equilibrate for 45 minutes under a traction of one gram. During this equilibration period, the tissue was allowed to adjust to the experimental conditions. The change in isometric contraction of the tissue was then recorded using the forced displacement transducer (Model: MLT 0402) which was connected to a PowerLab system, that allowed accurate measurement and recording of the contraction force of the tissue (Model: PL26T04) data acquisition system (AD Instruments, Sydney, Australia). Concentration response curves were drawn, median effective concentrations (EC50 values) and percentage inhibition were calculated.

Group I served as the control group in which tracheal smooth muscles were stabilised in Kreb’s solution with 1 g tension. Group II was the positive control, in which tracheal smooth muscle was stimulated by acetylcholine (Ach) 1µm and then later with KCl 25 mM and 80 mM respectively to get maximum concentration response curves. Theophylline with three molar concentrations of 0.2 mM solutions was mixed in the organ bath after every 5 minutes for the formation of solution having 0.2, 0.4, 0.6, and 0.8 mM concentrations, and cumulative dose response curves were formed. In Group III, the tracheal smooth muscles were stimulated by acetylcholine (Ach) 1µm, followed up by low KCl (25 mM) and high KCl (80 mM), and then by increasing concentration of ASE (0.04-2.50 mg/ml). In Group IV, tracheal smooth muscle was stimulated by acetylcholine (Ach) 1µm, KCl (25mM) and glibenclamide (3µM). Then, the tissue was treated with increasing concentration of ASE.

The data obtained from the experiment was presented as the mean ± standard error of the mean (SEM). The half maximal responses of ASE (EC50 values) along with their 95% confidence intervals (CI) were also estimated. Concentration response curves were drawn using the non-linear regression analysis in the statistical software GraphPad Prism version 5. The means between groups was compared by using one-way analysis of variance (ANOVA), and Tukey’s multiple comparison test with post hoc analysis was used to compare the groups. A p-value less than 0.05 was taken as statistically significant, indicating the difference between the groups.

**RESULTS**

**Astragalus sarcocolla (ASE)** extract was able to relax the sustained contractions caused by low KCl (25 mM) induced consistent contractions in smooth muscles of trachea in a dose-dependent manner with an EC50 value of 0.38 ± 0.021 mg/ml (95% CI: 0.04-0.38, n = 6), (Figure 1a and 2a). ASE was able to only
partially relax the sustained contractions caused by high KCl (80 mM) induced sustained contractions in smooth muscles of trachea, up to 26% only at very high doses. The EC50 value could not be calculated as there was not a 50% reduction in the response (Figure 1b and 2b). Theophylline, a standard bronchodilator when tested against the sustained contraction caused by elevated K+ (80 mM) and reduced K+ (25 mM) concentrations, was able to relax the sustained contractions caused by low K+ (25 mM) in smooth muscles of trachea (Figure 1c).

When smooth muscles were pretreated with a potassium channel closing agent (glibenclamide 3 µM) for few minutes and then stimulated with K+ (25 mM) concentrations for continuous contractions of smooth muscles, ASE was unable to relax the smooth muscles of trachea at the concentrations that ASE reduced the contractions when stimulated with low K+ (25 mM) alone (Figure 2c).

The relaxing effect of ASE on low K+ (25 mM) stimulated tracheal smooth muscle contractions was compared with theophylline. Theophylline significantly reduced the low K+ induced smooth muscle contractions (0.3 ± 0.04) (p < 0.001) in comparison to the control group. ASE also significantly reduced (p < 0.001) the smooth muscle contractions at 0.4 mg/ml, as compared to the control group (Figure 3).

DISCUSSION

To evaluate the bronchodilator effect of ASE in vitro, extract was used to counter the contractions induced by high potassium (K+) concentrations (80 mM) and low potassium (K+) concentrations (25 mM). It has been documented that persistent contractions induced by elevated potassium concentrations are primarily mediated by voltage-gated calcium (Ca2+) channels, which are activated by membrane depolarisation. Another mechanism involves the association of depolarisation with the discharge of sarcoplasmic calcium release channels. In this investigation, ASE demonstrated minimal inhibitory effects on contractions triggered by high levels of K+, implying that its spasmylytic impact was likely mediated through an alternative mechanism. However, when tested against contractions induced by low K+ levels, ASE displayed almost complete inhibition (88%). Agents that selectively relax contractions caused by K+ concentrations below 25 mM are considered potassium channel openers.

Only a few studies have evaluated the in-vitro effects of ASE. However, several other plants have also been shown to possess bronchodilatory effects. Wahid et al. reported bronchodilatory effect of Cucumis sativus seeds against high K-induced contractions. This differs from the present result since ASE significantly reduced the smooth muscle contractions against low K-induced contractions only. Similarly, another study documented that the smooth muscle relaxant effect of Dryopteris ramose was mediated through calcium channel blockade, as evident by inhibition of contractions against high KCl, whereas the current study had elucidated the potassium channel opening activity of ASE.

With glibenclamide, a specific blocker of ATP-dependent K+ channels, ASE was unable to effectively bring any change suggesting that the spasmylytic effect on tracheal smooth muscles, leading to bronchodilation, may be mediated through the activation of ATP-dependent K+ proteins. In comparison,
the bronchodilator effect of a phosphodiesterase inhibitor, was also tested for its bronchodilator effect. It exhibited inhibitory effects on both reduced and high K+ causing contractions at very low doses, indicating its ability to activate ATP-dependent K+ channels. Theophylline acts as a functional antagonist and inhibits the contractile response to various spasmodens. The bronchodilatory action of ASEX on reduced K+ causing contractions was significant when compared to the control group and was also comparable to that of theophylline. However, theophylline demonstrated superiority at lower doses, while ASEX exhibited a relaxant effect at higher doses compared to theophylline.

Potassium channel opening plays a crucial role in smooth muscle relaxation. The opening of potassium channels leads to an efflux of potassium ions from the smooth muscle cell, resulting in hyperpolarisation. Hyperpolarisation is a state where the membrane potential becomes more negative, moving further away from the threshold for excitation. This inhibits the generation and propagation of action potentials, reducing the contractile activity of the smooth muscle. The observed spasmodic effect on tracheal smooth muscles, mediated through potassium channel activation, supports the potential use of ASEX as a future anti-asthma medication.

This study had a few limitations. The authors could not use different types of blockers against low K+ induced contractions including 4-aminopyridine and TEA which are used to identify the different types of potassium channels involved.

CONCLUSION

The bronchodilator effect of Astragalus sarcocolla extract is mediated through the activation of ATP-sensitive potassium channels. Additional research is advised to observe the potential benefits of ASEX in humans.

ETHICAL APPROVAL:
An approval was obtained from the Ethics Review Committee of CMH Lahore Medical College and Institute of Dentistry, Lahore, before the commencement of the study (ERC number: 708/ER-C/CMH/LMC).

PATIENTS’ CONSENT:
The consent statement was not required since it was an in vitro animal study.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS’ CONTRIBUTION:
WAS: Conceptualised, designed, and interpreted the study.
MQ: Supervised and approved the final version to be published.
AQK, MK: Contributed to draft work, data acquisition, and analysis.
SZ: Contributed to manuscript writing and data interpretation.
RB: Critically revised the article for intellectual content.

All authors approved the final version of the manuscript to be published.

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