Relaxant Effect of *Urginea maritima* on Tracheal Smooth Muscle Mediated by the Effect on Beta-2 Adrenergic, Muscarinic Receptors and Calcium and Potassium Channels

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*Urginea maritima* (*U. maritima*) showed anti-inflammatory, antioxidant, antibacterial, diuretic, vasodilatation, and wound-healing effects on fungal infections, cardiac disorders, digestive disorders, rheumatoid disease, and respiratory disorders such as bronchitis, bronchial nosocomial infections, and severe cough. To examine the bronchodilatory effect of *U. maritima*, the relaxant effect of its extract on rat tracheal smooth muscle (TSM) and its possible mechanism was examined in this study. Male Wistar rats’ TSM were divided into eight groups (*n* = 8 in each group). Four of these groups were TSM tissues, contracted with KCl (60mM) incubated with atropine, glibenclamide, and indomethacin and nonincubated TSM, while the other four groups were TSM tissues contracted with methacholine (10μM) for 5 min, incubated with propranolol, chlorpheniramine, and diltiazem and nonincubated TSM. Cumulative concentrations of *U. maritima* extract (12.5, 25, 50, 100, 200, and 400 μg/ml) were then added to organ bath every 5 min. (A) 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 nonincubated tissues. A concentration-dependent relaxant effect of *U. maritima* on nonincubated TSM contracted with KCl (60mM) or methacholine (10μM) (*p* < 0.01 and *p* < 0.001) was observed. The relaxant effects of *U. maritima* extract in the incubated tissues with glibenclamide, propranolol, diltiazem, atropine, and chlorpheniramine were significantly lower than those in the nonincubated tissues (*p* < 0.05 to *p* < 0.001). EC50 values of *U. maritima* extract in the incubated TSM with glibenclamide, propranolol, diltiazem, and atropine were significantly higher than those in the nonincubated tissues (EC50 values for diltiazem-incubated tissues and *p* < 0.001 for other cases). *U. maritima* extract displayed considerable relaxant effect on TSM comparable to the effect of theophylline. Beta-2 adrenoceptor stimulation and muscarinic receptor inhibition as well as potassium opening and calcium channels blocking effects are the possible mechanisms for the relaxant effects of the plant.

1. Introduction

One of the most important chronic inflammatory diseases in the world is asthma with considerable morbidity. Asthma is characterized by pathological changes in the lung, like increased mucosa secretion, airway hyperresponsiveness, infiltration of inflammatory cells, and smooth muscle hyperplasia [1]. Over the past 30 years, there has been an increase in the number of patients with asthma, and 250,000 people die from this disease each year. The treatment of this disease is very costly and the direct and indirect costs of the asthma are globally on the rise [2]. The precise mechanism of asthma pathophysiology and the role of biochemical intermediates involved in asthma are not yet known, but,
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2. Materials and Methods

2.1. Preparation of the Extract. *U. maritima* was purchased from a market in Mashhad, Iran, in October 2018 and identified by Dr. Rakhshandeh, Pharmacological Research Center of Medicinal Plants and Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. *U. maritima* extract was prepared by peeling, weighed (50 g), and soaked in 70% ethanol (ethanol 96°, Taghtir Khorasan Co., Iran) at 40°C for 72 hours while shaking constantly. The extract was dried by rotary evaporator at 50°C to obtain a yield of 12% and the required concentrations were prepared.

2.2. Animals and Experimental Groups. Sixty-four male Wistar rats (weight, 200–250) were kept in a standard condition, 22 ± 2°C temperature, 12 h light/dark cycles, and free access to standard diet and tap water in the Animal House, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (#961800). All experiments on animals were done according to National Laws regarding the use and care of laboratory animals. Animals were divided into eight groups (*n* = 8 in each group) as shown in Table 1.

2.3. Tissue Preparation. The rats were sacrificed after anesthetizing by 1.6 g/kg intraperitoneal (i.p.) administration of urethane and their chests opened. Tracheal rings of rats containing three cartilages were prepared from the middle section of trachea as previously described [24] and mounted in a 10 ml organ bath containing Krebs-Henseleit solution supplied with 95% O2 and 5% CO2, and tissue responses were measured using an isometric transducer (MLT0202, AD Instruments, Australia) connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia) exactly as previously described [24–26].

2.4. Examination of Smooth Muscle Relaxant Effect of Plant Extract. TSM was contracted by KCl (60 mM) (Merck Chemical Ltd., Germany) or methacholine (10 μM) (Sigma Chemical Ltd., UK) [26]. It was well established that KCl contracts tracheal smooth muscles by depolarizing the smooth muscle cells and methacholine contracts them by muscarinic receptor stimulation [24, 26]. After 5 minutes, cumulative concentrations of extract of *U. maritima* (12.5, 25, 50, 100, 20, and 400 μg/ml) [27] and theophylline (0.2, 0.4, 0.6, and 0.8 mM) as positive control or saline (1 ml) as negative control were added to organ bath every 5 minutes [25, 26].

The reduction of contraction produced contractile agents (KCl or methacholine) due to the fact that each concentration of *U. maritima* extract and theophylline in proportion to maximum contractile response was calculated and considered as percent relaxation response [24]. The relaxation concentration response curves were prepared, and the extract concentration causing 50% of maximum relaxation effect (EC50) was measured from concentration response curve as previously defined [24–26, 28–31]. In incubated tissues with rightward shift in the concentration-response curve of the relaxant effect of the plant, the concentration ratio minus one (CR-1) was
Table 1: Experiment groups. Trachea of incubated groups subjected to different channel blocker or antagonists in organ bath first followed by contraction of TSM by KCl or methacholine after 10 min.

| Contractile agent | Condition       | Incubating agent     | Mechanisms                          | n  |
|-------------------|-----------------|----------------------|-------------------------------------|----|
|                   | Nonincubated tissues | —                   | —                                   | —  |
| KCl (60 mM)       | Incubated tissues| Atropine (1 μM)      | Muscarinic receptor inhibition       | n = 8 |
|                   |                  | Indomethacin (1 μM)  | Cyclooxygenase inhibition           | n = 8 |
|                   |                  | Chlorphenamine (1 μM)| Histamine (H1) receptor inhibition  | n = 8 |
| Methacholine (10 μM) | Nonincubated tissues | —                   | —                                   | n = 8 |
|                   | Incubated tissues| Diltiazem (5 μM)     | Calcium channel blocking            | n = 8 |
|                   |                  | Glibenclamide (1 μM) | Potassium channel opening           | n = 8 |
|                   |                  | Propranolol (1 μM)   | B₂-adrenoceptor stimulation         | n = 8 |

2.5. Statistical Analysis. Statistical comparisons were performed using InStat software. The data was presented as mean ± standard error of the mean (SEM). Comparisons were performed using ANOVA followed by Tukey’s multiple comparisons test and p < 0.05 was considered as a significant criterion.

3. Results

3.1. The Relaxant Effect of U. maritima Extract on TSM Contraction Induced by Methacholine in Nonincubated and Incubated Tissues. Concentration-dependent and significantly relaxant effects of the extract of U. maritima and theophylline were seen on TSM contracted by methacholine (p < 0.05 for the second extract concentration and p < 0.001 for all theophylline and higher extract concentrations).

The extract effects of two higher concentrations of U. maritima extract (200 and 400 μg/ml) were significantly less than the relaxant effects of the two higher concentrations of theophylline (p < 0.05 for both cases) (Figure 1(a)).

Different concentrations of U. maritima extract showed significant relaxant effects on TSM in incubated tissue with glibenclamide (p < 0.001 for 5 last concentrations). However, in incubated tissues with glibenclamide, the relaxant effects of 100 and 200 μg/ml concentrations of the extract were significantly lower than those in the nonincubated TSM (p < 0.001 and 0.05 for 100 and 200 μg/ml concentrations, respectively) (Figure 1(b)). EC50 values of the U. maritima extract for its relaxant effect in incubated TSM with glibenclamide were significantly higher than those in nonincubated tissues (Figure 2(a)).

A rightward shift in the concentration-response relaxation curve of the U. maritima extract was observed; in glibenclamide-incubated TSM compared to nonincubated tissues, a maximum response was achieved. The (CR-1) value of the extract in incubated TSM with glibenclamide was 0.6 ± 0.2.

The relaxant effects of different concentrations of U. maritima extract on incubated tissue with propranolol were significantly higher than that of control tissue (p < 0.01 for both cases) (Figure 2(a)). A rightward shift in concentration-response relaxation curve of the U. maritima extract was observed in propranolol-incubated TSM compared to nonincubated tissues but the maximum response was not achieved. The (CR-1) value of the extract in incubated TSM with propranolol was 2.3 ± 0.4.

Different concentrations of U. maritima extract caused significant relaxant effects in incubated tissues with diltiazem compared to the effect of saline (p < 0.001 for 5 last concentrations). The relaxant effects of 50 and 100 μg/ml of the extract in incubated tissue with diltiazem were significantly lower than those in the nonincubated TSM (p < 0.05 for both cases) (Figure 3(b)).

EC50 values of the U. maritima extract for its relaxant effect in incubated TSM with diltiazem were significantly higher than those in the nonincubated tissues (p < 0.001) (Figure 2(a)). A rightward shift in concentration-response relaxation curve of the extract was observed in diltiazem-incubated TSM compared to nonincubated tissues and the maximum response was achieved. The (CR-1) value of the extract in incubated TSM with diltiazem was 0.43 ± 0.1.

3.2. The Relaxant Effect of U. maritima Extract on TSM Contraction Induced by KCl in Nonincubated and Incubated
**Figure 1:** Concentration-response relaxant effects (mean ± SEM) of theophylline and *U. maritima* extract in nonincubated TSM contracted by 10 μM methacholine (*n* = 7). 1, 2, 3, 4, 5, and 6 in X-axis display six concentrations of the extract (12.5, 25, 50, 100, 200, and 400 μg/ml) and 3, 4, 5, and 6 display theophylline concentrations (0.2, 0.4, 0.6, and 0.8 mM) and (b) concentration-response relaxant effects (mean ± SEM) of theophylline and *U. maritima* extract in glibenclamide-incubated TSM (1 μM, *n* = 8). * * * * * * * p < 0.01 and * * * * * * * p < 0.001 compared to the effect of saline (NS). * * p < 0.05 in panel (a) indicates comparison between the effect of theophylline and that of the extract. * * p < 0.05 and * * * p < 0.01 in panel (b) show the comparison of the effect of the extract between incubated and nonincubated tissues. ANOVA with the Tukey–Kramer post hoc test was used for statistical comparison.

**Figure 2:** EC₅₀ values of *U. maritima* extract-induced TSM relaxation in nonincubated and incubated TSM with various agents and contracted with methacholine (a) or KCl (b). * * p < 0.05 and * * * * * * * p < 0.001 compared to nonincubated tissues. ANOVA with the Tukey–Kramer post hoc test was used for statistical comparison.
In nonincubated TSM contracted by KCl, the relaxant effects of all concentrations of *U. maritima* extract and theophylline were higher than the effect of saline (\( p < 0.001 \) for all cases except the low extract concentration). The relaxant effects of 0.2, 0.6, and 0.8 mM theophylline were significantly higher than those of the corresponding concentrations of the extract (\( p < 0.05 \) to \( p < 0.001 \)) (Figure 4(a)).

The extract of *U. maritima* showed significant and concentration-dependent relaxant effects on incubated TSM with atropine (\( p < 0.05 \) for 25 \( \mu \)g/ml and \( p < 0.001 \) for higher extract concentrations). The relaxant effects of four higher concentrations of the extract in incubated tissue with atropine were significantly lower compared to those in the nonincubated TSM (\( p < 0.001 \) for all cases) (Figure 4(b)).

**EC\(_{50}\) values of the extract** for its relaxant effect in incubated TSM with atropine were significantly higher compared to those in the nonincubated tissues with atropine (\( p < 0.001 \)) (Figure 2(b)).

A rightward shift in concentration-response curve of the extract was seen in atropine-incubated TSM compared to nonincubated tissues and the maximum response was achieved. The (CR-1) value of the extract in incubated TSM with atropine was 1.6 ± 0.2.

The relaxant effects in 5 higher concentrations of extract in incubated TSM with chlorpheniramine and indomethacin were significantly higher compared to the effect of saline (\( p < 0.05 \) for 25 \( \mu \)g/ml in chlorpheniramine-incubated tissues and \( p < 0.001 \) for higher extract concentrations). There was no significant difference between the effects of different concentrations of extract in incubated TSM with chlorpheniramine and indomethacin and nonincubated tissues (Figures 5(a) and 5(b)).
was no significant difference between the relaxant effects of different concentrations of *U. maritima* extract between the TSM contracted by methacholine or KCl (Figure 6).

3.4. Correlations between Concentrations of the Extract of *U. maritima* and Theophylline with Relaxant Effects

The relaxant effects of theophylline and the extract were significantly correlated with their concentrations in all experimental groups (\(p < 0.001\) for all cases) (Table 2).

4. Discussion

This study showed concentration-dependent relaxant effect of *U. maritima* extract in nonincubated TSM contracted by methacholine and KCl comparable to the effect of theophylline. The relaxant effect of *U. maritima* extract in nonincubated TSM contracted by methacholine and KCl was not significantly different. These results indicate a potent relaxant effect of the plant on TSM, which indicates its bronchodilatory effect in patients with obstructive pulmonary diseases. In fact, the effect of *U. maritima* in the treatment of respiratory diseases was indicated previously [23].

To examine the effect of *U. maritima* on \(\beta_2\)-receptor [29], muscarinic [32], histamine (H1) [33] receptors, calcium channels [30], and potassium channels [34], ATP-sensitive potassium channels [34] and arachidonic acid metabolism [35] and their contribution in the relaxant effect of the plant were examined on tracheal smooth muscle incubated with propanolol, atropine, chlorpheniramine, diltiazem, glibenclamide, and indomethacin, respectively.

The relaxant effects of the extract in incubated tissues with propanolol, atropine, diltiazem, and glibenclamide
were significantly lower than those in the nonincubated TSM. These results indicated the stimulatory effect of the plant on β2-adrenoceptor, inhibitory effect on muscarinic receptors, calcium channel blocking, and potassium channel opening effects, respectively. The EC50 values of the extract inducing relaxant effect in the incubated tissues with propranolol, atropine, diltiazem, and glibenclamide were also significantly higher compared to those in the nonincubated TSM. The higher EC50 values of the extract inducing relaxant effect in the incubated tissue also support the β2-adrenoceptor stimulation, muscarinic receptors inhibition, calcium channel blocking, and potassium channel opening properties of the plant. However, the maximum relaxant response was not obtained in incubated tissues with propranolol, which may indicate nonselective effect of the plant on β2-adrenoceptor [28]. The reason for the absence of maximum relaxant effect of the plant in incubated tissues with propranolol could be due to its effect on muscarinic receptors as well as calcium and potassium channels. The effect of another species of *U. maritima* extract on muscarinic receptors was demonstrated previously, which may support the effect of *U. maritima* on muscarinic receptor of TSM [24, 32]. Also, Memarzia and colleagues showed that the most important mechanism involved in relaxant effects of *Allium cepa* (*A. cepa*) extract was β2-adrenergic stimulatory and/or calcium channel [24], which may support the results of this study. A former study showed the relaxant activity of the extract of *Urginea indica* (another plant from Asparagaceae family) with the possible anticholinergic and Ca2+ antagonist mechanisms [27], which also supports the findings of the present study. However, the relaxant effects of *U. maritima* extract and its EC50 values in incubated tissues with chlorpheniramine and indomethacin were not significantly different from those in the nonincubated tissues. These results indicated the absence of the effect of the plant on histamine (H1) receptor [33] and arachidonic acid metabolism [35] pathways, ATP-sensitive potassium channels [34], and the contribution of these mechanisms to the relaxant effect of *U. maritima* extract on TSM.

This result showed relatively potent relaxant effect of *U. maritima* extract on TSM and the possible mechanisms of this effect for the first time. The mechanisms responsible for

![Figure 5: Concentration-response relaxant effects (mean±SEM) of *U. maritima* extract on 60 mM KCl-induced contraction of TSM in nonincubated and chlorpheniramine-incubated (1 μM) (a) and indomethacin-incubated TSM (1 μM) (b) (n=8 for all groups). ** p < 0.01 and *** p < 0.001, compared to the effect of saline (NS), + p < 0.05 compared to the effect of the extract on nonincubated tissues. ANOVA with the Tukey–Kramer post hoc test was used for statistical comparison.](image-url)
the relaxant effect of *U. maritima* extract on TSM are β2-adrenergic receptor stimulation, muscarinic receptors inhibition, calcium channel blocking, and potassium channels pathway opening effects or combinations of these mechanisms. The significant relaxant effect of *U. maritima* extract on TSM may indicate a bronchodilator effect for the *U. maritima* extract on obstructive pulmonary diseases.

### 5. Conclusions

In conclusion, this study displayed the potent relaxant effect of *U. maritima* on TSM comparable to the effect of theophylline, indicating its possible bronchodilatory property. Based on the results of this study, the possible mechanisms responsible for the relaxant effect of the plant on TSM are β2-adrenoceptor stimulation, muscarinic receptors inhibition, potassium channel opening, and calcium channel blocking properties.

### Data Availability

No data were used to support this study.

### Conflicts of Interest

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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