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Original Research Article

Tracheal smooth muscle relaxant effect of linalool involves muscarinic receptors and NO formation

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Abstract

Objective: Linalool, a monoterpene compound and a principal component of several aromatic herbs, possesses antispasmodic and smooth muscle relaxant properties. This study aimed to examine the relaxant effect of linalool on the tracheal smooth muscle (TSM) of rats and the mechanisms involved.

Materials and Methods: Tracheal rings (3–4 cartilaginous rings per trachea) from Wistar rats (200–250 g) of both sexes were used. After anesthesia using ketamine plus xylazine, followed by cervical dislocation, the trachea was removed. Cumulative concentrations of linalool (0.1, 0.2, 0.4 and 0.8 mg/ml) were added every 5 minutes to non-incubated tissues precontracted with methacholine or KCl for 7 minutes. The same concentrations were added to precontracted tissues pre-incubated (10 min) with propranolol, glibenclamide, diltiazem, papaverine, chlorpheniramine, indomethacin, L-NAME and atropine. Theophylline and saline served as positive and negative controls, respectively (n = 6–7 per group).

Results: Linalool showed concentration-related relaxant responses in non-incubated TSM precontracted by methacholine and KCl ($p<0.01$ to $p<0.001$). The relaxant responses of tissues incubated with propranolol, glibenclamide, diltiazem, papaverine, chlorpheniramine and indomethacin were not significantly different from non-incubated tissues. However, in L-NAME- and atropine-incubated tissues, some concentrations showed significantly lower relaxant effects than non-incubated tissues ($p<0.05$ to $p<0.001$). The highest concentration of linalool had a relaxant effect comparable to theophylline.

Conclusion: Linalool showed a pronounced relaxant action on TSM, similar to that of theophylline. The involvement of muscarinic receptor blockade and/or enhanced nitric oxide generation is proposed. Its bronchodilatory effect should be investigated in future clinical studies.

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Introduction

Obstructive pulmonary diseases are among the most prevalent chronic inflammatory airway diseases worldwide (Saadat et al. 2019b). Airway smooth muscle (ASM) is critically involved in asthma pathophysiology by contributing to acute bronchoconstriction, which is intensified by airway hyper-responsiveness and impaired bronchodilation (Doeing and Solway 2013). Although airway hyper-responsiveness to non-specific stimuli is a defining feature of asthma, its precise mechanisms remain incompletely understood (An et al. 2007). ASM is circumferentially arranged around the airway, and its contraction reduces lumen diameter, leading to airflow obstruction. In asthma, ASM exhibits exaggerated contractile responses and diminished responsiveness to bronchodilatory signals, while also contributing to airway remodeling and inflammation. Therefore, ASM is considered a critical therapeutic target in asthma management (Doeing and Solway 2013). Although current treatments may improve lung function and asthma symptoms such as bronchospasm, they do not definitively cure the disease and may cause several side effects. Therefore, medicinal plants and their ingredients, as multi-potential agents, could be used as combination or alternative drugs with fewer side effects and perhaps greater effectiveness (Saadat et al. 2019b).

Linalool ($C_{10}H_{18}O$) is a monoterpene compound found in several aromatic herbs. Plants containing linalool have served as remedies in folk medicine for managing various illnesses (Lis-Balchin and Hart 1999). This agent has shown analgesic and anti-inflammatory effects (Koto et al. 2006; Peana et al. 2002), as well as psychopharmacological (Piccialli et al. 2026), airway smooth muscle relaxant (Silveira et al. 2017), and antimicrobial activities (Carson and Riley 1995). A recent comprehensive review has summarized the various pharmacological properties of linalool and its parent plant (Hosseini et al. 2021).

Inhibitory effects of linalool on contractile responses to various agents, such as acetylcholine, histamine, 5-hydroxytryptamine, noradrenaline and oxytocin, have been reported. These effects may involve inhibition of smooth muscle postsynaptic G-protein-coupled receptor-evoked responses and presynaptic action potentials (Poyton et al. 2015). Linalool also shows antinociceptive and antihyperalgesic effects, which could be due to its action on cholinergic, opioidergic and dopaminergic systems (Peana et al. 2003). Inhibition of the build-up of nitrite in cultured of lipopolysaccharide (LPS)-stimulated cells by linalool has been shown to occur through an increase in the expression of inducible nitric oxide synthase (iNOS) (Peana et al. 2006; Poyton et al. 2015). In addition, linalool has been reported to have a competitive antagonistic action at the N-methyl-D-aspartate (NMDA) receptor (Peana et al. 2002).

Due to the potential of linalool in smooth muscle relaxation, this investigation was conducted to evaluate the relaxant action of linalool in tracheal smooth muscle (TSM) and the underlying mechanisms.

Materials and Methods

Animals

The study was performed on 100 Wistar rats of both sexes (200–250 g). The animals were maintained in the Animal House of the School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, in standard cages with a 12-hr light/dark cycle at 22 ± 2°C with free access to food and water. Both male and female rats were used because a previous study from our laboratory has shown no significant sex-related differences in the tracheal smooth muscle response to bronchodilator agents (Naghdi et al. 2022). Animals were randomly assigned to all experimental groups to minimize potential sex-related

bias. Ethical approval for this study was granted by the Ethics Committee of Mashhad University of Medical Sciences for Animal Experiments (approval number: 471127), Mashhad, Iran.

Trachea tissue preparation

Following anesthesia with ketamine (100 mg/kg) plus xylazine (10 mg/kg), the animals were humanely euthanized through cervical dislocation. After opening the chest, the trachea was gently freed from surrounding tissues and removed. The esophagus was completely separated from the trachea to prevent any interference of esophageal contractions with tracheal responses. The trachea was then cut into 3–4 cartilaginous rings. Two nichrome hooks were passed through each tracheal ring, and the ring was mounted in a 10-mL organ bath filled with Krebs solution of the composition previously described (Saadat et al. 2019b; Saadat et al. 2019c). The solution was continuously bubbled with 95% O_2/5% CO_2 at 37°C. Tracheal smooth muscle contractions were monitored with an isometric transducer (MLT0202, AD Instruments, Australia) linked to a PowerLab system (PowerLab 8/30, ML870, AD Instruments, Australia) at a resting tension of 1 g. An equilibration period of at least 1 h was permitted. The Krebs solution was refreshed every 15 min (Saadat et al. 2019b; Saadat et al. 2019c).

Protocol

After the stabilization period, each tracheal ring was contracted with methacholine or KCl (10 µM and 60 mM, respectively; Sigma Chemical Ltd, UK) for 7 minutes. Linalool was applied at four cumulative concentrations (0.1, 0.2, 0.4 and 0.8 mg/ml; Sigma Chemical Ltd, UK) at 5-minute intervals in order to produce concentration–response curves (Saadat et al. 2019a). Theophylline (0.2–0.8 mM; Sigma Chemical Ltd, UK) and saline (1 ml) were used as positive and negative controls, respectively. Tracheal smooth muscle relaxation was measured as the reduction in the contraction response induced by the contractile agent (methacholine or KCl). Tissue relaxation and the effective concentration of linalool producing 50% of the maximum response (EC_{50}) were evaluated as previously described (Memarzia et al. 2019; Saadat et al. 2019b).

Different substances, such as antagonists and inhibitors, were also used to investigate the mechanisms underlying the relaxant effect of linalool. These agents were introduced into the organ bath 10 min before the contractile agent, and the relaxant response to linalool was then assessed as previously described (Boskabadi et al. 2020; Naghdi et al. 2018).

Experimental groups

The present study consisted of 12 experimental groups, as described in previous studies (Boskabadi et al. 2020; Naghdi et al. 2018) and illustrated in Table 1**.** For each experiment one tissue was applied randomly.

Data analysis

One-way analysis of variance (ANOVA) was applied to compare data among multiple groups, followed by post hoc pairwise comparisons conducted via Tukey's test. Results are expressed as mean ± SEM (standard error of the mean). A p-value less than 0.05 was considered statistically significant. Analyses were carried out with SPSS software (version 20).

Results

Linalool-induced relaxation in methacholine-precontracted TSM

Linalool produced significant, concentration-dependent relaxation in non-incubated TSM precontracted with methacholine ($p<0.01$ to $p<0.001$). Theophylline at its three lower concentrations caused significantly greater relaxation than linalool ($p<0.05$ to $p<0.001$). However, at the highest concentration, linalool caused a degree of relaxation comparable to that of theophylline (Figure 1).

Table 1. Summary of the experimental groups used in this investigation

Groups	Contractile agent	Incubated agents	Possible mechanisms	Examined agent
	KCl			
1	*Non-incubated tissues*			*Theophylline*
2				*Linalool*
3	*Incubated tissues with*	*Atropine*	*Muscarinic receptor inhibition*	*Linalool*
4		*Chlorpheniramine*	*Histamine (H1) receptor inhibition*	*Linalool*
5		*Indomethacin*	*Cyclooxygenase inhibition*	*Linalool*
	Methacholine			
6	*Non-incubated tissues*			*Theophylline*
7				*Linalool*
8	*Incubated tissues with*	*Diltiazem*	*Calcium channel blocking*	*Linalool*
9		*Glibenclamide*	*Potassium channel opening*	*Linalool*
10		*Propranolol*	*β-adrenoceptor stimulation*	*Linalool*
11		*Papaverine*	*Phosphodiesterase inhibition*	*Linalool*
12		*L-NAME*	*Nitrite oxide (NO) production*	*Linalool*

For non-incubated TSM contracted by methacholine, n = 7; for other groups, n = 6. L-NAME: ω-nitro-L-arginine methyl ester.

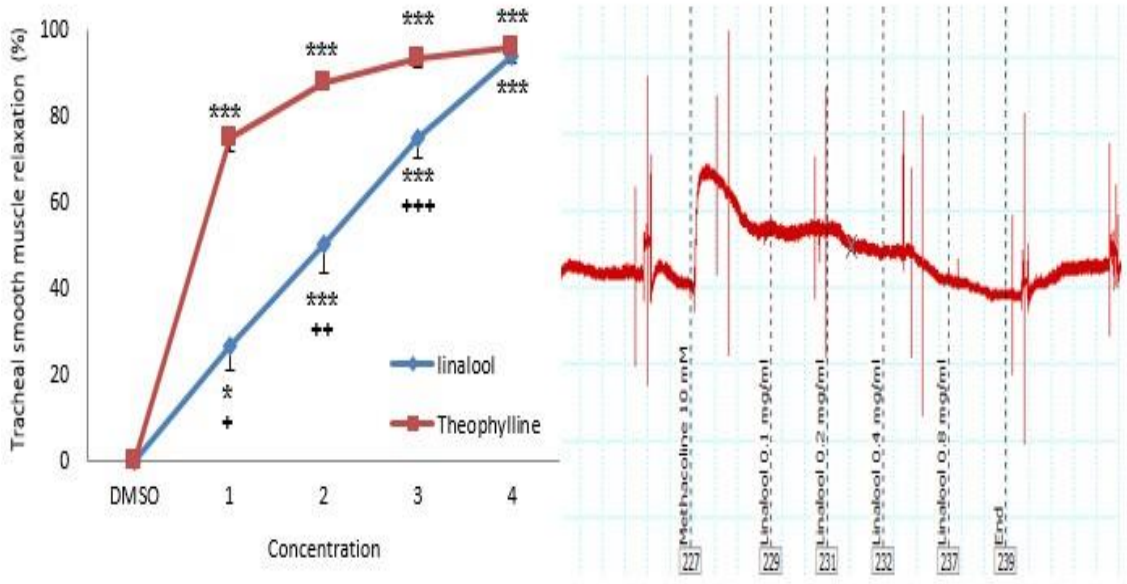


Figure 1. Linalool and theophylline concentration–response relaxant effect (mean ± SEM) on TSM contracted by methacholine (10 μM) in non-incubated tissues (n = 7 for each group). Numbers 1, 2, 3 and 4 on the X axis represent four concentrations of linalool (0.1, 0.2, 0.4 and 0.8 mg/ml) and theophylline (0.2, 0.4, 0.6 and 0.8 mM). TSM relaxation was expressed as a percentage of the maximum contraction induced by 10 μM methacholine. *p<0.05 and ***p<0.001 compared to the effect of dimethyl sulfoxide (DMSO). +p<0.05, ++p<0.01 and +++p<0.001 compared to the effect of theophylline. ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

In tissues pre-treated with propranolol, diltiazem, glibenclamide, and papaverine, the relaxation induced by various concentrations of linalool was not significantly different from that of non-incubated TSM (Figure 2). In TSM incubated with L-NAME, linalool at all concentrations caused significantly weaker relaxation compared with non-incubated tissues (p<0.05 to p<0.001) (Figure 3).

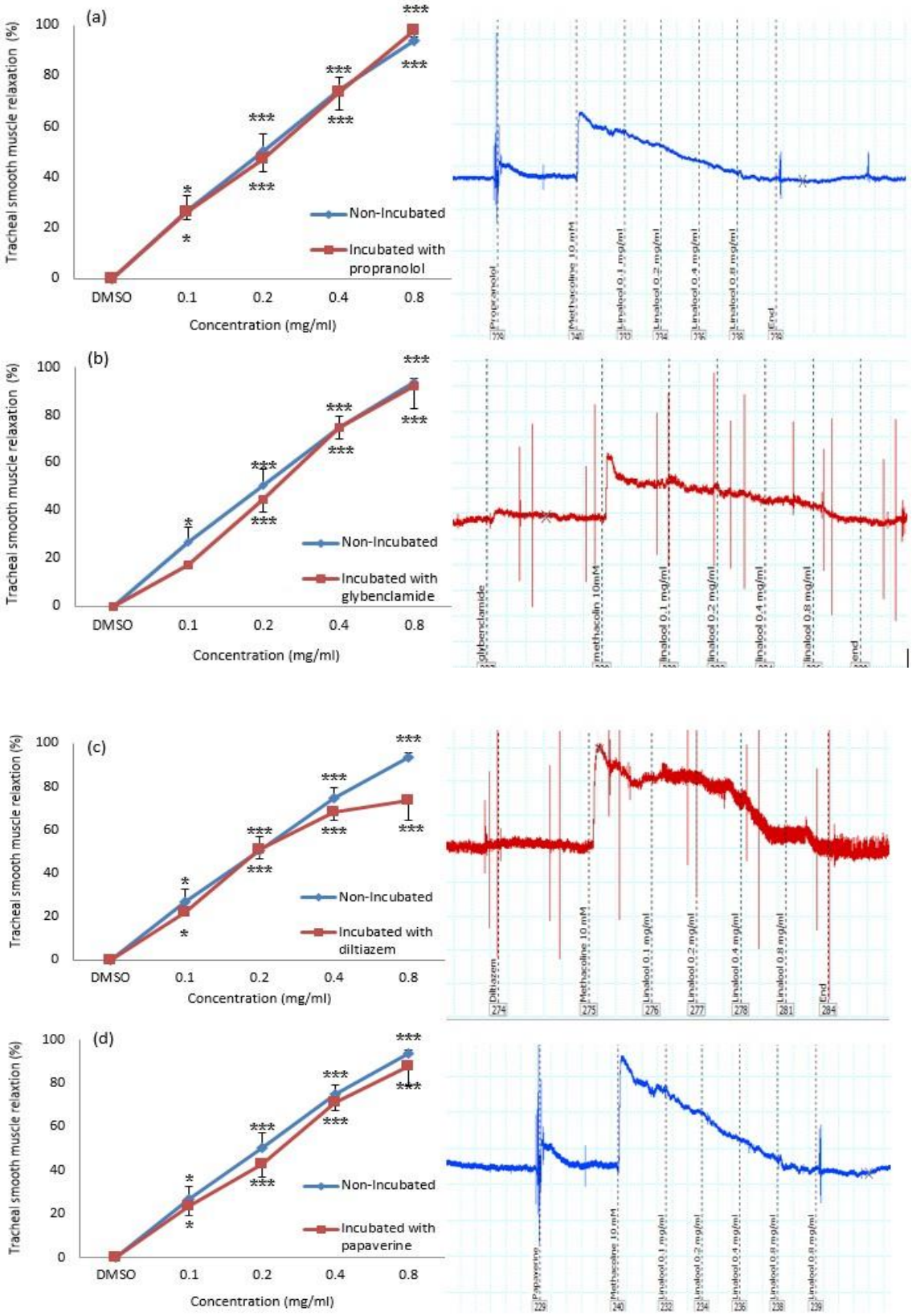


Figure 2. Linalool concentration–response relaxant effect (mean ± SEM) on TSM contracted by methacholine (10 μM) in non-incubated tissues (n = 7) and tissues incubated with (a) propranolol (1 μM, n = 6), (b) glibenclamide (1 μM, n = 6), (c) diltiazem (5 mM, n = 6), and (d) papaverine (50 μM, n = 6). TSM relaxation was expressed as a percentage of the maximum contraction induced by 10 μM methacholine. *p<0.05 and ***p<0.001 compared to the effect of dimethyl sulfoxide (DMSO). ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

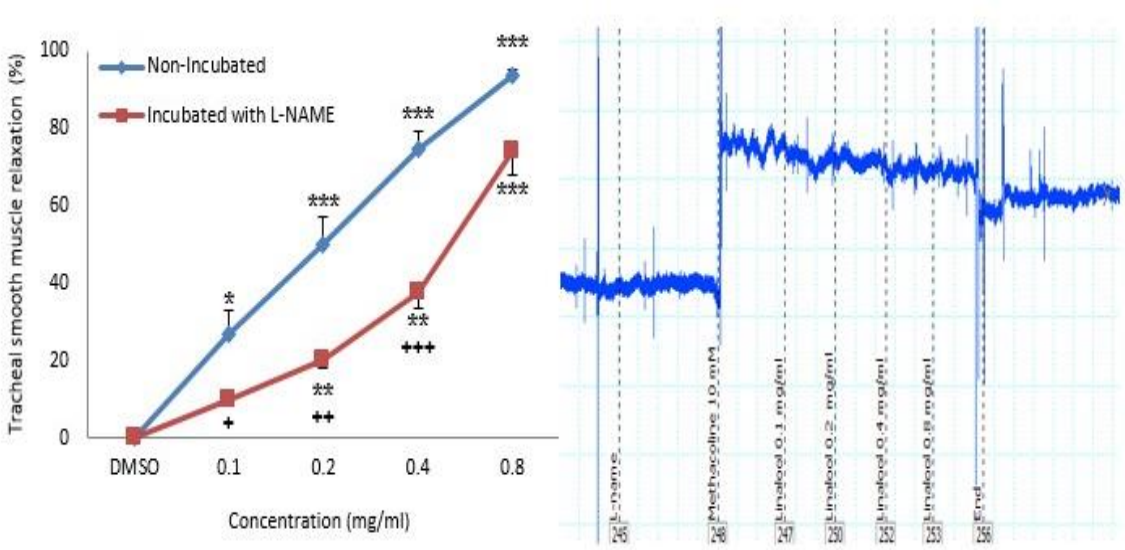


Figure 3. Linalool and theophylline concentration–response relaxant effect (mean ± SEM) on TSM contracted by methacholine (10 μM) in non-incubated (n = 7) and L-NAME-incubated tissues (300 μM, n = 6). TSM relaxation was expressed as a percentage of the maximum contraction induced by 10 μM methacholine. *p<0.05, **p<0.01 and ***p<0.001 compared to the effect of dimethyl sulfoxide (DMSO). +p<0.05, ++p<0.01 and +++p<0.001 compared to the effect of linalool in non-incubated tissues. ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

In TSM incubated with L-NAME, the EC_{50} values of linalool were significantly higher than those of non-incubated tissues pre-contracted by methacholine ($p<0.01$) (Figure 4).

The relaxant effects of linalool (the first three concentrations) in tissues incubated with L-NAME were significantly greater than those of TSM incubated with glibenclamide, propranolol, diltiazem and papaverine in methacholine-induced contraction ($p<0.05$ to $p<0.001$). Additionally, in propranolol-incubated TSM, the relaxant effect of the first concentration of linalool was significantly greater compared with tissues incubated with glibenclamide, and the same was true for its last concentration compared to tissues incubated with diltiazem ($p<0.05$ for both) (Table 2).

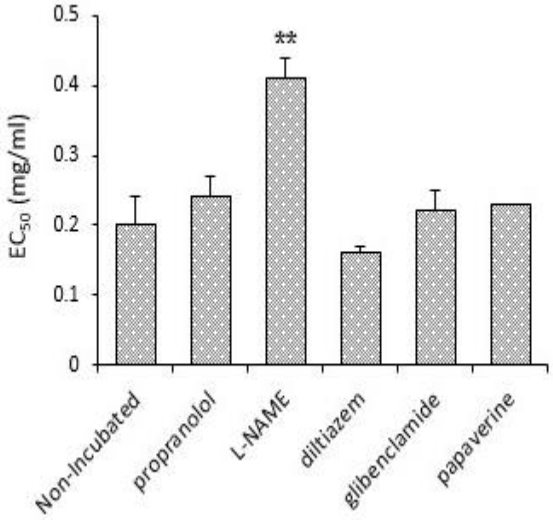


Figure 4. Effective concentration of linalool producing 50% of the maximum response (EC_{50}) values for linalool-induced relaxation in rat TSM contracted by methacholine (10 μM) in non-incubated tissues (n = 7) and tissues incubated with propranolol (n = 6), L-NAME (n = 6), diltiazem (n = 6), glibenclamide (n = 6), and papaverine (n = 6). **p<0.01 compared to non-incubated tissues. ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

Linalool-induced relaxation in KCl - precontracted TSM

Linalool elicited significant, concentration-dependent relaxation in non-incubated TSM precontracted with KCl ($p<0.01$ to $p<0.001$). At the highest concentration, linalool and theophylline showed comparable relaxant activity. Theophylline at its three lower concentrations produced greater relaxation than linalool ($p<0.01$ to $p<0.001$) (Figure 5).

Table 2. Relaxant responses to four concentrations of linalool in various incubated tracheal smooth muscle preparations contracted with methacholine

Incubating substance	**Concentration (mg/ml)**			
	0.1	**0.2**	**0.4**	**0.8**
Propranolol	26.36±2.84$^{\$\#\#\#}$	47.26±5.15$^{\#\#\#}$	73.70± 7.34$^{\#\#}$	97.79±3.95$^{\#\#+}$
Diltiazem	21.76±1.71$^{\#\#\#}$	50.68±4.04$^{\#\#\#}$	68.35±3.67$^{\#\#\#}$	73.43±8.89
Glibenclamide	17.42±1.92$^{\#}$	44.82±5.60$^{\#\#}$	74.72±4.62$^{\#\#}$	92.19±9.44
L-NAME	9.88±1.46	20.14±2.17	37.55±4.04	74±5.74
Papaverine	4.20±23.57$^{\#}$	5.73±42.65$^{\#\#}$	4.01±71.32$^{\#\#\#}$	87.55±8.67

Values are expressed as mean ± SEM. +$p<0.05$ versus diltiazem-treated preparations. #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ relative to the relaxant action of linalool in L-NAME-incubated tissues. \$ $p<0.05$ relative to the relaxant action of linalool in glibenclamide-treated tissues.

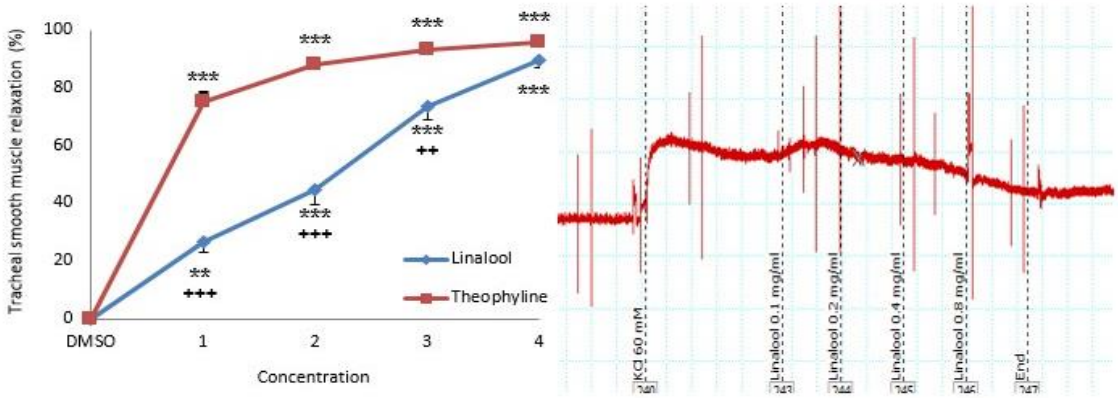


Figure 5. Linalool and theophylline concentration–response relaxant effect (mean ± SEM) on TSM contracted by KCl (60 mM) in non-incubated tissues (n = 7 for each group). Numbers 1, 2, 3 and 4 on the X axis represent four concentrations of linalool (0.1, 0.2, 0.4 and 0.8 mg/ml) and theophylline (0.2, 0.4, 0.6 and 0.8 mM). TSM relaxation was expressed as a percentage of the maximum contraction induced by 60 mM KCl. **$p<0.01$ and ***$p<0.001$ compared to the effect of dimethyl sulfoxide (DMSO), and ++$p<0.01$ and +++$p<0.001$ compared to the effect of theophylline. ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

Linalool induced significant relaxation in a concentration-dependent manner in TSM incubated with atropine, chlorpheniramine and indomethacin ($p<0.01$ to $p<0.001$).

Significantly lower relaxant effects of the three higher concentrations of linalool (0.2, 0.4 and 0.8 mg/ml) in tissues incubated with atropine were observed compared to non-incubated TSM ($p<0.05$ to $p<0.01$) (Figure 6a).

Linalool produced comparable relaxant responses in non-incubated tissues and in tissues incubated with chlorpheniramine or indomethacin (Figure 6b, c).

The mean EC_{50} value of linalool in tissues incubated with chlorpheniramine was significantly lower than that of non-incubated TSM contracted by KCl ($p<0.05$) (Figure 7).

In KCl-precontracted tissues, at the three lower concentrations, linalool caused significantly greater relaxation in tissues incubated with chlorpheniramine or indomethacin compared with atropine-incubated tissues (Table 3).

Table 3. Relaxant responses to four concentrations of linalool in various incubated tracheal smooth muscle preparations contracted with KCl

Incubating substance	**Concentration (mg/ml)**			
	0.1	**0.2**	**0.4**	**0.8**
Atropine	8.96±1.75	31.91±2.35	50.94±4.15	76.06±5.95
Chlorpheniramine	28±4.57**	49.86±4.64**	71.23±4.08**	88.58±1.57
Indomethacin	34.99±3.788***	56.42±3.23***	78.32±3.47***	89.2±4.16

Values are expressed as mean ± SEM. **p<0.01, ***p<0.001 relative to the relaxant action of linalool in atropine-treated preparations compared with other incubated tissues.

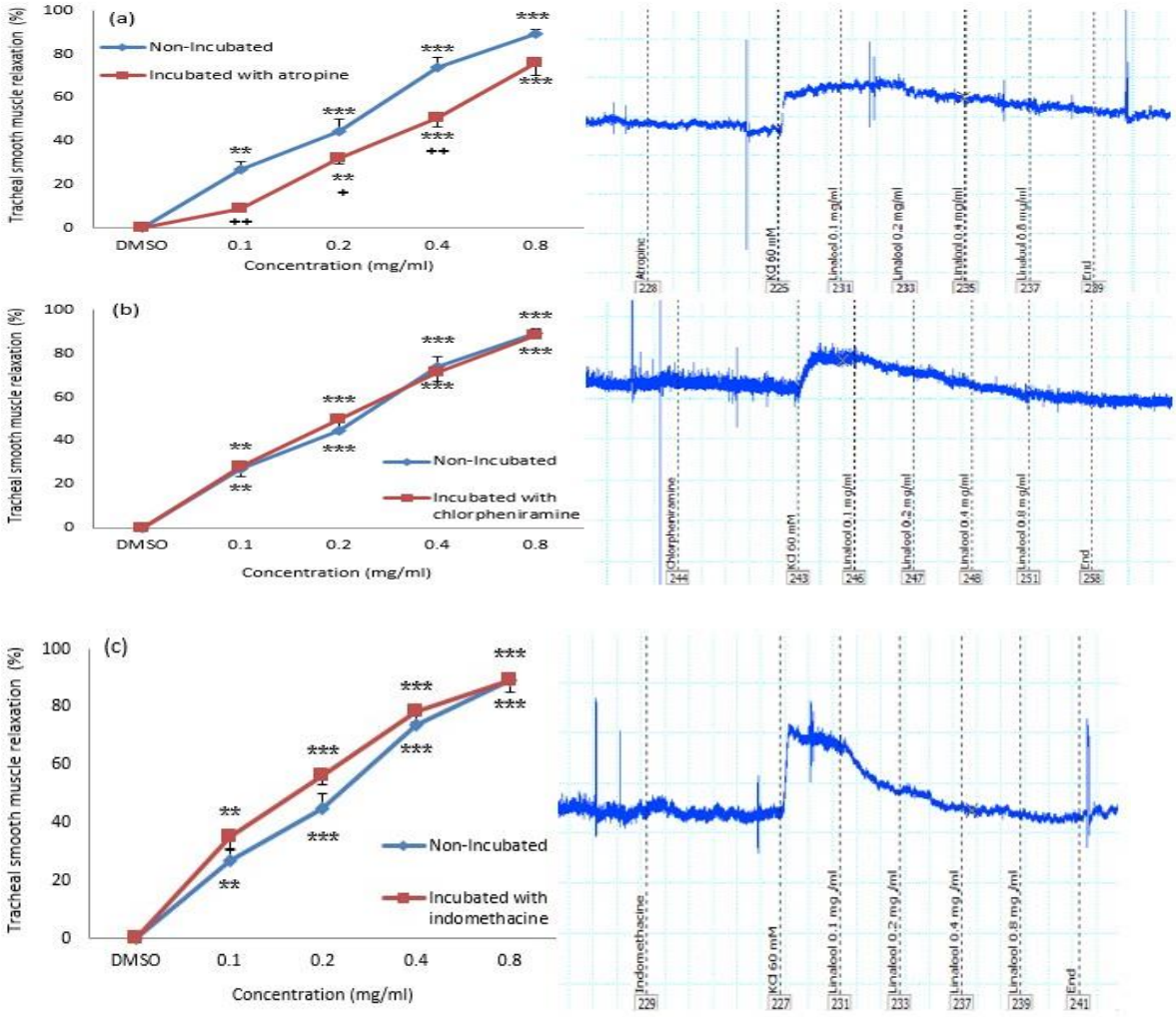


Figure 6. Linalool concentration–response relaxant effect (mean ± SEM) on TSM contracted by KCl (60 mM) in non-incubated tissues (n = 7) and tissues incubated with (a) atropine (1 μM, n = 6), (b) chlorpheniramine (1 μM, n = 6), and (c) indomethacin (1 μM, n = 6). TSM relaxation was expressed as a percentage of the maximum contraction induced by 60 mM KCl. **p<0.01, ***p<0.001 compared to the effect of dimethyl sulfoxide (DMSO), and +p<0.05, ++p<0.01 compared to the effect of linalool in non-incubated tissues. There was no significant difference in the relaxant effects of linalool between non-incubated tissues and tissues incubated with chlorpheniramine and indomethacin. ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

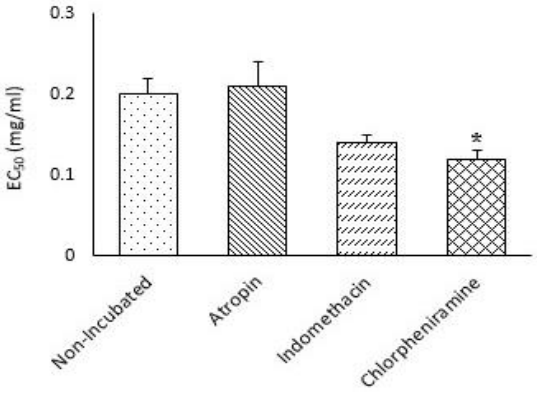


Figure 7. Effective concentration of linalool producing 50% of the maximum response (EC_{50}) values for linalool-induced relaxation in rat TSM contracted by KCl (60 mM) in non-incubated tissues (n = 7) and tissues incubated with atropine (n = 6), indomethacin (n = 6) and chlorpheniramine (n = 6). *p<0.05 compared to non-incubated tissues. ANOVA with Tukey Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

Comparison of linalool relaxant effect on KCl- and methacholine-induced contractions

Linalool at all concentrations produced comparable relaxant responses in methacholine- and KCl-induced contractions (Figure 8).

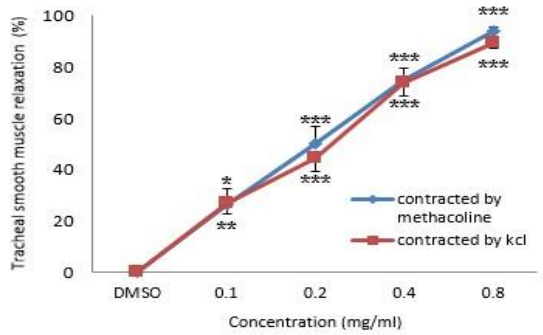


Figure 8. Linalool concentration–response relaxant effect (mean ± SEM) on TSM contracted by methacholine (10 μM) or KCl (60 mM) (n = 7 for each group). TSM relaxation was expressed as a percentage of the maximum contraction induced by 10 μM methacholine or 60 mM KCl. *p<0.05, **p<0.01, ***p<0.001 compared to the effect of dimethyl sulfoxide (DMSO). ANOVA with Tukey–Kramer post hoc test was used for statistical analysis. TSM: tracheal smooth muscle.

Discussion

Multiple receptors and ion channels are responsible for contraction and relaxant responses in airway smooth muscle. The relaxant effect of linalool on TSM and its possible mechanisms have not been fully elucidated. In this study, the effects of linalool on blockade of calcium channels, opening of potassium channels, muscarinic, β_2-adrenergic and histamine (H1) receptors, generation of nitric oxide, the cyclooxygenase cascade and phosphodiesterase function were evaluated by incubating TSM with diltiazem, glibenclamide, atropine, propranolol, chlorpheniramine, L-NAME, indomethacin and papaverine, respectively.

The findings of these experiments demonstrated linalool produced significant relaxation in a concentration-dependent manner in non-incubated TSM precontracted with KCl and/or methacholine. Moreover, linalool exerted a relatively potent relaxant action, similar to that of theophylline.

Earlier investigations have also demonstrated the relaxant action of linalool in guinea pig ileum and rat vas deferens. The mechanisms of action of linalool may depend on a number of factors that lead to smooth muscle relaxation in different tissue types (Poyton et al. 2015).

Several mechanisms have been proposed for the relaxation response induced by linalool in the presence of 5-hydroxytryptamine, acetylcholine, histamine, noradrenaline and oxytocin. These effects suggest that linalool affects the cell membrane through the opening of presynaptic voltage-gated calcium, potassium and sodium channels (Adsersen et al. 2006; Bradley et al. 2007; Lis-Balchin and Hart 1997; Rahmati et al. 2017).

In vitro studies have shown that nitric oxide (NO) may act directly as a neurotransmitter or bronchodilator agent in human respiratory tract. Guanylyl cyclase (GC) is a principal signal-transduction enzyme activated by NO and acts as a

relaxant agent in human airway smooth muscle (Barnes 1998a).

The relaxant action of linalool was assessed in TSM incubated with L-NAME to evaluate its effect on nitric oxide synthase (NOS). Under this condition, linalool at all concentrations produced significantly weaker relaxation compared with non-incubated preparations, suggesting that nitric oxide generation may contribute to the relaxant action of linalool on TSM. In addition, linalool showed a significantly greater EC_{50} in L-NAME-treated preparations compared with non-incubated TSM. These findings suggest that the relaxant effect of linalool on TSM may be mediated, at least in part, through nitric oxide formation.

It has been demonstrated that linalool might enhance the inhibitory effect of muscarinic receptors (Rahmati et al. 2017) and inhibit acetylcholinesterase enzyme activity, which has been considered as one of its other antispasmodic effects (Poyton et al. 2015). TSM preparations were exposed to atropine and precontracted with KCl in order to determine the influence of linalool on muscarinic receptors. In atropine-treated preparations, the higher concentrations of linalool produced significantly weaker relaxation compared with non-incubated tissues. A non-competitive effect of linalool on muscarinic receptors may be suggested by these results. Exposure of LPS-stimulated macrophage cell line J774.A1 to linalool induced an antinociceptive effect through m2 muscarinic receptors in rats, suggesting the probable involvement of the cholinergic system in this effect (Peana et al. 2006). It was also indicated that linalool reduced the number of writhing responses provoked by acetic acid in mice, while atropine antagonized the linalool effects. Thus, the antinociceptive action of linalool is mediated through muscarinic neurotransmission (Peana et al. 2003).

There were no significant differences between EC_{50} values of linalool in non-incubated and atropine-incubated tissues, which could be due to the lower maximum relaxation effect of linalool on atropine-incubated TSM. This effect also supports the non-competitive effect of linalool on muscarinic receptors (Barnes 1998b).

In this study, incubation of TSM with indomethacin was used to assess the effect of linalool on arachidonic acid metabolism and the cyclooxygenase pathway. Linalool showed no significant change in the relaxant response between non-incubated TSM and indomethacin-treated preparations. According to the findings of this part of the study, linalool did not act through this mechanism. There is controversy about the results of previous experiments in this regard. One study indicated that linalool did not improve PGE_2 formation and COX_2 expression (Peana et al. 2006), while another showed that linalool has a notable anti-inflammatory activity and a competitive inhibitory effect on the NMDA receptor (Peana et al. 2002).

Propranolol, chlorpheniramine, glibenclamide and papaverine were used to evaluate the possible involvement of β_2-adrenergic receptors, histamine (H1) receptors, potassium channels and phosphodiesterase activity, respectively. Linalool produced comparable relaxant responses in non-incubated tissues and in preparations treated with these agents. Therefore, these mechanisms are probably not implicated in the relaxant action of linalool on TSM.

The probable mechanisms accounting for the relaxation induced by linalool on TSM are illustrated in Figure 9. Future studies are needed to examine other possible mechanisms of the relaxant effect of linalool on TSM. The bronchodilatory effect of linalool should also be evaluated in clinical studies in patients with obstructive pulmonary diseases.

The present findings revealed a marked relaxant action of linalool on TSM, which has not been fully characterized in previous studies. These results also support the findings of previous studies on other experimental models, suggesting that linalool may induce smooth muscle relaxation through muscarinic receptor blockade and increased nitric oxide production.

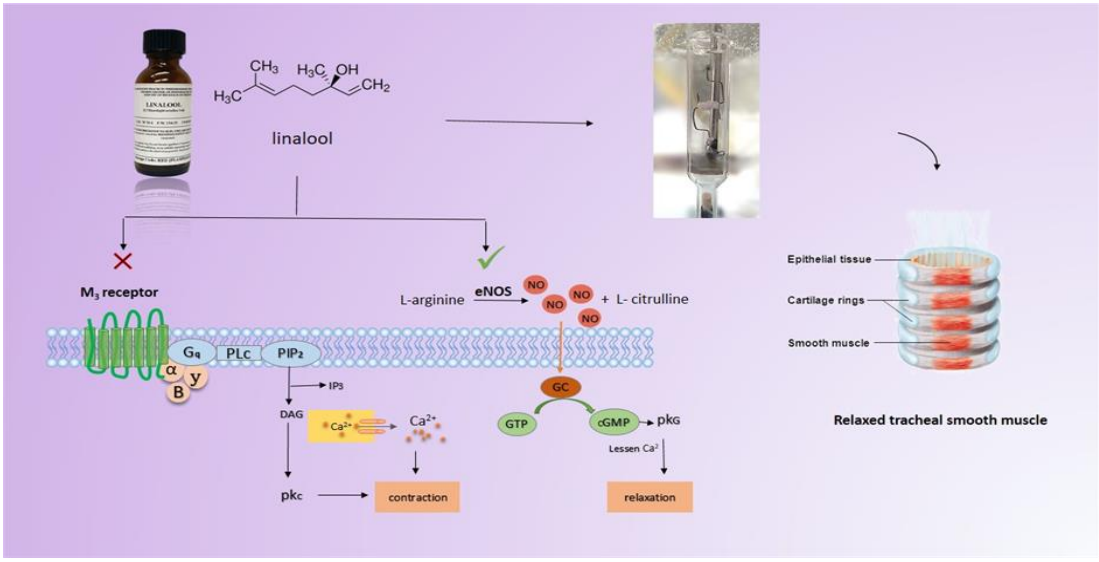


Figure 9. Molecular mechanisms of linalool in TSM relaxation. eNOS; endothelial nitric oxide synthase, NO; nitric oxide, PKC; protein kinase C, PLC; phospholipase C, PIP_2; phosphatidylinositol bisphosphate, GC; guanylyl cyclase, GTP; guanosine triphosphate, cGMP; cyclic guanosine monophosphate, IP_3; inositol trisphosphate, DAG; diacylglycerol. Muscarinic receptor blockade and involvement of NO production were suggested as the possible mechanisms of the relaxant effect of linalool on TSM according to the results of the present study.

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Conflicts of interest

No conflicts of interest are declared by the authors.

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Ethical Considerations

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences for Animal Experiments, Mashhad, Iran (approval number: 471127). All animal procedures were conducted in accordance with the guidelines for the care and use of laboratory animals.

Code of Ethics

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (approval number: 471127).

Authors' Contributions

M.H.B. and M.B. designed the experiments and supervised the research. F.N. and S.S. performed the experiments, analyzed the data, and wrote the paper. R.R. and H.S. helped in performing the experiments and preparing the paper. Z.GH. contributed to the interpretation of data and critical revision of the manuscript. All authors read and approved the final manuscript.

Abbreviations

ASM: airway smooth muscle; ANOVA: analysis of variance; DMSO: dimethyl sulfoxide; EC_{50}: effective concentration producing 50% of the maximum response; GC: guanylyl cyclase; iNOS: inducible nitric oxide synthase; L-NAME: ω-nitro-L-arginine methyl ester; NO: nitric oxide; NOS: nitric oxide synthase; SEM: standard error of the mean; SPSS: Statistical Package for the Social Sciences; TSM: tracheal smooth muscle.

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