

Possible mechanism(s) of the relaxant effect of asafoetida (*Ferula assa-foetida*) oleo-gum-resin extract on guinea-pig tracheal smooth muscle

Zahra Gholamnezhad¹, Goltaj Byrami¹, Mohammad Hossein Boskabady^{1*}, Mehrdad Iranshahi²

Abstract

Objective: Asafoetida (*Ferula assa-foetida*) is known as a valuable remedy for whooping cough, pneumonia, bronchitis in children and asthma treatment in folk medicine. In the present study the relaxant effects of the asafoetida on tracheal smooth muscle of guinea pigs and its probable mechanism(s) were examined.

Materials and Methods: The relaxant effects of three cumulative concentrations of the aqueous extract (2, 5 and 10 mg/ml), theophylline (0.25, 0.5 and 0.75 mM) and saline were examined on non-incubated tracheal smooth muscle of guinea pig precontracted by 10 μ M methacholine (group 1); preincubated tissues by propranolol and chlorpheniramine, contracted by methacholine (group 2) and preincubated tissues by propranolol, contracted by methacholine (group 3), (n=6 for each group).

Results: All concentrations of theophylline in group 1 and all concentrations of the extract in the other three groups showed significant relaxant effects compared to that of saline ($p < 0.001$ for all cases). There was not significant difference in the relaxant effect of the extract between three groups. The relaxant effects of two last concentrations of the extract (5 and 10 mg/ml) only in group 2 were significantly lower than that of theophylline ($p < 0.05$ for both case). There was no significant difference between relaxant effects of the extract and theophylline in group 2. There were significant positive correlations between the relaxant effects of the extract with their concentrations in all three groups ($p < 0.001$ for all cases).

Conclusion: These results showed a potent relaxant effect for the asafoetida extract on tracheal smooth muscle which is perhaps due to muscarinic receptor blockade.

Keywords: *Ferula Assafoetida*; Aqueous gum extract; Relaxant effects; Guinea pig; Tracheal smooth muscle

1- Department of Physiology and Pharmaceutical Research Centre, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I. R. Iran

2- Biotechnology Research Center and School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I. R. Iran

*Corresponding Author: Tel: +985118002221; Fax +985118828564

E-mail: Boskabadyhm@mums.ac.ir, MHBoskabady@hotmail.com

Introduction

Asafoetida is an oleo-gum-resin obtained from the exudates of the roots of the Iranian endemic medicinal plant, *Ferula assa-foetida*. This species is often considered to be the main source of asafoetida, although other *Ferula* species, such as *Ferula foetida*, *Ferula rubricaulis*, *Ferula rigidula*, *Ferula alliacea* and *Ferula narthex*, are also sources of asafetida. The oleo-gum-resin asafoetida is called “Anghouzeh”, “Khorakoma” and “Anguzakoma” in Iran (Iranshahy and Iranshahi, 2011). Asafoetida has been used as a spice and a folk phytomedicine for centuries. It is used as a flavoring spice in a variety of foods, particularly in India. In addition, Nepali people regularly consume it in their daily diets, and it is believed that asafoetida has aphrodisiac, sedative and diuretic properties (Bandyopadhyay et al., 2006). It is traditionally used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza (Zargari, 1996; Takeoka, 2001; Evans, 2002; Lee et al., 2009). Recent pharmacological and biological studies have also shown several activities, such as antioxidant (Dehpour, 2009), antiviral (Lee et al., 2009), antifungal (Angelini et al., 2009), cancer chemopreventive (Saleem et al., 2001), anti-diabetic (Abu-Zaiton, 2010), antispasmodic (Fatehi et al., 2004), hypotensive (Fatehi et al., 2004), and molluscicidal (Kumar and Singh, 2006) from this oleo-gum-resin.

One of the most important traditional uses of asafoetida is the treatment of asthma. In India,

Asafoetida is traditionally used as a useful symptomatic treatment for angina pectoris and asthma (Srinivasan, 2005). In Ayurveda, asafoetida is introduced as a valuable remedy for whooping cough, pneumonia and bronchitis in children; it is also a pulmonary stimulant (Kapoor, 2001).

In Iranian folk medicine, asafoetida is also used as a medicine for the treatment of asthma (Zargari, 1996). In Afghanistan and Fiji, the dried gum is taken for whooping cough (Ross, 2005). In ancient Rome, it was used as a culinary spice and as a replacement for Silphion cyrenaicum. The aforementioned plant also has been used for tuberculosis and incessant cough (Appendino et al., 2006). Several fractions such as gum fraction (25%, including glucose, galactose, l-arabinose, rhamnose and glucuronic acid), resin (40–64%, which contains ferulic acid esters (60%), free ferulic acid (1.3%), coumarin derivatives (e.g. umbelliferone), volatile oils (3–17%) including sulphur-containing compounds, and various monoterpenes have been isolated from this plant (Kajimoto et al., 1989).

Asthma is a chronic airway inflammatory disease which is characterized by airway thicknesses and obstruction due to mucus accumulation and bronchospasm. This inflammation can lead to airway hyper responsiveness (AHR) to many stimuli (Adamek-Guzik et al., 1996; Bjornsdottir and Cypcar, 1999). Two types of drugs are used in the treatment of asthma including anti-inflammatory drugs such as steroids, leukotriene modifiers, mast cell stabilizers and IgE blocking medicines as preventive treatment and bronchodilator drugs such as β -agonists and theophylline as relieving treatment. With regard to the effect of asafetida on asthma disease, it could be effective in asthma treatment as preventive or relieving mechanisms or both.

Although asafoetida has been traditionally used in treatment of asthma, there is not enough scientific data on its mechanism(s) of action. Therefore, in the present study the possible mechanism(s) of the relaxant effects of the asafoetida on tracheal chains of guinea pigs was examined.

Materials and Methods

Plant and extraction

Ferula assa-foetida was collected from the mountains of Gonabad region (South of Khorasan province, Iran) during summer. The plant was identified at the Botany Department, Faculty of Science, Mashhad University, Mashhad, Iran. A voucher specimen number was kept in record (293-0606-2) at the Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences. The powdered dried gum (10 g) was soaked in distilled water (50 ml) and boiled for 5 min, allowing the decoction to stand for 30 min and filtering it through paper filter. Concentrations and doses of the aqueous extract were expressed as total amount of the dried gum used in preparing the extract (Fatehi *et al.*, 2004). The concentration of *asafoetida* in the stock solution was 0.2 g/ml.

Tissue preparation

Guinea pigs (400-700 g, both sexes) were killed by a blow on the neck and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut and open opposite the trachealis muscle, and sutured together to form a tracheal chain (Holroyde, 1986; Boskabady *et al.*, 2004). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent, U.K.) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Protocols

The relaxant effects of three cumulative concentrations of the extract of *asafoetida* (2, 5 and 10 mg/ml) and theophylline anhydrous (Sigma Chemical Ltd UK) (0.25, 0.5 and 0.75 mM) as positive control, and saline (1 ml) as negative control were examined. Substances were added to 10 ml organ bath in different volume to obtain final above concentration.

In each experiment, the effect of saline, three cumulative concentrations of the extract and theophylline on contracted tracheal smooth muscle was measured after exposing tissue to each concentration of the solution for 5 min. A decrease in tone was considered to be a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction. An increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change (Holroyde, 1986; Boskabady *et al.*, 2004).

The relaxant effect of different solutions was tested with three different experimental designs, as follows (n=6 for each group):

- 1- On tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 1 experiment).
- 2- On tracheal chains incubated with 1 μM propranolol and chlorpheniramine and contracted by 10 μM methacholine (group 2 experiments).
- 1- On tracheal chains incubated with 1 μM propranolol and contracted by 10 μM methacholine (group 3 experiments).

The relaxant effects in three groups of experiments were examined in three different series of tracheal chains. All of the experiments were performed randomly with a 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs-Henseliet solution. In all experiments contractions were measured using an

Relaxant effect of *Ferula Assa-foetida* on tracheal smooth muscle

isometric transducer (MLT0202, AD Instruments, Australia) which was connected to a power lab system (PowerLab 8/30, ML870, AD Instruments, Australia). The effect of theophylline was tested only in group 1.

Statistical analysis

All data were expressed as mean±SEM. Data of relaxant effects of different concentrations of each solution in each group were compared with the results of negative and positive control using paired t test. The data of relaxant effects obtained in three groups of experiments were compared using one way analysis of variance (ANOVA) and Tukey Cramer post test. The relation between relaxant effect of the extract and theophylline concentrations was analysed using least square regression method. Significance was accepted at $p < 0.05$.

Results

Relaxant effect

All concentrations of theophylline in group 1 (non-incubated smooth muscle contracted by methacholine) and all concentrations of the extract in the remaining three groups showed significant relaxant effects compared to that of saline ($p < 0.001$ for all cases), (Table 1).

Comparison of the relaxant effect of theophylline with that of the extract

The relaxant effects of two last concentrations of the extract (5 and 10 mg/ml) only in group 2 were significantly lower than those of theophylline ($p < 0.05$ for both case), (Table 1).

Table 1. Relaxant effect (%) of asafoetida extract in comparison with negative control (saline) and positive control (theophylline) in group 1, 2 and 3 experiments (contracted tracheal chains by 10 μ M methacholine on non incubated tissues, incubated tissues with propranolol and chlorpheniramine and incubated tissues with propranolol, n=6 for each groups) .

Different Concentration	Saline	Theophylline	Group1	Group 2	Group 3
1	0.6±0.23	43±3.28 ***	32.26±6.6 ***	34.14±3.0 ***	42.1±4.06 ***
2	0.43±0.21	79.±5.8 ***	63.79±6.65 ***	59.00±4.4 ***+	75.73±6 ***
3	0.9±0.27	96.81±3.44 ***	91.43±2.83 ***	80.27±4.5 ***+	100.5±8.81 ***

Values are presented as mean±SEM. St. Dif.: Statistical difference. The three different concentrations for the extract were 2, 5 and 10 mg/ml; and for theophylline, 0.25, 0.50 and 0.75 mM respectively. The effect of theophylline was tested only in group 1.

Statistical differences between the effect of theophylline and extract with that of saline; *** $p < 0.001$.

Statistical differences between the effect of theophylline with those of the extract; + $p < 0.05$.

Comparison of the relaxant effect between three groups

There was no significant difference between relaxant effects of the extract and theophylline in group 2 (Figure 1).

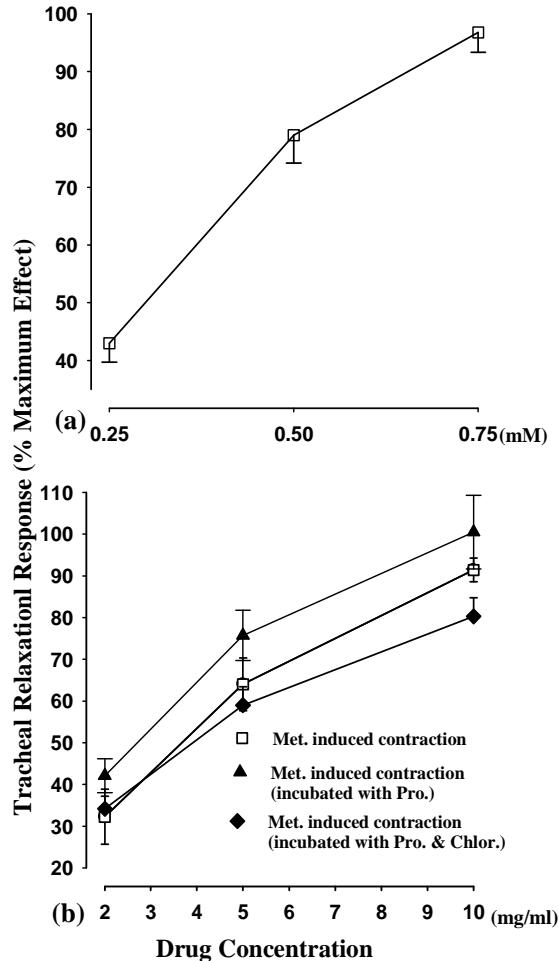


Figure 1. Concentration response curves of the relaxant effect of theophylline (a) and the extract of asafoetida (b) in three groups of experiments (n=6 for each group). group 1; methacholine induced contraction of tracheal chains (□), group 2; methacholine induced contraction of incubated tracheal chains with propranolol and chlorpheniramine (◆) and group 3; methacholine induced contraction of incubated tracheal chains with propranolol (▲). The effect of theophylline was tested only in group 1. There was not any statistical difference in the relaxant effect of different concentrations of the extract between three groups.

Correlation between the relaxant effect and the concentration

There were significant positive correlations between the relaxant effects of the extract with their concentrations in all three groups (p<0.001 for all cases), (Table 2).

Table 2. Correlation (r) between the relaxant effect and the concentration of the extract.

Groups	r	p
Group1	0.8750	0.001
Group2	0.8851	0.001
Group3	0.8298	0.001

Discussion

In the present study, the relaxant effects of the asafoetida on tracheal smooth muscle of guinea pigs and its possible mechanism(s) was investigated.

In group 1 experiment (contracted tracheal chains with methacholine), all concentrations of theophylline and the extract showed relaxant effect in comparison with saline which was not significantly different with that of theophylline.

To examine the contribution of histamine (H₁) receptor inhibitory and/or β-adrenoceptor stimulation effect of the asafoetida on its relaxant effect, the relaxant effect of the extract on incubated tissues with propranolol and chlorpheniramine in group 2 was also evaluated. The relaxant effects of different concentrations of the extract in group 2 were significantly higher compared to saline. However, the results of group 2 showed a non significant lower relaxant effect of the extract compared to group 1. In addition, the relaxant effect of two higher concentrations of the extract was significantly lower than theophylline. These

Relaxant effect of *Ferula Assa-foetida* on tracheal smooth muscle

results may indicate a small or no contribution of histamine (H_1) receptor inhibitory and/or β -adrenoceptor stimulation property of the asafetida on its relaxant effect. However, the significant relaxant effect of the extract in this group showed that the main mechanism of the relaxant effect of asafetida is its inhibitory effect on muscarinic receptors.

To examine that the findings observed are due to histamine (H_1) receptor inhibitory and/or β -adrenoceptor stimulation, the relaxant effect of asafetida was also examined on incubated tissues with propranolol in group 3. In group 3 of experiment (incubated smooth muscle with propranolol and contracted by methacholine), relaxant effects of all concentrations of the extract differ significantly compared to saline. The relaxant effect of the extract was not statistically different with that of theophylline in this group and was not significantly higher than those of group 2. The results of group 3 showed that β -adrenoceptor stimulation property of the plant do not contribute to its relaxant effect and the findings of group 2 are due to the inhibitory effect of the extract on histamine (H_1) receptor.

The relaxant effect of asafetida was concentration dependent and there was significant correlation between the relaxant effect and the concentration of the extract. The observed relaxant effect for the *asafetida* in the present study was supported by previous studies demonstrating antispasmodic (Fatehi et al., 2004) and hypotensive (Fatehi et al., 2004) effects for this oleo-gum-resin.

The results of the present study showed that the therapeutic effect described for *asafetida* on asthma disease (Srinivasan, 2005; Kapoor, 2001; Zargari, 1996) may be due to its relaxant effect causing bronchodilation and can be used as a relieving drug for the treatment of this disease.

In conclusion, the results of the present study showed a relaxant effect for the asafetida extract on tracheal smooth muscle which was comparable to that of theophylline. A muscarinic receptor blockade was also suggested for the extract. A small contribution of histamine (H_1) receptor inhibitory property of asafetida on its relaxant effect was also suggested. However, β -adrenoceptor stimulation effect of the extract did not contribute to its relaxant effect.

Acknowledgments

This study was financially supported by Vice Chancellery of Research of Mashhad University of Medical Sciences.

References

- Abu-Zaiton AS. 2010. Anti-diabetic activity of *Ferula assafoetida* extract in normal and alloxan-induced diabetic rats. Pak J Biol Sci, 13: 97-100.
- Adamek-Guzik T, Czerniawska-Mysik G, Guzik T. 1996. [Bronchial asthma--a chronic inflammatory disorder]. Przegl Lek, 53: 12-19.
- Angelini P, Pagiotti R, Venanzoni R, Granetti B. 2009. Antifungal and allelopathic effects of *Asafoetida* against *Trichoderma harzianum* and *Pleurotus* spp. Allelopathy Journal, 23: 357-368.
- Appendino G, Maxia L, Bascope M, Houghton P J, Sanchez-Duffhues G, Munoz E, et al. 2006. A meroterpenoid NF-kappaB inhibitor and drimane sesquiterpenoids from *Asafetida*. J Nat Prod, 69: 1101-1104.
- Bandyopadhyay D, Basak B, Chatterjee A, Lai T K, Banerji A, Banerji J, et al. 2006. Saradaferin, a new sesquiterpenoid coumarin from *Ferula assafoetida*. Nat Prod Res, 20: 961-965.
- Bjornsdottir US, Cypcar DM. 1999. Asthma: an inflammatory mediator soup. Allergy 54 Suppl, 49: 55-61.
- Boskabady MH, Khatami A, Nazari A. 2004. Possible mechanism (s) for relaxant effects of

- Foeniculum vulgare* on guinea pig tracheal chains. *Pharmazie*, 59: 561-564.
- Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. 2009. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas y Aceites*, 60: 405-412
- Evans WC. 2002. *Trease and Evans Pharmacognosy*. London, W.B. Saunders. Fifteenth ed: 286.
- Fatehi M, Farifteh F, Fatehi-Hassanabad Z. 2004. Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract. *J Ethnopharmacol*, 91: 321-324.
- Holroyde MC. 1986. The influence of epithelium on the responsiveness of guinea-pig isolated trachea. *Br J Pharmacol*, 87: 501-507.
- Iranshahy M, Iranshahi M. 2011. Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-a review. *J Ethnopharmacol*, 134: 1-10.
- Kajimoto T, Yahiro K, Nohara T. 1989. Sesquiterpenoid and disulphide derivatives from *Ferula assa-foetida*. *Phytochemistry*, 28: 1761-1763.
- Kapoor LD, Ed. 2001. *Handbook of Ayurvedic Medicinal Plants*. Washington, D.C., CRC Press.
- Kumar P, Singh DK. 2006. Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. *Chemosphere*, 63: 1568-1574.
- Lee CL, Chiang LC, Cheng LH, Liaw CC, Abd El-Razek MH, Chang FR, et al. 2009. Influenza A (H1N1) antiviral and cytotoxic agents from *Ferula assa-foetida*. *J Nat Prod*, 72: 1568-1572.
- Ross IA. 2005. *Medicinal plants of the world : chemical constituents, traditional, and modern medicinal uses*. Totowa, N.J., Humana Press.
- Saleem M, Alam A, Sultana S. 2001. Asafoetida inhibits early events of carcinogenesis: a chemopreventive study. *Life Sci*, 68: 1913-1921.
- Srinivasan K. 2005. Spices as influencers of body metabolism: an overview of three decades of research. *Food Research International*, 38: 77-86.
- Takeoka G. 2001. Volatile Constituents of Asafoetida. *Aroma Active Compounds in Foods*, American Chemical Society, 794: 33-44.
- Zargari A, Ed. 1996. *Medicinal Plants*, Tehran University Publications, Tehran.