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# Histamine ($H_1$) Receptors, Cyclooxygenase Pathway and Nitric Oxide Formation Involved in Rat Tracheal Smooth Muscle Relaxant Effect of Berberine

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## ABSTRACT

In this study we aimed to examine the relaxant effect of berberine, a compound extracted from a variety of herbs, on rat tracheal smooth muscle (TSM) and its possible mechanism(s).

Cumulative concentrations of berberine (20, 65, 200 and 600 µg/mL) were added on pre-contracted TSM by methacholine or KCl in non-incubated or incubated tissues with atropine, chlorpheniramine, propranolol, diltiazem, glibenclamide, indomethacin, L-NG-nitro arginine methyl ester (L-NAME) and papaverine. The relaxant effects of theophylline (0.2, 0.4, 0.6 and 0.8 mM) as positive control and saline (1 mL) as negative control were also examined in non-incubated tissues.

Berberine showed significant and concentration-dependent relaxant effects in non-incubated tissues contracted by KCl and methacholine ($p<0.01$ to $p<0.001$). There was no significant difference in the relaxant effects of berberine between non-incubated and incubated tissues with atropine, propranolol, diltiazem, glibenclamide, and papaverine. The relaxant effects of second concentrations of berberine in incubated tissues with L-NAME, its three lower concentration in incubated tissues with chlorpheniramine and its all concentrations in incubated tissues with indomethacin were significantly lower than non-incubated tissues ($p<0.05$ to $p<0.001$). The EC50 values of berberine in incubated tissues with chlorpheniramine was significantly higher than the non-incubated condition ($p<0.05$).

Our findings reveal a relatively potent relaxant effect of berberine that is lower than the effect of theophylline. Proposed mechanisms for the relaxant effect of berberine are histamine ($H_1$) receptor blockade, inhibition of cyclooxygenase pathways and/or nitric oxide formation.

**Keywords:** Berberine; Cyclooxygenase; Histamine ($H_1$) receptor; Nitric oxide; Relaxation; Smooth muscle; Trachea

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## INTRODUCTION

The pharmacology of airway smooth muscle focuses on the influence of various agents that these



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agents may have therapeutic property such as bronchodilatory effect.[1] Great attention has recently been paid to recognize the relaxation mechanism of various agents which usually showed multiple mechanisms.[1]

Berberine has been detected and isolated from rhizomes, roots, and stem bulk[2] of some plant families such as the Annonaceae, Berberidaceae, Fumariaceae, Menispermaceae, Papaveraceae, Ranunculaceae, and Rutaceae.[3] Berberine, (2, 3-methylenedioxy-9, 10-dimethoxyprotoberberine chloride), is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids.[4] (Figure 1A). It has long been known for its anti-inflammatory and antimicrobial activities.[5] Other pharmacological actions of berberine include antidiarrheal, antineoplastic, antiarrhythmic,[6] anti-colitic,[7] inhibition of intestinal ion secretion and smooth muscle contraction.[8] Four major metabolites were identified for berberine including berberrubine, thalifendine, demethyleneberberine and jatrorrhizine[4] (Figure 1B-E).

Berberine can evoke endothelium-dependent relaxation of vascular smooth muscle which dilates blood vessels and decreases blood pressure by blocking $\alpha_1$-receptors of vascular smooth muscle cells, inhibiting choline phospholipid enzymes activity, and enhancing acetylcholine activity.[4] Berberine showed potent relaxant effect on rat isolated mesenteric arteries. Moreover, berberine-induced a concentration-dependent relaxation in phenylephrine-precontracted corpus cavernosum in the rabbit. Exposure to L-NG-nitro arginine methyl ester (L-NAME) reduced the relaxant effect of berberine. While, atropine, indomethacin, phentolamine, and propranolol did not affect the relaxant effect of berberine.[9] Berberine also showed the relaxation response in guinea-pig tracheal smooth muscle (TSM) with an $EC_{50}$ of 34.2±0.6 μg/mL. The relaxant effect of berberine was not antagonized by xanthine amine congener or timolol.[10]

Several preclinical and clinical studies showed pharmacological and therapeutic effects of berberine on inflammatory conditions, hyperlipidemia, diabetes, cardiovascular diseases, osteoporosis, cancer, cerebral ischemia and trauma, Alzheimer disease, mental disease, bacterial and viral infections.[11] In spite of extensive applications and multiple effects, the mechanism of action in most of its effects is not exactly clear. Therefore, the present study set up to evaluate the relaxant effect of berberine and its possible mechanisms on the TSM in rats.

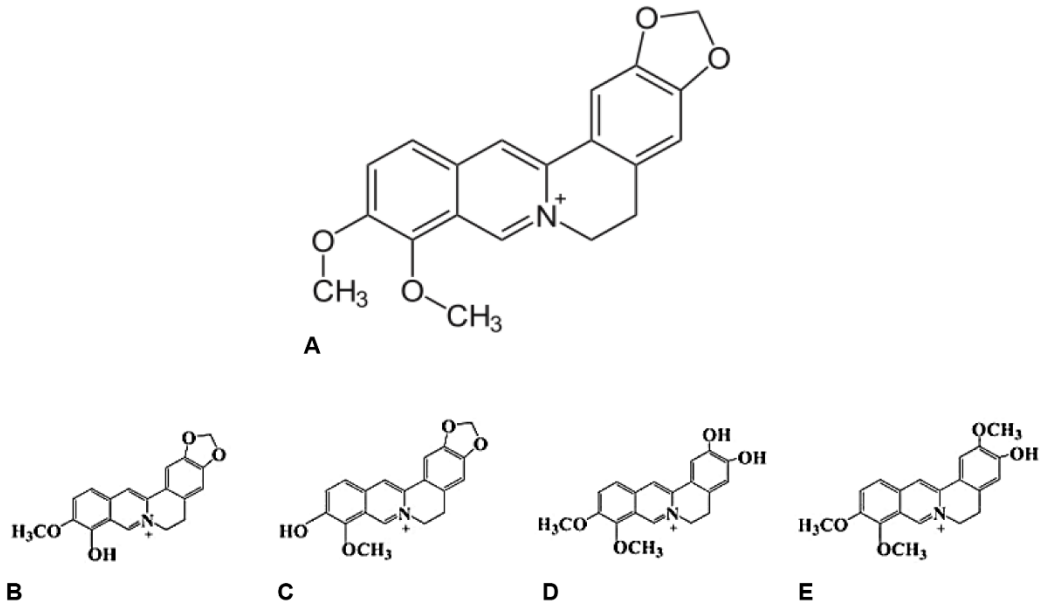


**Figure 1. The chemical structural formula of berberine, $C_{20}H_{18}NO_4$ (A) berberrubine, $C_{19}H_{16}ClNO_4$ (B), thalifendine, $C_{19}H_{16}NO_4$ (C), demethyleneberberine, $C_{19}H_{18}NO_4$, (D), jatrorrhizine $C_{20}H_{20}ClNO_4$ (E)**

## MATERIALS AND METHODS

### Materials

Berberine chloride ($C_{20}H_{18}ClNO_4$) with CAS Number 633-65-8 and EC Number 211-195-9 was purchased from Sigma Chemical Co. (Dorset, UK). Potassium chloride (KCl) was obtained from Merck (Darmstadt, Germany). Methacholine, atropine, chlorpheniramine, indomethacin, diltiazem, glibenclamide, propranolol, L-NAME, and papaverine were also purchased from Sigma Chemical Co. (Dorset, UK).

### Animals

One hundred male or female Wistar rats weighing approximately 200 to 250 g were purchased from Animal House, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran and maintained under standard condition at 12 h light/dark cycle, 22±2°C and humidity of 54±2% with food and water available *ad libitum*. The study was approved by the ethics committee of Mashhad University of Medical Sciences for Animal Experiments (N. 951625).

### Preparation of Tracheal Ring

A piece of the trachea with 5-6 cartilage rings was isolated from sacrificed rats and mounted between two stainless steel hooks in 10 mL organ bath containing Krebs-Henseliet solution (KHs) under 1 g resting tension as previously described.[12,13] The contractile responses of isolated tissue were recorded using an isometric transducer (MLT0202, AD Instruments, Australia) which was linked to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia).

### Measurement of TSM Relaxation

After a 60 min equilibrium period, the TSM was contracted by KCl (60 mM) or methacholine (10 μM) for 5 or 7 min, respectively.[12,13] Berberine (20, 65, 200 and 600 μg/mL) were applied cumulatively on KCl or methacholine-contracted TSM at 5 min interval. At the end of the intervals, the relaxation response was recorded in each experiment. Theophylline (0.2, 0.4, 0.6 and 0.8 mM) as positive control and saline (1 mL) as negative control, were also examined. The percent relaxation per each concentration of berberine or theophylline was plotted relative to the maximum contraction resulted from KCl or methacholine to make concentration-response curve. Furthermore, the effective concentration of berberine causing 50% of maximum response ($EC_{50}$) was also measured from concentration-response curve.[13]

### Experimental Groups

The relaxant effects of berberine were examined on non-incubated and incubated tissues with different substance for 10 minutes before contraction of TSM (Table 1). The effect of theophylline as a positive control was only evaluated on non-incubated tissues (n=6).

**Table 1. Different experimental groups of study on smooth muscle relaxant effect of berberine**

| Agents inducing contraction | Agents Incubating TSM | Possible Mechanisms |
|---|---|---|
| KCl (60 mM) | | |
| Non-incubated TSM (n=5) | | |
| Incubated TSM | Atropine (n=5) | Muscarinic receptor inhibition |
| | Indomethacin (n=5) | COX inhibition |
| | Chlorpheniramine (n=5) | Histamine ($H_1$) receptor inhibition |
| Methacholine (10 μM) | | |
| Non-incubated TSM (n=7) | | |
| Incubated TSM | Diltiazem (n=6) | Calcium channel blocking |
| | Glibenclamide (n=7) | Potassium channel opening |
| | Propranolol (n=5) | β-adrenoceptor stimulation |
| | Papaverine (n=5) | Phosphodiesterase inhibition |
| | L-NAME (n=5) | NO formation |

Abbreviations: TSM, tracheal smooth muscle; COX, cyclooxygenase; L-NAME, L-NG-nitro arginine methyl ester; NO, nitrite oxide.

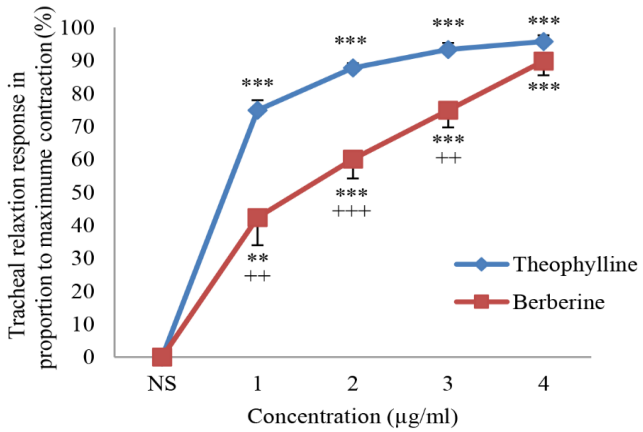


**Figure 2. Relaxant effects of cumulative concentrations of berberine (n=5) and theophylline (n=6) on rat tracheal smooth muscle (TSM) contractions induced by KCl (60 mM). 1, 2, 3 and 4 in X-axis represent four concentration of berberine (20, 65, 200 and 600 µg/mL) and theophylline (0.2, 0.4, 0.6 and 0.8 mM). **: *p*<0.01 ***: *p*<0.001 compared to saline (NS), ++: *p*<0.01 ++: *p*<0.001 compared to the effect of theophylline. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

### Data Analysis

Tracheal contractions induced by KCl or methacholine were assumed as 100% and the relaxation response (%) after applying berberine was calculated. Statistical analyses were performed using SPSS software version 16 (Inc, Chicago, IL, USA). Data were analyzed by the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test and results were presented as mean ± SEM. Values of $p<0.05$ were considered statistically significant.

## RESULTS

### The Relaxant Effect of Berberine in Non-Incubated TSM Contracted by KCl

A concentration-dependent and significant relaxant effect was seen for berberine in the tissues contracted by KCl ($p<0.01$ to $p<0.001$). The relaxant effects of three lower concentrations of berberine were significantly lower than theophylline ($p<0.01$ to $p<0.001$), but there was no any significant difference between the highest concentration of berberine and theophylline (Figure 2).

### The Relaxant Effect of Berberine in Incubated TSM Contracted by KCl

Berberine showed concentration-dependent and significant relaxant effect in tissue incubation with atropine ($p<0.05$ to $p<0.001$). There was no significant difference in the relaxant effects of berberine between non-incubated and incubated tissue with atropine (Figure 3A).

In incubated tissues with chlorpheniramine, only two higher concentrations of berberine showed significant relaxant effects ($p<0.01$ and $p<0.001$, respectively). The relaxant effects of three lower concentrations of berberine in incubated tissues with chlorpheniramine were significantly lower than non-incubated TSM ($p<0.01$ to $p<0.001$, Figure 3B).

Berberine showed concentration-dependent and significant relaxant effect in tissues incubation with indomethacin ($p<0.01$ to $p<0.001$). The relaxant effects of all concentrations of berberine in incubated tissues with indometacin were significantly lower than non-incubated TSM ($p<0.05$ to $p<0.001$, Figure 3C).

There was no significant difference in $EC_{50}$ values of berberine between non-incubated tissues concentrated by KCl (47.4±23.83) and incubated tissue with indomethacin (23.2±5.32) or atropine (97.6±75.87). The $EC_{50}$ values of berberine in incubated tissue with chlorpheniramine (193.2±58.44) were significantly higher than the non-incubated condition ($p<0.05$, Figure 4).

The relaxant effect of 65 µg/mL of berberine in incubated tissues with indomethacin or chlorpheniramine was significantly lower than those of

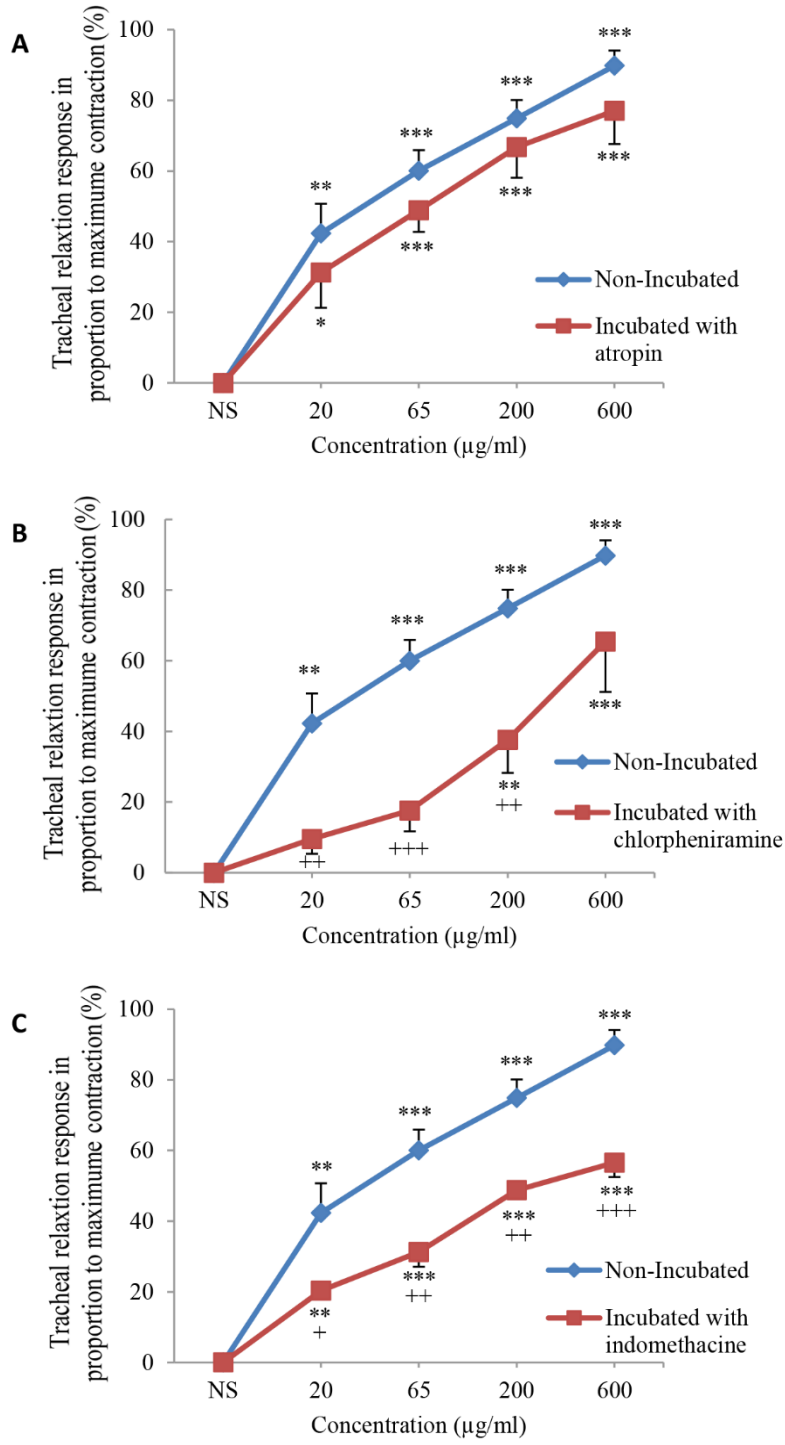


**Figure 3. Relaxant effects of cumulative concentrations of berberine on rat tracheal smooth muscle (TSM) contractions induced by KCl (60 mM) in non-incubated and incubated tissues with (A) atropine (1µM), (B) chlorpheniramine (1µM) and (C) indomethacin (1µM), (n=5 for each group) *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$, compared to saline (NS), +: $p<0.05$ ++: $p<0.01$ +++:$p<0.001$ compared to non-incubated tissues. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

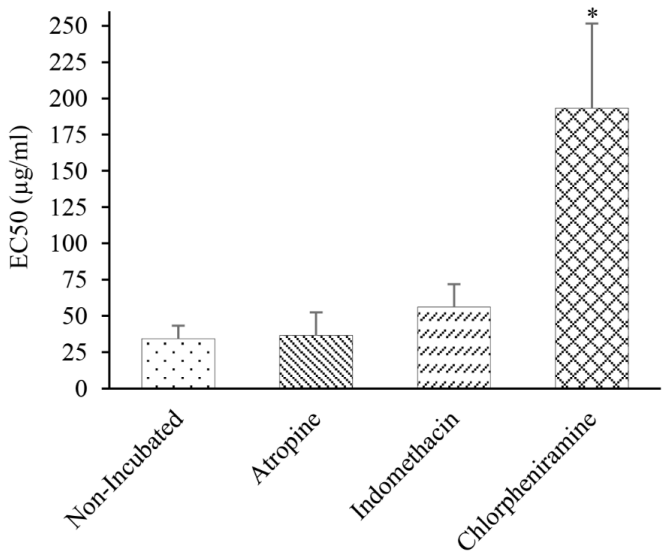


**Figure 4. $EC_{50}$ values of berberine-induced relaxation in rat trachea contractions induced by KCl (60 mM) in non-incubated and incubated tissues with atropine, indomethacin, and chlorpheniramine (n=5 for each group). Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

incubated tissues with atropine ($p<0.05$ and $p<0.01$, respectively) in KCl-induced contraction (Table 2).

### The Relaxant Effect of Berberine in Non-Incubated TSM Contracted by Methacholine

Concentration-dependent and significant relaxant effects were seen for berberine in the tissues contracted by methacholine ($p<0.01$ to $p<0.001$). The relaxant effects of two lower concentrations (20 and 65 µg/mL) of berberine were significantly lower than those of corresponding theophylline concentrations ($p<0.001$ and $p<0.01$, respectively; Figure 5).

### The Relaxant Effect of Berberine in Incubated TSM Contracted by Methacholine

Berberine showed concentration-dependent and significant relaxant effects in incubated tissues with diltiazem, glibenclamide, propranolol, papaverine and L-NAME ($p<0.05$ to $p<0.001$). There was no significant difference in the relaxant effects of berberine between non-incubated and incubated tissues with diltiazem, glibenclamide, propranolol, and papaverine, (Figure 6). In incubated tissues with L-NAME, the relaxant effect of 65 µg/mL of berberine was significantly lower than non-incubated TSM ($p<0.05$, Figure 7).

There was no significant difference in $EC_{50}$ values of berberine between non-incubated tissues concentrated by methacholine (82.57±15.94) and incubated tissue with diltiazem (66.33±18.39), glibenclamide (79.28±41.77), propranolol (44.00±4.94), papaverine (53.00±21.26) and L-NAME (97.40±26.61, Figure 8).

**Table 2. Comparison of the relaxant effects of four concentrations of berberine (percentage change in proportion to the maximum contraction) in different incubated tracheal smooth muscle (TSM) contracted by 60 mM KCl**

| Incubating substance | Concentration (µg/mL) | | | |
|---|---|---|---|---|
| | 20 | 65 | 200 | 600 |
| Atropine | 31.26±9.99 | 48.88±6.14$^{++\,*}$ | 66.72±8.63 | 77.04±9.42 |
| Chlorpheniramine | 9.53±4.17 | 17.58±5.88 | 37.67±9.40 | 65.46±14.27 |
| Indomethacin | 20.32±2.14 | 31.25±4.17 | 48.77±1.78 | 56.58±4.09 |

Data were presented as mean±SEM. *: $p<0.05$ compared to incubated tissues with indomethacin. ++: $p<0.01$ compared to incubated tissues chlorpheniramine. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.

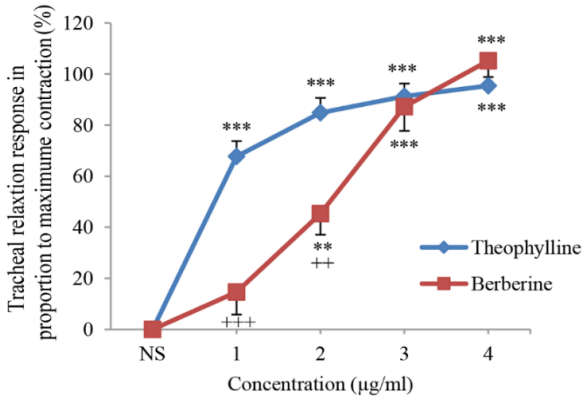


**Figure 5. Effects of cumulative concentrations of berberine (n=5) and theophylline (n=6) on rat tracheal smooth muscle (TSM) contractions induced by methacholine (10 µM). 1, 2, 3 and 4 in X-axis represent four concentration of berberine (20, 65, 200 and 600 µg/mL) and theophylline (0.2, 0.4, 0.6 and 0.8 mM). **: *p*<0.01 ***: *p*<0.001 compared to saline (NS). ++: *p*<0.01 +++: *p*<0.001 compared to the effect of theophylline. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

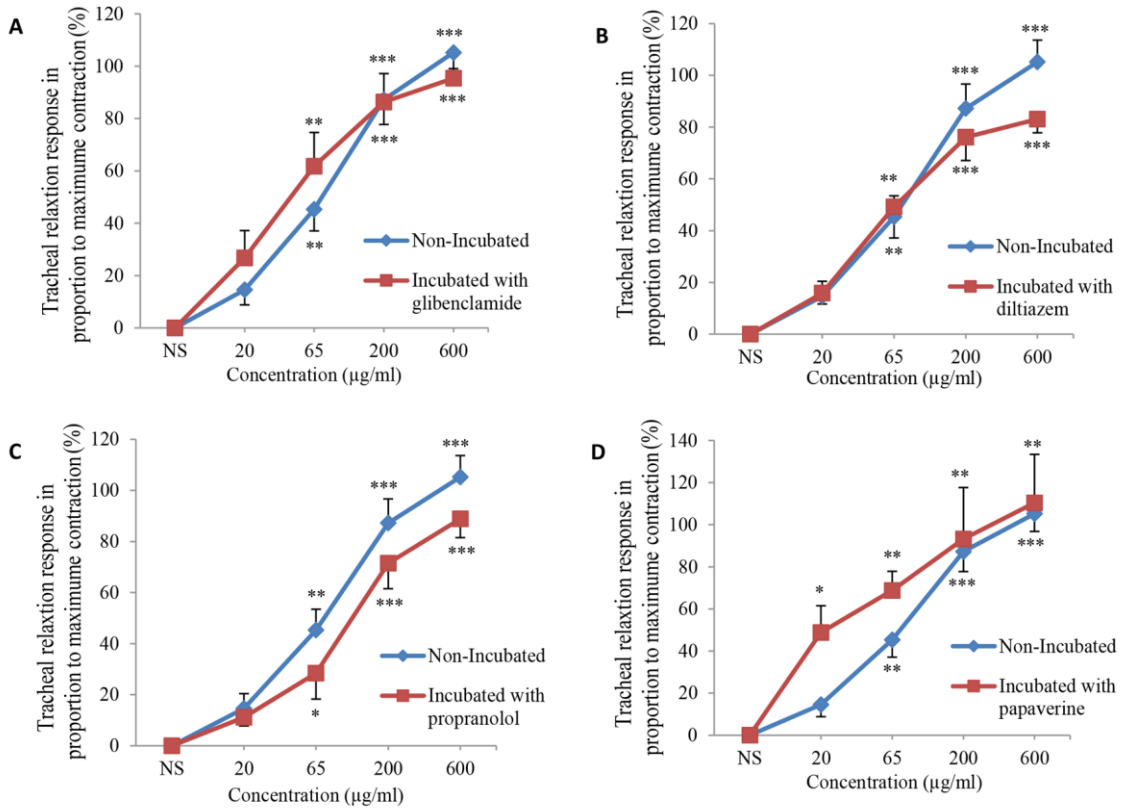


**Figure 6. Relaxant effects of cumulative concentrations of berberine on rat tracheal smooth muscle (TSM) contraction induced by methacholine (10 µM) in non-incubated (n=7) and incubated tissues with (A) glibenclamide (1 µM, n=7), (B) diltiazem (5 µM, n=6), (C) propranolol (1 µM, n=5), and (D) papaverine (50 µM, n=5). *: *p*<0.05 **: *p*<0.01 ***: *p*<0.001 compared to saline (NS). There was no significant difference in the relaxant effect between incubated tissues with various agents and non-incubated TSM. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

**Table 3. Comparison of the relaxant effects of four concentrations of berberine (percentage change in proportion to the maximum contraction) in different incubated tracheal smooth muscle (TSM) contracted by 10 mM methacholine**

| Incubating substance | Concentration (µg/mL) | | | |
|---|---|---|---|---|
| | 20 | 65 | 200 | 600 |
| Propranolol | 8.94±4.02 | 30.64±8.76 | 71.5±9.98 | 88.82±7.31 |
| Diltiazem | 15.79±4.15 | 49.10±11.94 | 76.14±9.04 | 83.10±5.27 |
| Glibenclamide | 25.29±10.74 | 63.24±11.89 # | 86.30±10.87 | 95.37±3.69 |
| L-NAME | 9.39±5.36 | 18.53±7.77 | 69.39±7.37 | 100.42±9.08 |
| Papaverine | 48.80±12.71 *#+ | 59.87±15.20 # | 98.63±19.11 | 113.96±19.53 |

Data were presented as mean ± SEM. *: $p<0.05$ compared to incubated tissues with propranolol. +: $p<0.05$ compared to incubated tissues diltiazem. #: $p<0.05$ compared to incubated tissues with L-NG-nitro arginine methyl ester (L-NAME). Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.

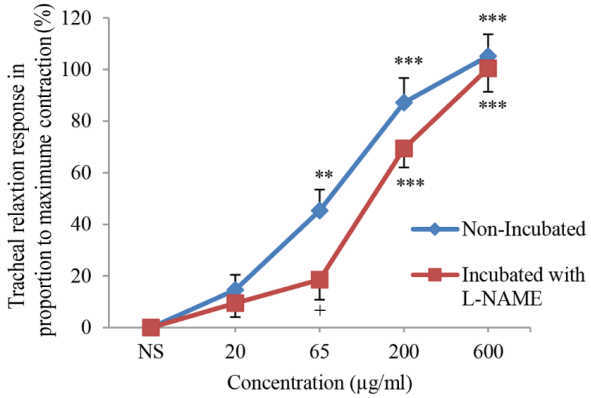


**Figure 7. Relaxant effects of cumulative concentrations of berberine on rat tracheal smooth muscle (TSM) contractions induced by methacholine (10 µM) in non-incubated (n=7) and incubated tissues with L-NG-nitro arginine methyl ester (L-NAME, 300 µM, n=5). **: $p<0.01$, ***: $p<0.001$ compared to saline (NS). +: $p<0.05$ compared to non-incubated tissues. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

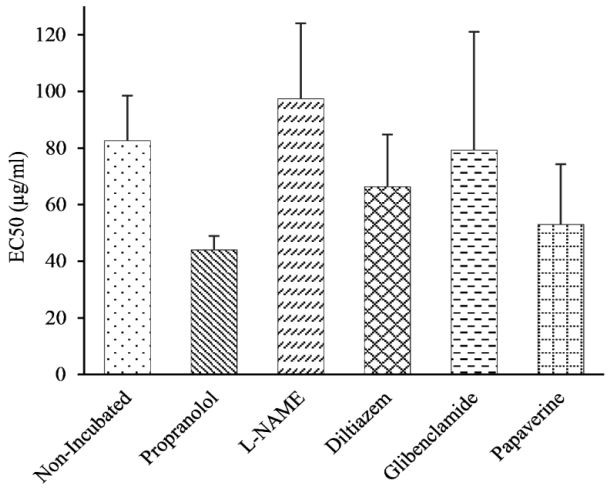


**Figure 8. $EC_{50}$ values of berberine-induced relaxation in rat tracheal smooth muscle (TSM) contractions induced by methacholine (10 mM) in non-incubated (n=7) and incubated tissues with diltiazem (n=6), glibenclamide (n=7), propranolol (n=5), papaverine (n=5), and L-NG-nitro arginine methyl ester (L-NAME, n=5). There was no significant difference in $EC_{50}$ values between different groups. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

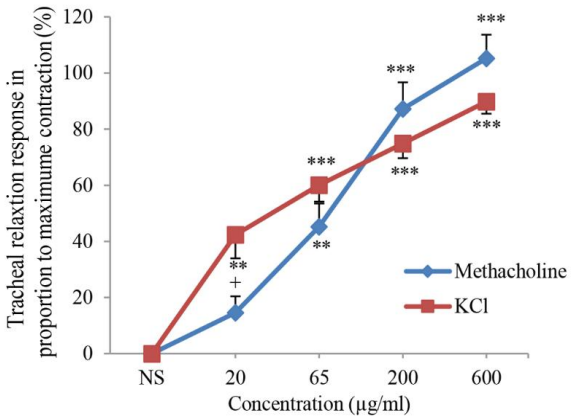


**Figure 9. Relaxant effects of cumulative concentrations of berberine on rat tracheal smooth muscle (TSM) contraction induced by KCl (60 mM, n=5) and methacholine (10 µM, n=7) of non-incubated tissues. **: $p<0.01$ ***: $p<0.001$ compared to saline (ns). +: $p<0.05$ compared to KCl induced contraction. Statistical comparisons were performed using ANOVA with Tukey Kramer post-test.**

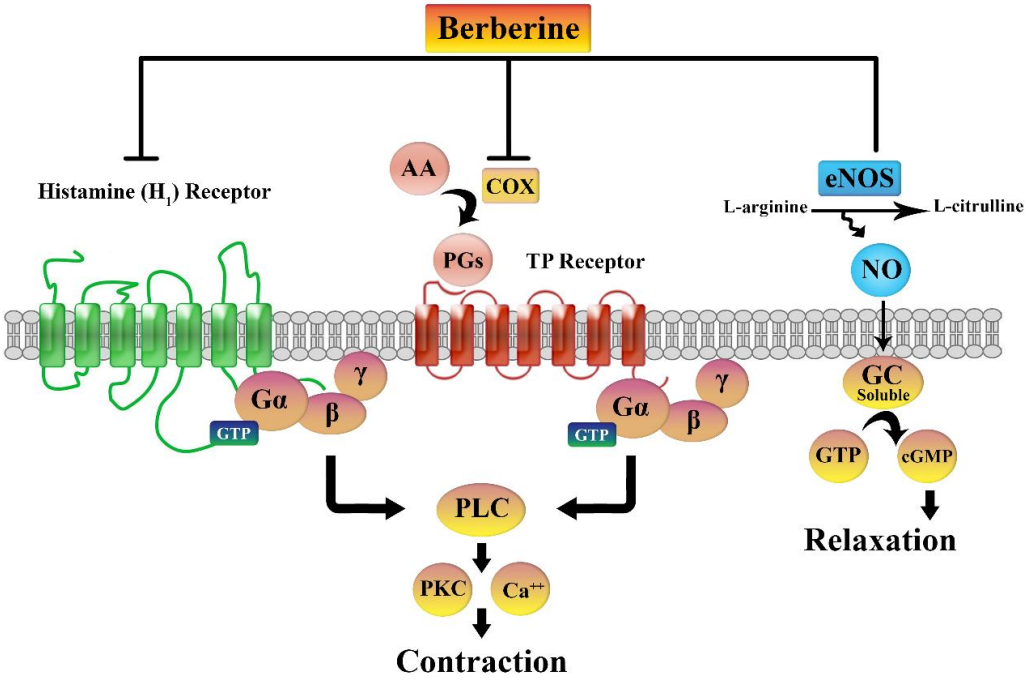


**Figure 10. Possible molecular mechanisms of berberine in tracheal smooth muscle (TSM) relaxation. AA; Arachidonic acid, COX; Cyclooxygenase, eNOS; Endothelial nitric oxide synthase, NO; Nitric oxide, PGs; Prostaglandins, PKC; Protein kinase C, PLC; phospholipase C, GC; Guanylyl cyclase, GTP; Guanosine triphosphate, cGMP; Cyclic guanosine monophosphate. The results of the present study suggest histamine ($H_1$) receptors blockade, inhibition of COX pathways and/or involvement of NO formation are the possible mechanisms of the relaxant effect of berberine on TSM.**

The relaxant effects of two lower concentrations (20 and 65 µg/mL) of berberine in incubated tissues with papaverine were significantly higher than those of incubated tissues with L-NAME ($p<0.05$) and only the effect of its first concentration was significantly higher than that of incubated tissues with diltiazem and propranolol ($p<0.05$ for both) in methacholine-induced-contraction. Additionally, the relaxant effect of the second concentration of berberine in incubated tissues with glibenclamide was significantly higher than that of incubated tissues with L-NAME ($p<0.05$; Table 3).

**Comparison of the Relaxant Effects of Berberine between TSM Contracted with KCl and Methacholine**

The relaxant effect of the first concentration (20 µg/mL) of berberine in TSM contracted by methacholine was significantly lower than that in tissues contracted with KCl ($p<0.05$; Figure 9).

## DISCUSSION

Due to the effects of the various receptors and ion channels on TSM and the complex post-receptor mechanisms involved in the contractile and relaxant responses, the pharmacology of TSM is complex. This causes various potential pharmacological mechanisms for agents to induce the relaxation of TSM.[14]

The results of this study showed concentration-dependent and significant relaxant effect of berberine in non-incubated TSM contracted by KCl and methacholine. These results indicated a relatively potent relaxant effect of berberine which was comparable to the effect of theophylline.

To evaluate the effect of berberine on muscarinic receptors, potassium channel opening, calcium channel-blocking, ß$_2$-adrenergic receptors and phosphodiesterase activity, the relaxant effect of berberine was examined on TSM incubated with atropine, glibenclamide, diltiazem, propranolol, and papaverine respectively. There was no significant difference in the relaxant effects of berberine between non-incubated and incubated tissues with these agents. Berberine probably does not induce TSM relaxation by the above-mentioned mechanisms.

Berberine is known to block $Ca^{2+}$ channels,[15] while it did not act through this mechanism on TSM, in the current study. Some data indicated that mechanisms other than inhibition of $Ca^{2+}$ channels may underline the endothelium-independent relaxant response to berberine. However, berberine was reported to inhibit both L- and T-type voltage-gated $Ca^{2+}$ currents in guinea pig ventricular myocytes.[16] Alternatively, the effect of berberine on $Ca^{2+}$ channel may be tissue-dependent.

To assess the effect of berberine on histamine ($H_1$) receptors, the relaxant effect of berberine was examined on TSM incubated with chlorpheniramine. The high concentration of berberine showed significant relaxant effects in incubated TSM incubated with chlorpheniramine. The relaxant effects of three lower concentrations of berberine in incubated TSM with chlorpheniramine were significantly lower than non-incubated tissues. In addition, $EC_{50}$ berberine in incubated tissues with chlorpheniramine was significantly higher than that of non-incubated TSM. These findings suggested the possible inhibitory effect of berberine on histamine ($H_1$) receptors. The lower relaxant effect of three lower concentrations of berberine on incubated tissues with chlorpheniramine compared to the effects obtained in tissues incubated with another agent also support this mechanism of action for berberine. It was shown that berberine at 25-200 µM is able to inhibit ephedrine and histamine-induced aortic contractions in a reversible manner but it failed to inhibit contractions from high potassium or caffeine[17] which support the results of the present study.

To evaluate the effect of berberine on arachidonic acid metabolism and cyclooxygenase (COX) pathways, the relaxant effect of berberine was examined on TSM incubated with indomethacin, a nonselective COX inhibitor. Berberine showed concentration-dependent and significant relaxant effect in tissue incubated with indomethacin. The relaxant effects of all concentrations of berberine in incubated TSM with indomethacin were significantly lower than non-incubated tissues which may indicate the possible inhibitory effect of berberine on the COX pathway.

To assess the effect of berberine on nitric oxide (NO) production and the role of this mechanism, the relaxant effect of berberine was examined on TSM incubated with L-NAME, a selective inhibitor of nitric oxide synthase (NOS). The relaxant effect of the second concentration of berberine in incubated tissues with L-NAME was significantly lower than non-

incubated tissues which may indicate the possible enhancing effect of berberine on NO formation. A lower relaxant effect of three lower concentrations of berberine on incubated tissues with L-NAME compared to incubated tissues with other agents also support this mechanism of action for berberine. The ability of berberine on releasing NO plays a major role in its vasodilating activity,[9,15,18] and could be relevant also for the relaxation of guinea pig trachea.[19] A study indicated that the relaxation of berberine involved the NO-cGMP signal transduction pathway. The enhancing effect of berberine on endothelial NOS (eNOS) mRNA expression might associate with its relaxation of corpus cavernosum smooth muscle.[20] The findings of these studies support the results of the present study regarding the effect of berberine on NO formation.

Generally, several mechanisms have been proposed to explain the relaxant effect of berberine on vascular smooth muscle including enhancing the effect of acetylcholine,[21] muscarinic receptor exciting,[22] α-adrenoceptor blocking,[21,23] inhibition of intracellular $Ca^{2+}$ release,[15,21] activation of 4-aminopyridine and $Ba^{2+}$-sensitive $K^+$ channels,[21] and release of NO.[18,21] Furthermore, its proposed that mechanism of action of berberine in corpus cavernosal tissues is due to the release of NO from sinusoidal endothelium[9] which was in accordance with the findings of the present study. However, some studies on TSM indicated that berberine may be interacting with adrenergic, adenosinic[10] and/or muscarinic acetylcholine receptors[24,25] which were not in line with the results of the current study. Figure 10 illustrates various mechanisms responsible for the relaxant effect on TSM and the possible mechanisms of berberine-induced TSM relaxation effect.

There are the following potential limitations in this study. The other possible mechanisms responsible for the relaxant effect of berberine including the activation of rectifying $K^+$ channels, $Ca^{2+}$-and voltage-activated $K^+$ channels, glucocorticoid receptor activation, stimulation/ potentialization of soluble guanylyl cyclase, and inhibition of non-adrenergic non-cholinergic (NANC) system should be also evaluated in further studies.

This study reported a potent relaxant effect of berberine on TSM which is a novel finding and has not been reported previously. The possible mechanisms for the relaxant effect of berberine on TSM are histamine ($H_1$) receptors blockade, inhibition of COX pathways and/or NO formation.

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