Research Paper

Portulaca oleracea Affects Muscarinic Receptors of Guinea Pig Tracheal Smooth Muscle

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The relaxant effect of *Portulaca oleracea* L. on tracheal smooth muscles has been shown. The effect of the plant on muscarinic receptors of tracheal smooth muscles as one possible mechanism responsible for its relaxant effect was examined. Concentration response curves to methacholine were performed in the presence of three concentrations of aqueous-ethanol extract from aerial part of the plant (0.25, 0.50 and 1.00 mg/ml), 10 nM atropine, and saline. The values of EC_{50} (effective concentration causing 50% of maximum response) and CR-1 ((EC_{50} of test solutions/EC_{50} of saline)-1) were measured. The study was done using three designs as non-incubated (group 1), incubated tissues with propranolol and chlorpheniramine (group 2) and incubated tissues with propranolol (group 3). Concentration-response curves to methacholine were shifted to the right and EC50 methacholine were significantly higher in the presence of atropine, medium and highest extract concentrations in all groups and its low concentration in group 3 compared to saline. In addition, EC_{50} methacholine in the presence of high extract concentration (27.00±2.12) in group 2 was greater than its low (10.33±1.54) and medium (16.83±2.52) concentrations ($p<0.05$). The values of the CR-1 obtained in the presence of all extract concentrations in all groups were lower than that of atropine ($P<0.05$ to $P<0.001$). An inhibitory effect for the extract of *P. oleracea* on muscarinic receptors of tracheal smooth muscles was shown and a histamine (H_1) receptor blockade was also suggested.

Key words: *Portulaca oleracea*, muscarinic receptor, tracheal smooth muscle, inhibitory effect, aqueous-ethanol extract

Portulaca oleracea L. is an annual succulent which may reach 15.75 inches in height with alternate leaves clustered at stem joints; the yellow flower plant grows in different areas of the world including north and northwest of Iran[1]. Main active constituents of *P. oleracea* are omega-3 fatty acid, α-linolenic acid and eicosa-pentaenoic acid. This plant also contains vitamins (mainly vitamins A, C and some vitamin B) [2,3]. The plant also contains organic acids such as citric, maleic, cyanuric, caffeic acid as well as flavonoids, coumarins, and glycosides[4].

P. oleracea has several therapeutic effects including diuretic, antiascorbic, antipyretic, antiinflammatory[5-7]. The plant is also used for treatment of type-2 diabetes mellitus patients[8]. Different pharmacologic effects of this plant are antiinflammatory, analgesic[6], antioxidant[9-11], potassium channel opener and blood pressure modifier[12], as well as neuropharmacological[13,14], wound healing[15], antibacterial[16] and antipyretic effects[17]. The relaxant effect of this plant was studied on skeletal muscles[18] and smooth muscle of small intestine[19]. Our previous studies also showed relaxant effect of the plant on tracheal smooth muscle[20] and the possible mechanism of this effect for this plant[21]. Antitussive effect of the plant in guinea pigs[7] and its bronchdilatory in asthmatic patients[5] were also demonstrated in our previous experiments.

In the present study, the inhibitory effect of aqueous-ethanol extract of *P. oleracea* on muscarinic receptors of guinea pigs' tracheal smooth muscles, as one possible mechanism responsible for its relaxant effect was examined.

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MATERIALS AND METHODS

P. oleracea was purchased from the local market in July of 2014, in Mashhad. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences (Herbarium No:240-1615-12) which was authenticated at Department of Pharmacognosy.

The aqueous ethanol extract was prepared by using maceration method; 100 g of powdered aerial part of the plant was subjected to extraction with 50% ethanol by 3 times for 72 h. The hydro-alcoholic maceration extracts were dried in rotary apparatus with 35°. The yields extract was 21% w/w which stored in -20° away from light. For preparation of concentrations, the plant extract was dissolved in normal saline.

Animals and tissue preparations:

Guinea pigs of both sexes (600-800 g) were used in this study (Razi Institute, Mashhad, Iran). Animals were kept in a temperature controlled room with access to standard food and water *ad libitum* and maintained at 22±2° on a 12 h light/dark cycle during the study period. Experiments were performed in compliance with the rulings of the Institute of Laboratory Animals Resources, Commission on Life Sciences[22].

Tracheal chains of Dunkin-Hartley guinea pigs were prepared as previously described[23] and suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent, UK) containing Krebs-Henseleit solution with known composition[23] which was maintained at 37° and gassed with 95% O_2 and 5% CO_2.

Tissue was suspended under isotonic tension (1 g) and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min. In each experiment, a contraction was measured using an isotonic transducer (Harvard APP LTD, 50-6360 SINO. 0210) and measured by using a software by a computer (Acer Model No. G781) recording[24]. The study was approved by the University's Ethics Committee. The allowance number of the relevant ethical committee for the animal experiments is 910690.

Protocols:

The effect of *P. oleracea* on muscarinic receptors was examined as previously described[21]. Briefly, tracheal smooth muscle was exposed to 10 nM atropine maleate (Sigma Chemical Ltd UK, Catalogue No. C4915), three concentrations of aqueous-ethanol extract from *P. oleracea* (0.25, 0.50 and 1.00 mg/ml) or saline for 10 min and then cumulative log concentration response curve to methacholine (10^{-7} to 10^{-3} µmol) was performed in each cases. The EC_{50} value (effective concentration of methacholine causing 50% of maximum response), the maximum responses to methacholine and the slope of the methacholine-response curve of each experiment were measured. In experiments with parallel shift in methacholine-response curve, the concentration-ratio minus one (CR-1) as an index of the competitive antagonism effect was calculated by Eqn. 1[24].

$$CR\text{-}1=(EC_{50} \text{ of test solution}/EC_{50} \text{ of saline})\text{-}1 \quad (1)$$

The study was performed in three experimental designs as, (1) non-incubated tracheal chains (group 1, n=6), (2) incubated tissues with 1 µM chlorpheniramine (Sigma Chemical Ltd, UK) and 1 µM propranolol hydrochloride (Sigma Chemical Ltd, UK) 30 min prior to the beginning and while obtaining methacholine-response curve (group 2, n=6) and (3) incubated tissues with 1 µM propranolol hydrochloride (Sigma Chemical Ltd UK) 30 min prior to the beginning and while obtaining methacholine-response curve (group 3, n=7). All of the experiments were performed randomly with 1h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution.

Statistical analysis:

All data were expressed as mean±SEM. The EC_{50}, the slope, and maximum response obtained in the presence of extract and atropine were compared with those obtained in the presence of saline and (CR-1) obtained in the presence of extract with those obtained in the presence of atropine using the paired t-test. The comparisons of the data of different concentrations of the extract were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer posttest. The values of EC_{50}, the slope, (CR-1), and maximum response obtained in three groups were also compared using ANOVA with Tukey-Kramer post-test. Significance was accepted at $P<0.05$. Statistical analyses were performed with GraphPad Instat version 3.00 (GraphPad Software, San Diego California USA).

RESULTS AND DISCUSSION

Cumulative log concentration-response curves of methacholine obtained in the presence of all concentrations of the extract and atropine showed clear rightward shift compared to that of saline in all three groups of experiments (fig. 1).

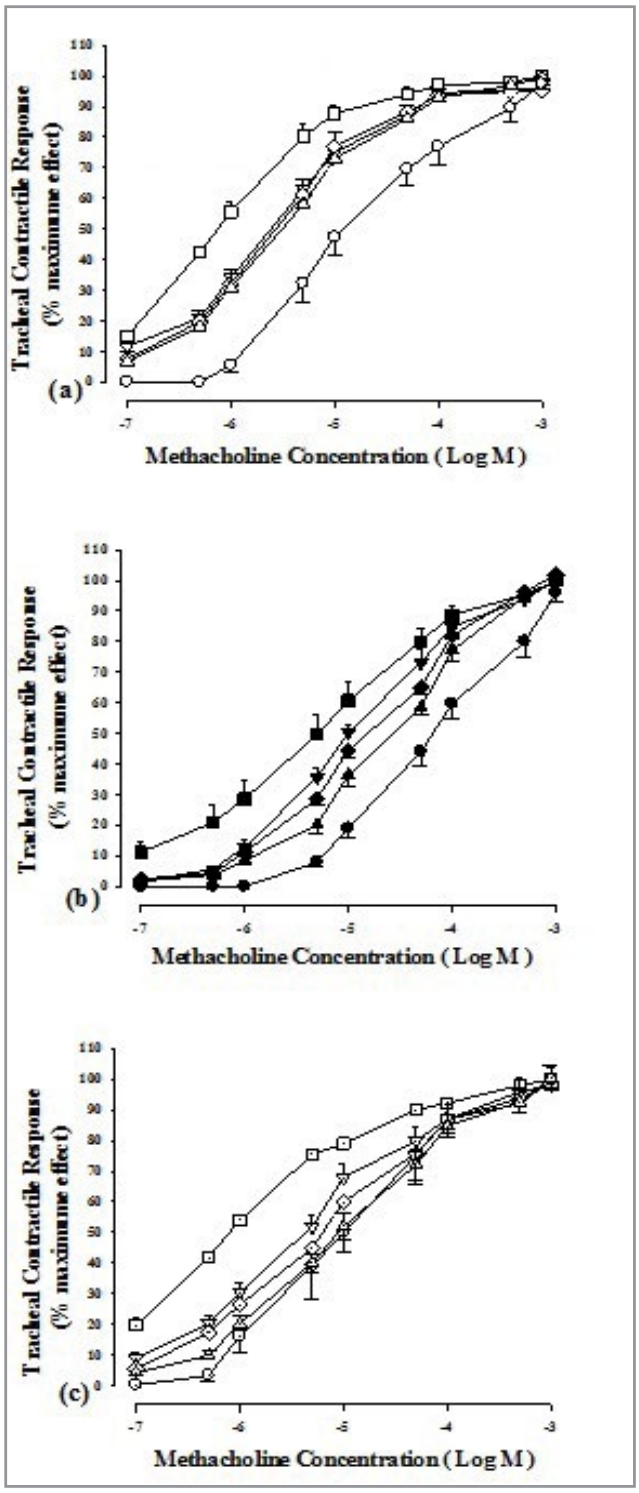

Fig. 1: Cumulative log concentration-response curves of methacholine-induced contraction of guinea pig tracheal smooth muscle.

Cumulative log concentration-response curves of methacholine-induced contraction of guinea pig tracheal smooth muscle in the presence three concentrations of aqueous-ethanol extract from *P. oleracea*, 10 nM atropine (□) and saline (○) in (a) non incubated trachea (group 1, open symbols, n=6), (b) incubated tissues with 1 µM chlorpheniramine and 1 µM propranolol (group 2, filled symbols, n=6) and (c) on tissues incubated with propranolol (Group 3, open symbols with a dot,n=7). Extract 0.25 mg/ml (▽); extract 0.50 mg/ml, (◊); extract1 mg/ml (Δ)

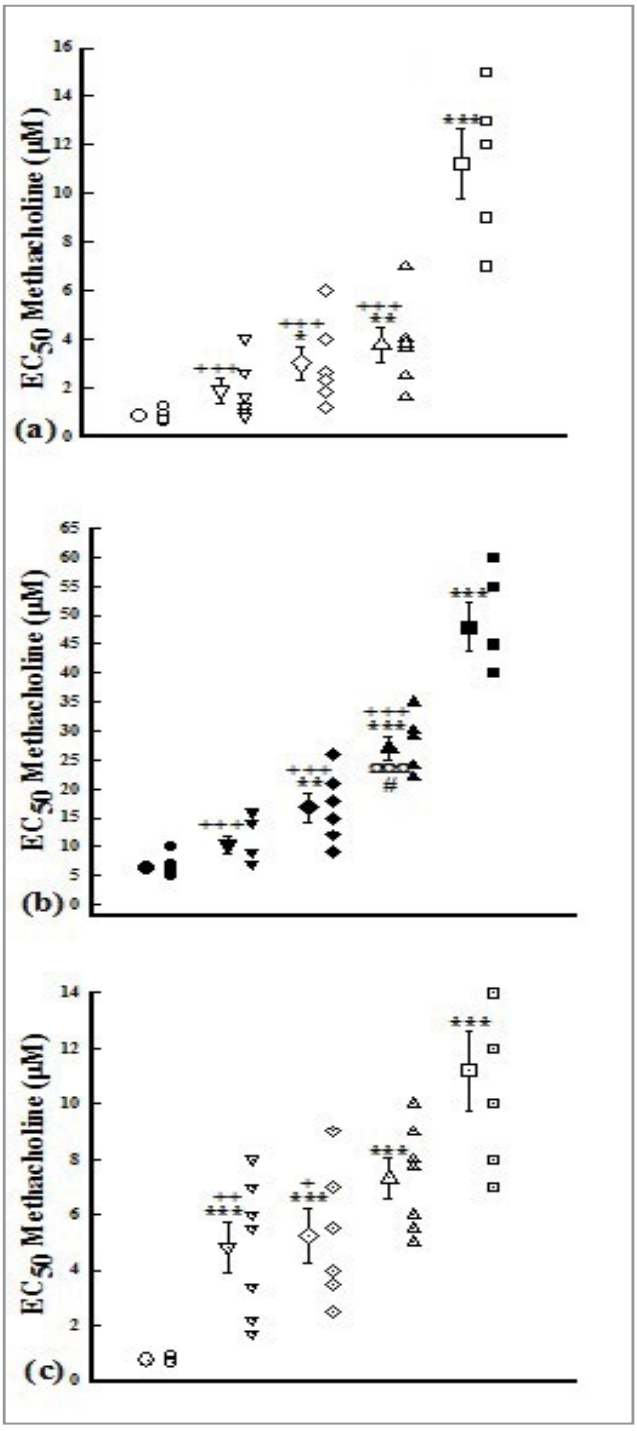

Fig. 2: EC_{50} values obtained in the three groups.

EC_{50} obtained in the presence of three concentrations of *P. oleracea* extracts, 10 nM atropine and saline in (a) non-incubated tissues, (b) incubated tissues with 1 µM chlorpheniramine and 1 µM propranolol n=6; and (c) with propranolol, n=7. *: *P*<0.05, Compared between saline and other solutions. +: *P*<0.05, compared between atropine and other solutions. Ω: *P*<0.05, compared between extract 0.25 vs. 1.00. #: *P*<0.05, compared in EC_{50} between extract 0.50 vs. 1.00. Atropine (□); saline (○); extract 0.25 mg/ml (▽); extract 0.50 mg/ml (◊); extract1 mg/ml (Δ).

The values of EC_{50} of methacholine obtained in the presence of atropine, medium and high extract concentrations (0.50 and 1.00 mg/ml) in all groups and its low concentration (0.25 mg/ml) in group 3 were significantly higher than that of saline ($P<0.05$). In addition, the values of EC_{50} of methacholine obtained in the presence of high extract concentration in group 2 was significantly higher than its low and medium concentrations ($P<0.05$, fig. 2).

There was no statistical difference in maximum response among saline, atropine and extract in three groups of experiment (Table 1). There was no statistical difference in the slope of concentration response curveamong saline, atropine and extract in three group of experiment (Table 2).

The values of CR-1 obtained in the presence of all extract concentrations in three groups were significantly lower than that of atropine ($P<0.05$). In addition, the values of CR-1 obtained in the presence of low concentration of the extract in groups 2 and 3 were significantly lower than its high concentration ($P<0.05$). The values of CR-1 obtained in the presence of medium concentration of the extract in group 3 were also significantly lower than high concentration of the extract ($P<0.05$, fig. 3).

There were significant correlations between the values of EC_{50} and extract concentrations in all three groups ($r=0.442$, $P<0.05$, $r=0.740$, $P<0.05$ and $r=0.4772$, $P<0.05$ in groups 1, 2 and 3, respectively).

In previous studies, the relaxant effect of *P. oleracea* on tracheal smooth muscle was shown[20,21]. This effect could be due to several mechanisms including the inhibitory effect of the plant on muscarinic receptors[25]. Therefore, in the present study, the inhibitory effect of the extract of the plant on muscarinic receptors of guinea pig tracheal smooth muscle was examined for possible mechanism responsible for its relaxant effect on tracheal smooth muscle. In group 1 experiments (non-incubated tissues) parallel rightward shifts in methacholine log concentration-response curves in the presence of three concentrations of *P. oleracea*, but higher rightward shift in the presence of atropine were shown. EC_{50} of methacholine obtained in the presence of medium and high concentrations of the extract and atropine was significantly higher than that of saline which may confirm the inhibitory effect of plant concentration at muscarinic receptors. The maximum response to methacholine and slope of methacholine concentration response curves obtained in the presence of the extract concentrations and atropine were not significantly different with those obtained in the presence of saline. These finding may suggested a possible competitive antagonistic effect of the plant at muscarinic receptors of guinea pig trachea smooth muscle[26]. However, the values of CR-1 obtained in the presence of three concentrations of the plant were smaller than atropine. The smaller CR-1 values obtained in the presence of the extract concentrations compared to that of atropine indicate lower competitive antagonist effect for the extract than atropine at concentrations used.

TABLE 1: MAXIMUM RESPONSE TO METHACHOLINE IN THREE GROUPS OF EXPERIMENTS

Solutions	Concentration	Group 1	Group 2	Group 3
Saline		100.00±0.00	100.00±0.00	100.00±0.00
Extract	0.25 mg/ml	97.25±2.413	100.25±1.702	98.55±5.611
	0.50 mg/ml	96.29±5.611	101.74±2.459	98.54±3.617
	1 mg/ml	100.80±2.835	99.82±1.256	100.37±4.041
Atropine		97.37±1.546	95.75±2.658	95.592±2.146

Data are presented as mean±SEM. Group 1, experiments in non-incubated tracheal smooth muscle (n=6); Group 2, experiments in tracheal smooth muscle incubated with propranolol and chlorpheniramine (n=6); Group 3, experiments in tissues incubated with propranolol (n=7). There was no significance difference observed between the data of different solution and different groups.

TABLE 2: SLOPE OF METHACHOLINE RESPONSE CURVES IN THE THREE GROUPS OF EXPERIMENTS

Solutions	Concentration	Group 1	Group 2	Group 3
Saline		0.98±0.006	0.96±0.008	0.95±0.014
Extract	0.25 mg/ml	0.92±0.012	0.97±0.008	0.97±0.004
	0.50 mg/ml	0.96±0.006	0.98±0.002	0.98±0.004
	1 mg/ml	0.95±0.008	0.98±0.005	0.96±0.009
Atropine		0.97±0.01	0.95±0.02	0.92±0.039

Data are presented as mean±SEM. Group 1, experiments in non-incubated tracheal smooth muscle (n=6); Group 2, experiments in tracheal smooth muscle incubated with propranolol and chlorpheniramine (n=6); Group 3, experiments in tissues incubated with propranolol (n=7). There was no significance difference observed between the data of different solution and different groups.

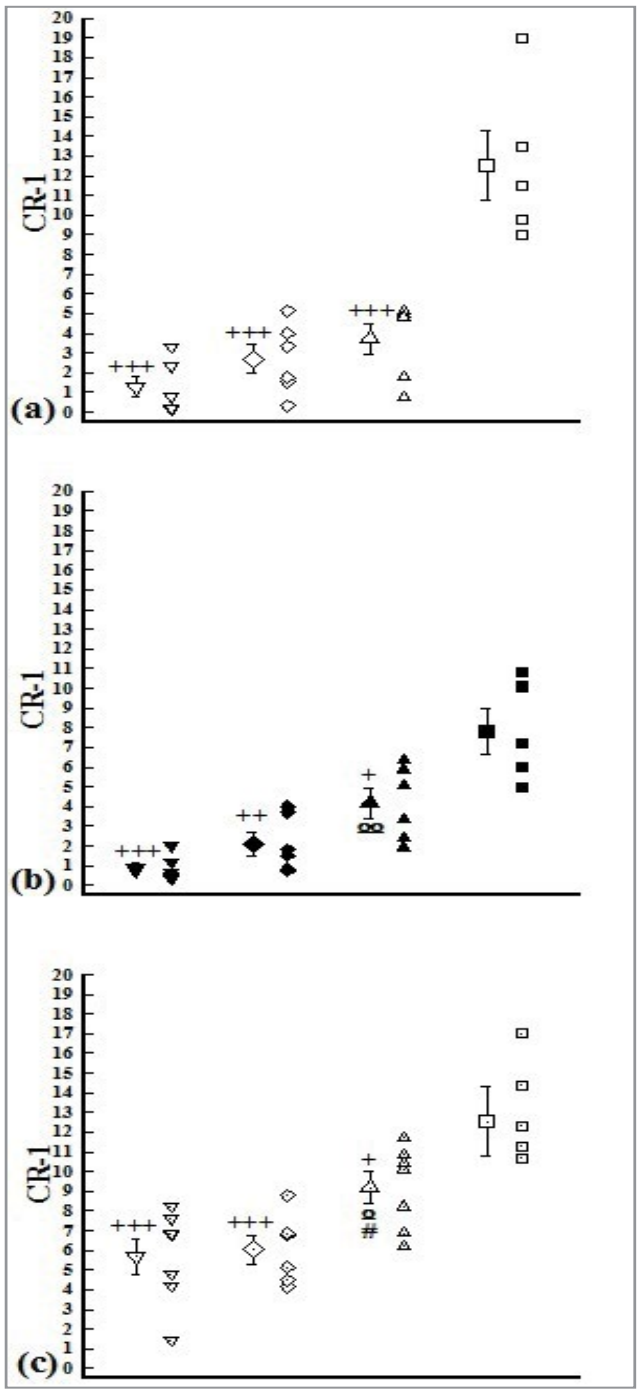

Fig. 3: CR-1 values obtained in the three groups

Values of (CR-1) obtained in the presence of three concentrations of *P. oleracea* extracts and 10 nM atropine in (a) non incubated tissues, n=6; (b) incubated tissues with 1 µM chlorpheniramine and 1 µM propranolol, n=6 and (c) tissues incubated with propranolol, n=7. +: *P*<0.05, compared between atropine and the extract. Ω: *P*<0.05, compared between extract 0.25 vs. 1.00. #: *P*<0.05, compared between extract 0.50 vs. 1.00. Atropine (□); saline (○); extract 0.25 mg/ml (▽); extract 0.50 mg/ml (◊); extract 1 mg/ml (Δ).

To evaluate the contribution of beta-adrenergic stimulatory and/or histamine (H_1) blocking effect on muscarinic receptor antagonism have seen for the plant in group 1, the inhibitory effect of the plant was also examined in incubated tissues with chlorpheniramine and propranolol to inhibit beta-adrenergic and H_1 receptors (group 2 experiments). In group 2 experiments also, parallel rightward shifts in methacholine log concentration-response curves in the present of three concentration of *P. oleracea* and higher rightward shift in the present of atropine were observed. EC_{50} of methacholine obtained in the presence of medium and high concentrations of extract and atropine were also significantly greater than that of saline. The maximum response to methacholine and slope of methacholine concentration response curves obtained in the presence of the extract concentrations and atropine were also not significantly different with those obtained in the presence of saline. These findings support the possible competitive antagonistic effect of the plant at muscarinic receptors and indicate the absence of major beta-adrenergic stimulatory and/or H_1 blocking effect on muscarinic receptor antagonism have seen for the plant in group 1. The values of EC_{50} of methacholine obtained in the presence of all solutions in group 2 were significantly higher than group 1. These results may be due to some interaction(s) among the beta-adrenergic stimulatory and/or H_1 and muscarinic receptors. However, the values of CR-1 obtained in the presence of all examined solution were not significantly different with those of group 1 confirming the absence of beta-adrenergic stimulatory and/or H_1 blocking effect on muscarinic receptor antagonism seen for the plant.

The inhibitory effect of the plant on muscarinic receptors was also examined on incubated tissues with propranolol only; to evaluate the contribution of beta-adrenergic stimulatory alone on antagonism has seen for the extract at muscarinic receptors seen in groups 1 and 2. The results of this group also showed parallel rightward shifts in methacholine log concentration-response curves in the present of three concentrations of *P. oleracea* and atropine. EC_{50} of methacholine obtained in the presence of all extract concentrations and atropine were also significantly higher than that of saline. The maximum response to methacholine and slope of methacholine concentration response curves obtained in the presence of the extract concentrations and atropine were also not significantly different with those obtained in the presence of saline. The values of EC_{50} of methacholine obtained in the presence of all solutions in group 3 were not significantly different compared to group 1. These non-significant differences

in EC_{50} values between groups 3 and 1 may suggest that higher EC_{50} values has seen in group 2 is not due to interaction(s) between beta adrenergic stimulatory and muscarinic receptors. The results of group 3 experiments also confirmed a competitive antagonism effect of *P. oleracea* on muscarinic receptor of tracheal smooth muscle.

In all three groups the effect of *P. oleracea* was concentration dependent and there were significant positive correlations between the values of EC_{50} and the extract concentrations. These findings also confirm the concentration dependent effect of the plant on muscarinic receptors and may support the competitive antagonistic effect of the plant on this receptor type.

These results suggested a competitive antagonistic effect the extract of *P. oleracea* on muscarinic receptors. The results also suggest the absence of beta-adrenergic stimulatory and/or histamine (H_1) blocking effect on muscarinic receptor antagonism seen for the plant.

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Conflicts of interest:

There are no conflicts of interest.

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