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The Relaxant Effect of *Plantago Major* on Rat Tracheal Smooth Muscles and Its Possible Mechanisms

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ABSTRACT

This study was conducted to evaluate the possible mechanisms of the relaxant effects of hydroalcoholic extract of *Plantago major* (*P. major*) on tracheal smooth muscle (TSM) in rats.

The effects of cumulative concentrations of *P. major* (5, 10, 20 and 40 mg/mL) and theophylline (0.2, 0.4, 0.6 and 0.8 mM) were evaluated on pre-contracted TSM with 10 µM methacholine or 60 mM KCl. To determine the possible mechanisms, the relaxant effect of the plant was also examined on incubated TSM with atropine, indomethacin, chlorpheniramine, glibenclamide, diltiazem, papaverine, and propranolol.

The results indicated concentration-dependent relaxant effects for *P. major* in non-incubated TSM contracted by methacholine or KCl. There was no statistically significant difference in the relaxant effects of *P. major* between non-incubated and incubated tissues with indomethacin, papaverine, and propranolol. However, the relaxant effects of *P. major* in incubated tissue with atropine ($p<0.01$ to $p<0.001$), chlorpheniramine ($p<0.05$ to $p<0.001$), glibenclamide ($p<0.05$), and diltiazem ($p<0.01$) were significantly lower than non-incubated TSM.

P. major indicated relatively potent relaxant effects which were lower than those of theophylline. Muscarinic and histamine (H_1) receptors inhibition, as well as calcium channel blocking and potassium channel opening effects are suggested to contribute to the TSM relaxant effect of the plant.

Keywords: Histamine (H_1) receptor; Muscarinic receptor; *Plantago*; Smooth muscle; Trachea

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INTRODUCTION

Plantago major L. (*P. major*), broadleaf plantain, is an amazing herb that belongs to the Plantaginaceae family.[1] The seeds of the *P. major* are ovate shape and small size (0.4–0.8_0.8–1.5 mm) with a bit bitter taste.[2] It grows in compressed soils and is plentiful beside routes, roadsides, and other areas. It propagates by seeds, which are held on the long, narrow spikes that rise well above the foliage.[3] This plant grows in regions that weed is common and widely spread and naturalized in

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the world. *P. major* is native to northern and central Asia and most of Europe. In 4000 years ago, *P. major* was spread by the man from Europe throughout the world.[4]

P. major is a medicinal plant that used for the treatment of various conditions (e.g., skin problems, respiratory diseases, digestive tracts, and urinary infection).[2] A lot of beneficial effects have been reported for *P. major* leaves extracts including anti-inflammation, antitumor, antibacterial, antifungal[5], antiviral, analgesic, antioxidant, anti-ulcer, renal andhepatoprotective[6,] and immune-modulating activities.[7-9] Chemical studies on various *P. major* extracts indicated that phenol groups are major compounds of the plant. Terpenoids, flavonoids as organic acids, alkaloids and glycosides are observed in *P. major* extracts (Figure 1).[10] Also, Ursolic acid (0.22%) and oleanolic acid (0.07%) are terpenoids compounds of *P. Major*.[10,11]

The above-mentioned compounds found in almost all parts of the plant. Bioactivity of *P. major* leaves contained the secondary metabolites is attributed to these chemical constituents.[12] It has been demonstrated that the anti-inflammatory effect of the plant may relate to ursolic acid. This terpenoid compound is a selective inhibitor of cyclooxygenase-2 (COX-2), that responsible for prostaglandins biosynthesis; so, *P. major* have an anti-inflammatory effect by COX-2 inhibition.[12]

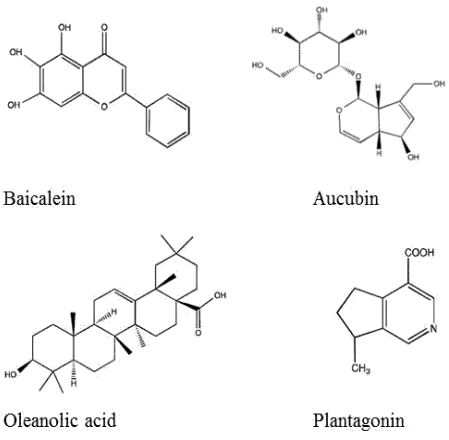

Figure 1. Some chemical structural of *Plantago major (P. Major)* compounds including baicalein (flavonoid), aucubin (irioid glycoside), oleanolic acid (terpenoid) and plantagonin (alkaloid)

Leaves of the plant are rich sources of essential fatty acids (18:26ω and 18:33ω) and also of carotenes.[13] *P. major*, also contain some other effective components including ferulic acid, ursolic acid, baicalein, apigenin, benzoic acid, chlorogenic acid, ascorbic acid, citric acid, oleanolic acid, salicylic acid.[14] It is believed that the most important chemicals ingredients in *P. major* are baicalein (a flavonoid) and aucubin (an irioid glycoside) which are biologically active agents (Figure 1).[2]

In recent years, in both non-developed and developed countries, chemical synthetic drugs have been extensively substituted with herbal medicines.[15] This plant can relax smooth muscles for example, in clinical practice, many brands of *P. major* are used to decrease gastrointestinal movements and diarrhea management.[10] On the other hand, the effect of *P. major* on respiratory and allergic diseases was shown.[16]

Based on previous studies, the present study was designed to assess the possible mechanisms responsible for the relaxant effect of hydroalcoholic extract of *P. major* on rat tracheal smooth muscle (TSM). Additionally, to evaluate the effect of *P. major* on ß2-adrenergic, muscarinic and histamine (H_1) receptors, prostacyclin and COX-2 pathway, potassium and calcium channels, as well as phosphodiesterase activity, the relaxant effect of *P. major* was examined on TSM incubated with propranolol, atropine, chlorpheniramine, glibenclamide, diltiazem, indomethacin, and papaverine respectively.

P. major indicated concentration-dependent relaxant effects in non-incubated TSM contracted by potassium chloride (KCl) and methacholine which was reduced in incubated tissue with atropine chlorpheniramine, glibenclamide, and diltiazem. The results indicated a potent relaxant effect of *P. major* which suggested to be due to muscarinic and histamine (H_1) receptors inhibition, calcium channel blocking, and potassium channel opening effects.

MATERIALS AND METHODS

Materials

P. major seeds were obtained from a grocery market in Mashhad, Khorasan Razavi province, Iran, October 2017. This plant was recognized and identified by Dr. Iranshahi (PharmD, Ph.D. in Pharmacognosy), a professor in the Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

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KCl was obtained from Merck (Darmstadt, Germany). Methacholine, atropine, chlorpheniramine, indomethacin, diltiazem, glibenclamide, propranolol and papaverine were also purchased from Sigma Chemical Co. Ltd (UK).

Preparation of *P. Major* Extract

P. major seeds were carefully dried and cleaned and powdered with mixer. Next, 100 g of powdered seeds were poured into Erlenmeyer flask and soaked up with a hydro-ethanolic solution (70%) for 3 days, with stirring and shaking at 37ºC (in shaker-stirrer).

To remove alcohol, the mixture was then filtered by Eyela (Heidolph, Germany) rotary evaporator and the resultant solution was concentrated under reduced pressure at 45ºC for 24 h in an oven (for drying). The yield extract was 13% and it was stored at -70ºC until use.

Animals

The experiments were performed on fifty-one male Wistar rats (7 weeks, weighting 200-250 g). The animals were purchased from the Animal House, Faculty of Medicine, Mashhad University of Medical Sciences (Mashhad, Iran) and maintained under controlled conditions with 12 h/ 12 h light/dark cycle at 22±2 °C. Water and food were always accessible *ad libitum* to animals. This study was approved by the Committee on Animal Research of the Mashhad University of Medical Sciences for Animal Experiments (Approval No. 930658).

Tissue Preparation

After sacrificing the animals, the chest was opened and segmental resection of the trachea was prepared including four rings. The tissues mounted in a 10 mL organ bath containing Krebs- Henseleitsolution (KHS), and maintained at 37±0.5 °C with isometric tension of 1 gas previously described[17]. The isometric transducer (MLT0202, AD Instruments, Australia) linked to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia) was used to measure contraction and relaxation responses in all experiments.

Experimental Design

In various groups, the relaxant effect of *P. major* extract was measured according to previous studies[18,19] on incubated or non-incubated tissues with different drugs for 10 minutes as described in Table 1.

To examine the relaxant effect of *P. major*, rat TSM was contracted by methacholine for 7 minutes or KCl for 5 minutes in the non-incubated (n=10) and incubated tissues with 1 µM atropine (n=11), 1 µM indomethacin (n= 8), 1 µM chlorpheniramine (n=8), 5 µM diltiazem (n=8), 1 µM glibenclamide (n=8), 1 µM propranolol (n= 8) or 50 µM papaverine (n=8).

Cumulative concentrations of *P. major* extract (5, 10, 20. and 40 mg/mL) were added on pre-contracted TSM with methacholine or KCl every 5 minutes and at the end of the intervals, the relaxation was recorded in each experiment. Concentrations of *P. major* were chosen based on previous studies.[18] The percent relaxation of each concentration of *P. major* or theophylline was plotted relative to the maximum contraction due to methacholine or KCl to make a concentration-response graph. Theophylline (0.2, 0.4, 0.6, and 0.8 mM) and saline (1 mL) were also examined as positive control and negative control respectively. Furthermore, the effective concentration of *P. major* causing 50% of maximum response (EC_{50}) was also measured from concentration-response curves.[19]

Table 1. The protocol of the study and the methods of evaluating various mechanisms of the relaxant of the effect of *Plantago major* (*P. major)* on tracheal smooth muscle (TSM)

TSM Contracting agent	Condition	Incubating substance	Mechanisms
60 mM KCl	Non-incubated tissues (n=10)		
	Incubated tissues	1 µM atropine (n=11)	Muscarinic receptor inhibition
		1 µM indomethacin (n=8)	Cyclooxygenase inhibition
10 µM	Non-incubated tissues (n=10)		
methacholine		1 µM chlorpheniramine (n=8)	Histamine (H_1) receptor inhibition
	Incubated tissues	5 µM diltiazem (n=8)	Calcium channel blocking
		1 µM glibenclamide (n=8)	Potassium channel opening
		1 µM propranolol (n=8)	β-adrenoceptor stimulation
		50 µM papaverine (n=8)	Phosphodiesterase inhibition

Statistical Analysis

All data were presented as mean±SEM. Statistical comparisons of the results were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. Statistically significant was considered as $p<0.05$. The correlation between the relaxant effect of *P. major* and theophylline with their concentrations was assessed by Pearson's correlation coefficient.

RESULTS

***P. major* Induced Relaxant Effect in non-incubated TSM Contracted by KCl or Methacholine**

Concentration-dependent and significant relaxant effects were exhibited for *P. major* in non-incubated TSM contracted by KCl ($p<0.01$ to $p<0.001$ for the last three higher concentrations of the extract). However, the relaxant effect so falls concentrations of the *P. major* extract, were significantly lower than those of theophylline ($p<0.001$ for all concentrations, Figure 2a).

P. major extract demonstrated significant and concentration-dependent relaxant effects in non-incubated TSM contracted by methacholine ($p<0.001$ for three last concentrations, Figure 2b). In methacholine-induced contraction also, the relaxant effects of all concentrations of the extract were significantly lower than those of theophylline ($p<0.01$ for its high concentration and $p<0.001$ for other concentrations, Figure 2b).

***P. major* Induced Relaxant Effect in Incubated TSMs Contracted by KCl**

In tissues incubated with atropine, the relaxant effects of three first concentrations of *P. major* were significantly lower than non-incubated TSM ($p<0.01$ to $p<0.001$, Figure 3a). However, in incubated tissues with indomethacin, the relaxant effect of a high concentration of *P. major* was significantly higher than the non-incubated condition ($p<0.05$, Figure 3b).

No statistically significant difference was observed among EC_{50} values of the extract in non-incubated TSM contracted by KCl (11.06±3.87) and tissues incubated with atropine (18.75±5.63) and indomethacin (10.45±5.62, Figure 3c).

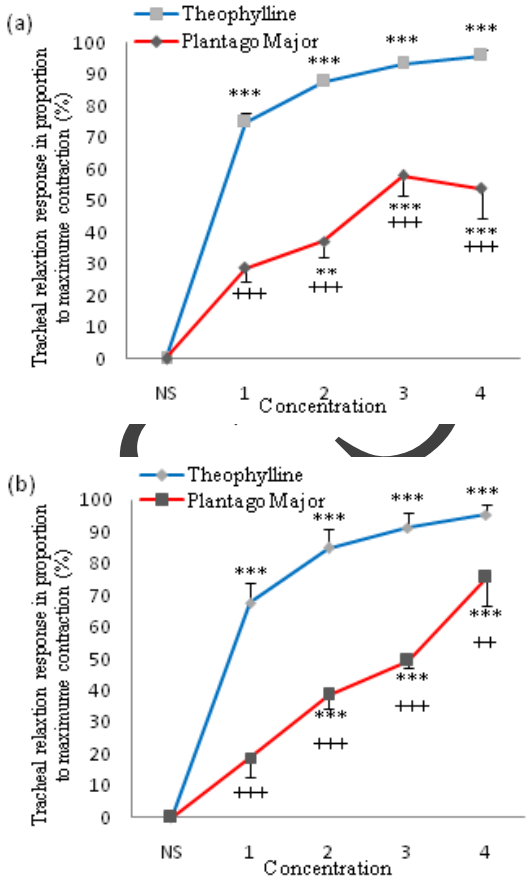

Figure 2. Concentration-response relaxant effect of hydroalcoholic extract of *P. major* (5, 10, 20, 40 mg/mL), (n=10) and theophylline (0.2, 0.4, 0.6, 0.8 mM) (n=6) on (a) KCl (60 mM) or (b) methacholine (10 µM) induced contraction of tracheal smooth muscle (TSM) in non-incubated tissues. Data are shown as mean ± SEM.*: $p<0.001$ compared to saline (NS). +++: $p<0.001$ and ++: $p<0.01$ compared to the effect of theophylline. The correlation between the relaxant effect of *Plantago major* (*P. Major)* and theophylline with their concentrations was assessed by Pearson's correlation coefficient**

***P. major* Induced Relaxant Effect in Incubated TSMs Contracted by Methacholine**

The relaxant effects of three last concentrations of *P. major* in incubated tissues with chlorpheniramine and the relaxant effect of its second concentration (10 mg/mL) in incubated tissues with glibenclamide and diltiazem were significantly lower compared to non-incubated TSM ($p<0.05$ to $p<0.001$, Figure 4a-c).

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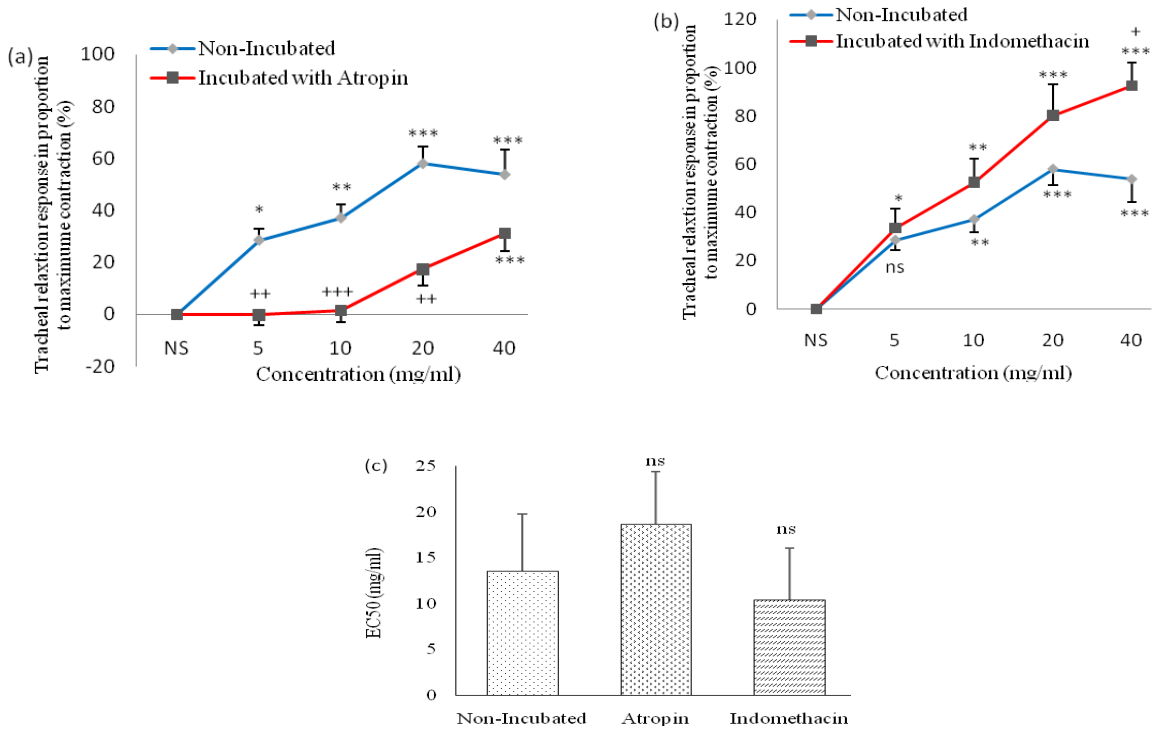

Figure 3. The concentration-response relaxant effect of hydroalcoholic extract of *Plantago major* (*P. major)* on KCl (60 mM) induced contraction of tracheal smooth muscle (TSM) in non-incubated (n=10) and incubated tissues with (a) atropine (1 µM, n=11) and (b) indomethacin (1 µM, n=8). (c) EC_{50} values of hydroalcoholic extract of *P. major* induced relaxation obtained on contracted TSMs of the rat with KCl in non-incubated and incubated tissues with atropine and indomethacin. Data are shown as mean ± SEM. ***:p<0.001, **:*p*<0.01 and *:*p*<0.05 compared to saline (NS). +++: *p*<0.001, ++: *p*<0.01 and +: *p*<0.05 compared to non-incubated tissues. Statistical Analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests

However, the relaxant effects of various concentrations of *P. major* in incubated tissues with papaverine and propranolol were not statistically different from non-incubated tissues (Figures 4dand 4e).

There was no statistically significant difference among EC_{50} values of the extract of the plant in non-incubated tissue contracted by methacholine (13.55±6.25) and TSM incubated with chlorpheniramine (16.90±6.20), glibenclamide (16.67±6.62), diltiazem (14.70±5.10), papaverine (14.17±3.47) and propranolol (16.05±6.64), (Figure 4f).

Also, there were no statistically significant differences in the relaxant effect of various concentrations of *P. major* obtained in KCl induced contraction with those in TSM contracted by methacholine (Figure 5).

Correlations of the Relaxant Effect of *P. major* or Theophylline with Their Concentrations

In non-incubated TSM contracted by KCl or methacholine, there were significant correlations between the relaxant effect of theophylline ($p<0.001$ and $p<0.01$, respectively) or *P. major* ($p<0.01$ and $p<0.001$, respectively) and their concentrations (Table 2).

Significant correlations were also seen between the relaxant effect of *P. major* and its concentrations in KCl or methacholine induced contraction of TSM, incubated with atropine (r=0.728, $p<0.001$), indomethacin (r=0.785, $p<0.001$), chlorpheniramine (r=0.721, $p<0.01$), diltiazem (r=0.750, $p<0.001$), glibenclamide (r=0.703, $p<0.01$), papaverine (r=0.910, $p<0.001$), and propranolol (r=0.828, $p<0.001$, Table 2).

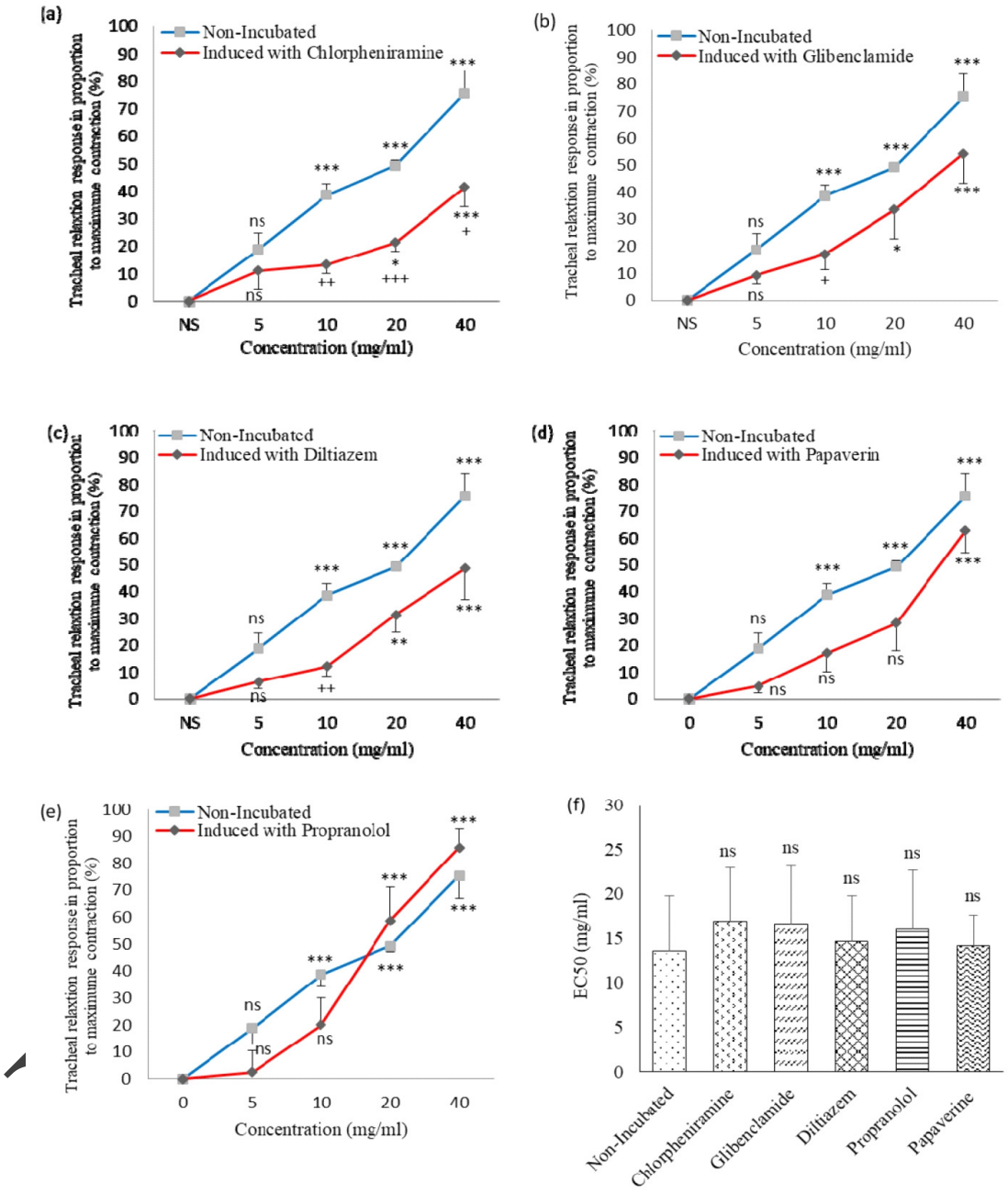

Figure 4. The concentration-response relaxant effect of hydroalcoholic extract of *Plantago major* (*P. major)* on methacholine (10 mM) induced contraction of tracheal smooth muscle (TSM) in non-incubated (n=10) and incubated tissues with (a) chlorpheniramine (1 µM, n=8), (b) glibenclamide (1 µM, n=8), (c) diltiazem (5 µM, n=8), (d) papaverine (100 µM, n=8) and (e) propranolol (1 µM, n=8). (f) EC_{50} values of hydroalcoholic extract of *P. major* induced relaxation obtained on contracted rat TSM with methacholine in non-incubated and incubated tissues with chlorpheniramine (n=8), glibenclamide (n=8), diltiazem (n=8), propranolol (n=8) and papaverine (n=8). ***:p<0.001, **: p<0.01 and *:p<0.05 compared to saline (NS). +++: p<0.001, ++: p<0.01 and +: p<0.05 compared to non-incubated tissues. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

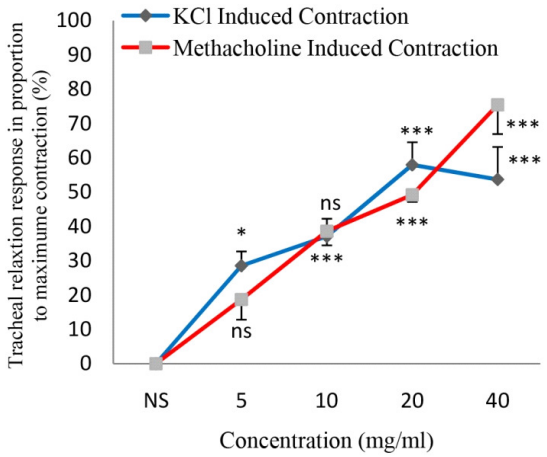

Figure 5. The concentration-response relaxant effect of hydroalcoholic extract of *Plantago major* (*P. major)* on methacholine (10 µM) and KCl (60 mM) induced contraction of non-incubated tracheal smooth muscle (TSM) (n=10). Data are shown as mean ± SEM. ***:p<0.001 and *:p<0.05 compared to saline (NS). Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Table 2. Relationship between the relaxant effect of *Plantago major* (*P. major)* and theophylline with their concentrations in different experimental groups

Contractile agents	Studied agents	Conditions	R	*p* value
		Non-Inc	0.601	$p < 0.01$
KCl	*P. major*	Atropine-Inc	0.728	$p < 0.001$
		Indomethacin-Inc	0.785	$p < 0.001$
	Theophylline	Non-Inc	0.790	$p < 0.001$
Methacholine		Non-Inc	0.873	$p < 0.001$
		Chlorpheniramine-Inc	0.721	$p < 0.01$
		Diltiazem-Inc	0.750	$p < 0.001$
	P. major	Glibenclamide-Inc	0.703	$p < 0.01$
		Papaverine-Inc	0.910	$p < 0.001$
		Propranolol-Inc	0.828	$p < 0.001$
	Theophylline	Non-Inc	0.607	$p < 0.01$

Data were presented as mean ± SEM.

DISCUSSION

This study examined the relaxant effect of *P. major* on pre-contracted TSM by methacholine and KCl and indicated a relatively potent relaxant effect for *P. major*.

The relaxant effects of the medicinal plants on TSM might be produced by various mechanisms such as ß2-adrenergic receptors stimulation,[20] histamines (H_1) receptors inhibition,[21] calcium channel-blocking,[22] potassium channel opening,[23] muscarinic receptors inhibition,[24] methylxanthine activity,[25] inhibition of nitric oxide synthase (NOS),[26] inhibition of the enzyme phosphodiesterase,[27] inhibition of cyclooxygenase (COX) pathway,[28] and inhibiting the ATP-sensitive potassium channels.[29] The previous study has shown that Flavonoid compounds of *P. major*, have a relaxant effect on smooth muscle via potassium channels opening and beta2-adrenergic receptor activation and histamine (H_1) receptors inhibition.[17]

In the present study, to evaluate the effect of *P. major* on muscarinic receptors, histamine (H_1)

receptors inhibition, prostacyclin, and COX-2 inhibitory mechanism, potassium channel opening, calcium channel-blocking, ß2-adrenergic receptors and phosphodiesterase activity, the relaxant effect of *P. major* was examined in TSM incubated with atropine, chlorpheniramine, indomethacin, glibenclamide, diltiazem, propranolol, and papaverine respectively.

In incubated TSM with atropine and contracted with KCl, the relaxant effect of hydroalcoholic extract of *P. major* was examined to assess the contribution of muscarinic receptor inhibitory effect on the relaxant property of the plant. The results of this experiment indicated significantly lower relaxant effects of three first concentrations of *P. major* in incubated tissues with atropine compared to non-incubated TSM.EC_{50} value obtained in TSM incubated with atropine was not statistically different compared to non-incubated conditions. These results indicated the inhibitory effect of *P. major* on muscarinic receptors which could be contributed in its relaxant effect of the extract on TSM.

The main muscarinic receptor in the smooth muscle is the M_3 receptor which activates phospholipase C and inositol trisphosphate to release calcium from intracellular stores.[30] This expresses that a combination of muscarinic receptor antagonists and β2-adrenoceptor agonists which used in obstructive respiratory diseases, have synergistic effects on airway caliber, so the dose of these drugs in comparison with monotherapy is reduced.[31] Muscarinic receptors also can induce the production of inflammatory mediators and pro-inflammatory cytokines[32] especially in cigarette-smokers and allergic conditions.[33] Lower relaxant effect of all concentrations of *P. major* on incubated tissues with atropine compared to the effects obtained in TSM incubated with other agents also support this mechanism of action for *P. major* (Table 3).

Table 3. Comparison of the relaxant effect *Plantago major* (*P. major)* (percentage change in proportion to the maximum contraction) in different incubated tracheal smooth muscles (TSMs) contracted by 10 µM methacholine (n=8) or 60 mM KCl (n=10)

Incubating substance	Concentration (mg/mL)			
	5	10	20	40
Atropine	0.24 ±4.11	1.35 ± 4.28	17.34 ± 6.40	31.17 ± 6.73
Indomethacin	33.55 ± 7.98 **	52.43 ± 9.82 **	80.17 ± 13.06 **	92.49 ± 9.52 ***
Chlorpheniramine	11.06 ± 6.88	13.29 ± 3.22	21.27 ± 3.10	41.48 ± 7.29
Diltiazem	6.34 ± 2.53	12.17 ± 3.59	31.33 ± 6.50	48.63 ±11.58
Glibenclamide	9.35 ± 3.42	17.04 ± 5.72	33.64 ± 10.77	54.39 ±11.09
Papaverine	4.62± 2.61	16.94±7.04	28.23 ± 10.38	62.73 ±8.43
Propranolol	2.52 ± 8.09	20.11 ± 10.05	58.81 ± 12.45 #	85.81 ±6.75 ## ∫ ^

Data were presented as mean ± SEM. ***:p<0.001 and **:p<0.01 compared to incubated tissues with atropine. ##:p<0.01 and #:p<0.05 compared to incubated tissues with chlorpheniramine. ∫:p<0.05 compared to incubated tissues with diltiazem. ^:p<0.05 compared to incubated tissues with glibenclamide.

To study the effect of *P. major* on histamine (H_1) receptor and the involvement of this mechanism in the relaxant effect of the plant, its relaxant effect was examined in TSM incubated with chlorpheniramine and contracted with methacholine. The results of this group expressed significantly lower relaxant effects of three higher concentrations of *P. major* in incubated tissues with chlorpheniramine compared to the non-incubated TSM. Histaminic receptors are involved in cell contraction. When these receptors (H_1) activated, intracellular Calcium levels were increased via phospholipase C cascade. On the other hand, histamine receptors have an inflammatory effect by inducing the release of cytokines that can activate Th1 and Th2 immune cells.[34] The results indicated the inhibitory (non-competitive) effect of the extract on histamine receptors which could be contributed in its relaxant effect on TSM. In a clinical trial also, *P. major* indicated antihistamine effect in urticarial, which support our finding.[35]

To evaluate the involvement of prostacyclin mechanism and COX pathways in *P. major* relaxation, its effect was examined in incubated tissues with indomethacin as nonselective COX inhibitor. As shown

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in the previous study, some compounds of *P. major* extract such as α-linolenic acid, triterpenic acid, ursolic acid, and oleanolic acid, have COX inhibitory effect.[36] The anti-inflammatory effect of *P. major* was also seen previously[6] which could be due to its cyclooxygenase inhibitory effect. The significant lower relaxant effect of the highest concentration of the extraction incubated tissues with indomethacin compared to non-incubated condition suggested that the COX inhibitory effect of *P. major* may contribute to its TSM relaxation with a non-competitive manner.

To examine the involvement of calcium channel blocking of *P. major*, the relaxant effect of the extract was studied in incubated TSM with diltiazem. The significant lower relaxant effects of the second concentration of the extract (10 mg/mL) were seen in incubated tissue with diltiazem compared to non-incubated tissues. Therefore, the calcium channel-blocking mechanism may also contribute to the relaxant effect of *P. major* on TSM.

In incubated TSM with glibenclamide, the relaxant effect of *P. major* was evaluated to determine the involvement of potassium channels in the relaxant effect of the plant. The significant lower relaxant effect of the second concentration of the extract (10 mg/mL) was observed. Potassium is an intracellular ion, therefore the opening of potassium channels, leads to cell hyperpolarization and potassium diffusion out of the cell. It is indicated that *P. major* causes potassium diffusion outside the cell[37] which suggests the other possible mechanism responsible for the relaxant effect of the plant could be the potassium channel-opening effect.

However, the relaxant effect of the extract in incubated TSM with propranolol and papaverine was not significantly different from those on non-incubated TSM. These results indicated that ß2-adrenergic stimulation and phosphodiesterase inhibitory mechanisms are not contributed to the relaxant effect of *P. major*.

One of the main limitations of this study is the lack of the characterization of the extract of the plant by HPLC which should be performed in a further study. In addition, the other possible mechanisms responsible for the relaxant effect of the plant including its effect on the non-adrenergic non-cholinergic nervous system should be examined in future studies. The clinical studies for evaluating the bronchodilatory effect of *P. major* in patients with obstructive pulmonary diseases should be also undertaken in the future.

The present study provided novel information about the TSM relaxant effect of *P. major*. Inhibition of muscarinic and histamine (H_1) receptors, calcium channel blocking, and potassium channel opening effects were suggested as possible mechanisms for the relaxant effect of *P. major*.

Taken together, hydroalcoholic extract of *P. major* indicated a relatively potent relaxant effect on TSM which was lower than the effect of theophylline at studied concentrations. Based on the results of this study and the pathophysiology of pulmonary disease, *P. major* can be used as an effective medicinal plant in the treatment of chronic inflammatory respiratory and obstructive pulmonary problems via expressed mechanisms. It is suggested that the efficacy of this plant be studied in future animal studies and clinical trials.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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