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RELAXANT EFFECT OF PORTULACA OLERACEA ON GUINEA PIG TRACHEAL CHAINS AND ITS POSSIBLE MECHANISM(S) OF ACTION

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RESEARCH ARTICLE

ABSTRACT. THERAPEUTIC EFFECT OF portulaca oleracea on respiratory diseases is indicated in Iranian ancient medical books. The relaxant effect of this plant has also been observed on smooth muscles in previous studies. In the present study, the relaxant effects of boiled and aqueous extracts of portulaca oleracea on tracheal chains of guinea pigs were examined. The relaxant effects of 5 cumulative concentrations of boiled and aqueous extracts (0.25, 0.5, 0.75, 1.0, and 1.25 w/v) in comparison with saline as a negative control and 4 cumulative concentrations of theophylline (0.25, 0.5, 0.75, and 1.0 mM) were examined by their relaxant effects in tracheal chains of guinea pig precontracted with 60 mM KCl (group 1, N = 6) and 10 µM methacholine under two different conditions: the non-incubated tissues (group 2, N = 6) and tissues incubated with 1 µM propranolol plus 1 µM chlorpheniramine (group 3, N = 4). In group 1 experiments, only the two higher concentrations of theophylline and boiled extract showed significant relaxant effects compared to that of saline ($P < 0.001$ and $P < 0.05$ for theophylline and boiled extract, respectively). The effects of two higher concentrations of theophylline in this group were significantly greater than those of boiled and aqueous extracts ($P < 0.01$). However, in group 2 and 3 experiments, both boiled and aqueous extracts and theophylline showed a concentration-dependent relaxant effect compared to that of saline ($P < 0.05$ to $P < 0.001$). There were no significant differences when comparing the relaxant effects of boiled and aqueous extracts with those of theophylline in group 2 experiments. The relaxant effects of boiled and aqueous extracts in group 1 were significantly lower than those of groups 2 and 3. However, the differences in he effects of extracts between group 2 and 3 and those of theophylline in group 1 and 2 were not statistically significant. Taken together, these results suggested a potent relaxant effect of portulaca oleracea on the tracheal chains of guinea pigs, and this effect was comparable to or even greater than theophylline at concentrations used.

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1. INTRODUCTION

Portulaca oleracea L. is a grassy and annual plant which grows in many areas of the world. The seeds of portulaca oleracea have several therapeutic effects including diuretic, anti-ascorbic, antipyretic, anti-asthma, anti-inflammatory, and antitussive effect [1,2,3]. Previous studies have shown different pharmacological effect for this plant including: Relaxant effect on skeletal muscle [4,5], relaxant effect on smooth muscle of small intestine and effect on blood pressure [6], anti-inflammatory and analgesic effect [7], antioxidant effect [8,9] and opening effect on potassium channel [10]. In the present study, the relaxant effect of boiled and aqueous extracts from portulaca oleracea on guinea pig tracheal chains was examined.

2. MATERIALS AND METHODS

2.1. PLANTS AND EXTRACTS

Portulaca oleracea was collected in the School of Pharmacy and identified by Ahei. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences (Herbarium No: 240-1615-12). The boiled extract of the plant was prepared as follow: 10 g of the chopped, dried plant was boiled with 100 ml distilled water for 15 min. The extract then filtered with a clean cotton cloth and the volume of extract adjusted to 100 ml by moderate heating. Therefore the final extract concentration was 10% w/v. For preparation of aqueous extract 50 g of plant was extracted with 300 ml distilled water by suxhelat apparatus. The solvent of aqueous extract was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final aqueous extract was 20% w/w in all extracts.

2.2. TISSUE PREOARATIOS

Male guinea pigs (400-700 g) were killed by a blow on the neck and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain [11]. Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

2.3. PROTOCOLS

The relaxant effects of five cumulative concentrations of boiled and aqueous extracts (0.25, 0.5, 0.75, 1.0, and 1.25 w/v), four cumulative concentrations of theophylline anhydrous (Sigma Chemical Ltd., UK) (0.25, 0.5, 0.75, and 1.0 mM), and saline as negative control were examined. To produce different concentrations of boiled and aqueous extracts, 0.25 ml of 10 w/v and 0.125 of 20 w/v concentrated extracts were added to a 10 ml organ bath respectively five times. For theophylline, 0.25 ml of 10 mM concentrated solution was added to organ bath four times. The consecutive volumes were added to organ bath at five minutes intervals.

In each experiment the effect of five cumulative volumes from each extract, four cumulative volumes from theophylline, or saline on contracted tracheal smooth muscle was measured after exposing tissue to the solution for 5 min. A decrease in tone was considered as a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction and an increase in tone was considered as a contractile (bronchoconstrictory) effect, which was expressed as negative percentage change [12].

The relaxant effect of different solutions was tested with two different experimental designs as follows:

1. On tracheal chains contracted by 60 mM of KCl (Group 1 experiments, N = 6).

2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd., UK) (Group 2 experiments, N = 6).

3. On incubated tracheal chains with 1 μM propranolol hydrochloride and 1 μM chlorpheniramine

TABLE 1. RELAXANT EFFECT OF TWO DOFFERENT EXTRACTS FROM PORTULACA OLERACEA IN COMPARISON WITH NEGATIVE CONTROL (SALINE) AND POSITIVE CONTROL (THEOPHYLLINE) IN GROUP 1 EXPERIMENTS (CONTRACTED TRACHEAL CHAINS WITH 60 mM KCl).

DIFFERENT CCONCENTRATION	SALINE	BOILED EXTRACT	AQUEOUS EXTRACT	THEOPHYLLINE
0.25	–	0.0 ± 0.0 NS, ns	0.0 ± 0.0 NS, ns, nS	-5.20 ± 2.15 NS
0.5	–	2.93 ± 2.93 NS, ns	0.0 ± 0.0 NS, ns, nS	13.65 ± 6.15 NS
0.75	–	12.41 ± 6.24 NS, ++	0.0 ± 0.0 NS, +++, nS	49.00 ± 5.77 ***
1	–	31.55 ± 12.10 *, +++	1.54 ± 1.06 NS, +++, ¶	85.83 ± 6.39 ***
1.25	1.19	39.55 ± 12.39 *	2.75 ± 5.04 NS, ¶¶	–

NOTE: Values are presented as mean ± SEM. Statistical differences between the effect of extracts and negative control (saline); NS: non-significant difference, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Statistical differences between the effect of extracts and positive control (theophylline); ns, non-significant difference, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$. Statistical differences between the effect of two extracts; nS, non-significant difference; ¶ $P<0.05$; and ¶¶ $P<0.01$. The unit of concentration for extracts was w/v and for theophylline was mM.

maleate (Sigma Chemical Ltd., UK) 30 min prior to beginning and during the testing relaxation of different solutions. In this series of experiments, tracheal chains were also contracted by 10 µM methacholine hydrochloride (Group 3 experiments, N = 4).

The relaxant effect of theophylline was examined only on groups 1 and 2. The relaxant effects in three groups of experiments were examined in three different series of tracheal chains. All of the experiments were performed randomly with a 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation. The study protocol was approved by ethical committee of Mashhad University of Medical Sciences.

2.4. STATISTICAL ANALYSIS

All data were expressed as mean ± SEM. Data of relaxant effects of different concentrations of extracts were compared with the results of negative and positive control using ANOVA. The data of relaxant effect obtained in three groups of experiments were also compared using ANOVA. The relaxant effect of two extracts and theophylline

TABLE 2. RELAXANT EFFECT OF TWO DOFFERENT EXTRACTS FROM PORTULACA OLERACEA IN COMPARISON WITH NEGATIVE CONTROL (SALINE) AND POSITIVE CONTROL (THEOPHYLLINE) IN GROUP 2 EXPERIMENTS (CONTRACTED TRACHEAL CHAINS BY 10 μM METHACHOLINE).

DIFFERENT CONCENTRATION	SALINE	BOILED EXTRACT	AQUEOUS EXTRACT	THEOPHYLLINE
0.25	–	8.94 ± 4.76 NS, ns	13.31 ± 5.80 NS, ns, nS	-1.86 ± 7.98 NS
0.5	–	42.51 ± 17.94 NS, ns	49.59 ± 11.44 *, ns, nS	11.97 ± 3.47 NS
0.75	–	72.31 ± 13.51 ***, ns	75.91±7.27 ***, ns, nS	33.56 ± 6.35 *
1	–	80.94 ± 12.31 ***, ns	86.83 ± 6.10 ***, ns, nS	69.99 ± 6.74 ***
1.25	-2.40 ± 0.99	91.93 ± 5.87 ***	92.68 ± 5.10 ***, nS	–

NOTE: For abbreviations see Table I.

were related to the concentrations using least square regression. Significance was accepted at $P<0.05$.

3. RESULTS

3.1. RELAXANT (BRONCHODILATORY) EFFECT

In group 1 experiments only, different volumes of theophylline and boiled extract showed significant relaxant effects compared to those of saline ($P < 0.05$ to $P < 0.001$). The effects of the last two concentrations of both extracts were significantly lower than those of theophylline ($P < 0.01$ to $P < 0.001$). In addition the effects of the last two concentrations of boiled extract was significantly higher than those of aqueous extract in this group (TABLE 1).

In groups 2 and 3 both extracts from Portulaca oleracea and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea pig. The relaxant effects of the most concentrations of extracts and theophylline were significantly higher than those of saline ($P < 0.01$ to $P < 0.001$). Only the first concentration of aqueous extract and two lower concentrations of boiled extract and theophylline did not show significant relaxant effects (TABLE 2 AND 3). In addition, the effects of all concentrations of both extracts in the group 2 were not significantly different from those of theophylline (TABLE 2). There were no significant differences in the effect of the different concentrations between two extracts in groups 2 and 3 (TABLE 2 AND 3).

TABLE 3. RELAXANT EFFECT OF TWO DOFFERENT EXTRACTS FROM PORTULACA OLERACEA IN COMPARISON WITH NEGATIVE CONTROL (SALINE) IN GROUP 3 EXPERIMENTS (INCUBATED PREPARATION WITH 1 μM PROPRANOLOL AND 1 μM CHLORPHENIRAMINE, CONTRACTED TRACHEAL CHAINS BY 10 μM METHACHOLINE).

DIFFERENT CONCENTRATION	SALINE	BOILED EXTRACT	AQUEOUS EXTRACT
0.25	–	27.26 ± 14.07 NS	25.37 ± 14.05 NS, nS
0.5	–	64.19 ± 10.33 **	69.10 ± 16.67 **, nS
0.75	–	81.70 ± 10.59 ***	84.47 ± 12.69 ***, nS
1	–	93.57 ± 6.61 ***	88.54 ± 10.31 ***, nS
1.25	-1.0 ± 0.52	99.38 ± 3.58 ***	92.60 ± 7.98 ***, nS

NOTE: For abbreviations see TABLE 1.

3.2. COMPARISON OF THE RELAXANT EFFECT BRTWEEN THREE GROUPS OF EXPERIMENTS

The relaxant effects of most concentrations of both extracts in group 2 and 3 were statistically greater than those of group 1 experiments ($P < 0.05$ to $P < 0.001$). The relaxant effect of most concentrations of both extract in group 3 were higher than those of group 2, but these differences were not statistically significant. In addition, there were no significant differences in the effect of all concentrations of theophylline between groups 1 and 2 (FIG. 1).

3.3. CORRELATION BETWEEN CONCENTRATIONS OF SOLUTIONS AND THEIR RELAXANT EFFECT

There were significant positive correlations between the relaxant effects of both extracts and theophylline with concentrations of the solutions in all three experimental groups except that for aqueous extract in group1 ($P < 0.05$ to $P < 0.001$) (TABLE 4).

4. DISCUSSION

In this study the relaxant (bronchodilatory) effects of boiled and aqueous extract from Portulaca oleracea in comparison with saline as negative control and theophylline as positive control were studied. In group 1 experiments (contracted tracheal chains by KCl) only the two last concentrations of theophylline and of boiled extract showed relaxant effect. The aqueous extract and saline did not show any relaxant effect in this group of experiments. However, both extracts from Portulaca oleracea showed relatively potent relaxant effects compared with the effect of saline in groups 2 and 3 experiments. The effects of all concentrations of boiled and aqueous extract in groups 2 and 3 were comparable with those of theophylline in group 2. However, the effect of theophylline was not examined in group 3 experiments.

The relaxant effect of different extracts from Portulaca oleracea on tracheal chains of guinea pigs might be produced due to several different mechanisms including stimulation of β-adrenergic receptors [12,13], inhibition of histamine H_1

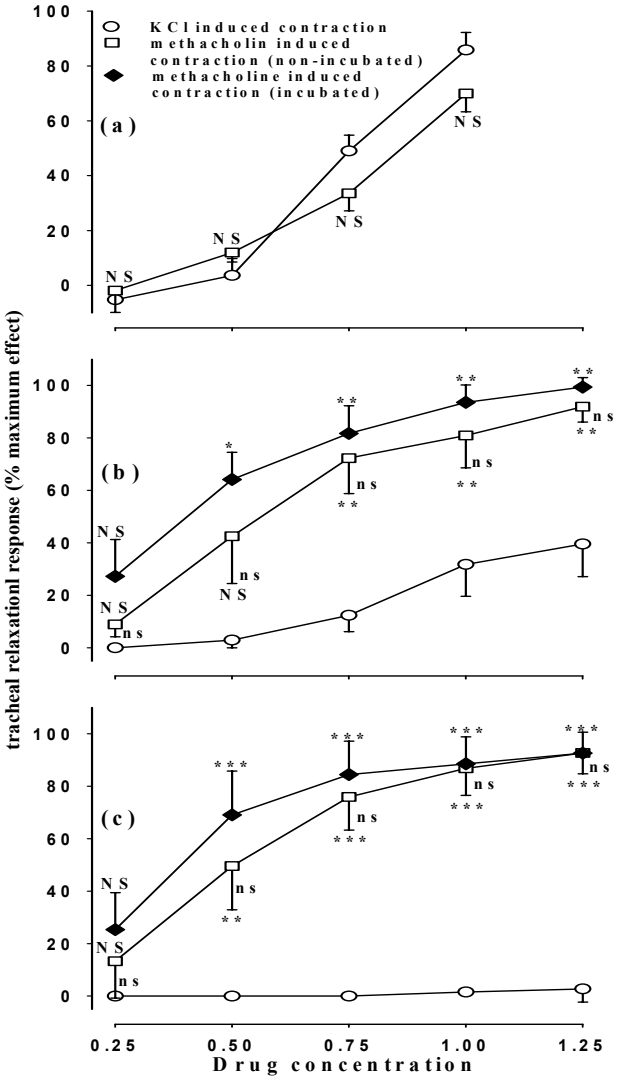

FIGURE 1. CONCENTRATION RESPONSE CURVES OF THE RELAXANT EFFECTS OF THEOPHYLLINE (*a*), BOILED EXTRACT (*b*), AND AQUEOUS EXTRACT (*c*) FROM PORTULACA OLERACEA IN THREE GROUPS OF EXPERIMENTS. Three different groups of experiments were as follows: group 1, KCl induced contraction on non-incubated tracheal chains (o N = 6); group 2, methacholine induced contraction on non-incubated tracheal chains (□ N = 6), and group 3, methacholine-induced contraction on incubated tracheal chains of guinea pig with propranolol and chlorpheniramine (♦ N = 4). Statistical differences in the relaxant effect of different substances between group 1 with those of group 2 and 3; NS, non-significant difference; * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.002$. Statistical differences in the relaxant effect of different substances between groups 2 and 3; ns, non-significant difference; + $P < 0.05$; and ++ $P < 0.01$.

receptors [14] or an anticholinergic property of this plant [15]. To evaluate the contribution of β-adrenergic stimulatory and/or H_1 histamine blocking effect of boiled and aqueous extracts from this plant on their bronchodilatory effects, the effects of these extracts on β-adrenergic and H_1 histamine receptors inhibited by propranolol and chlorpheniramine respectively, were re-examined in group 3 experiments. The relaxant effects of most concentrations of both extracts from Portulaca oleracea obtained in the group 3 experiments were non-significantly greater than those of group 2.

TABLE 4. CORRELATION (*r*) BETWEEN THE RELAXANT EFFECTS OF TWO DIFFERENT EXTRACTS FROM PORTULACA OLERACEA AND THEOPHYLLINE WITH CONCENTRATION IN THREE GROUPS OF EXPERIMENTS.

DIFFERENT SUBSTANCES	GROUP 1	GROUP 2	GROUP 3
BOILED EXTRACT	0.621 ***	0.720 ***	0.720 ***
AQUEOUS EXTRACT	0.321 ^{NS}	0.823 ***	0.651 ***
THEOPHY-LLINE	0.939 ***	0.940 ***	–

NOTE: Statistical significances; NS, non-significant difference; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

These findings suggest probable β-adrenergic stimulatory and/or histamine H_1 blocking properties of the plant extracts that may contribute to their relaxant effect on tracheal chains of guinea pig.

The relaxant effect of both extracts and theophylline was concentration dependent. There were positive correlations between increasing the concentrations of the boiled extracts and the relaxant effects in all three experimental groups, i.e., the aqueous extract in group 2 and 3 and theophylline in group 1 and 2. The relaxant effects of all concentrations of boiled extracts were very similar to those of aqueous extract in groups 2 and 3. In addition, the effects of both extracts in group 2 experiments were comparable to the effect of theophylline.

The absence of obvious relaxant effect of aqueous extract from Portulaca oleracea in group 1 and the relatively potent relaxant effect of this extract in group 2 and 3 experiments may indicate an opening effect of these fractions on potassium channels because the bronchodilatory effect of potassium channel opening has been demonstrated previously [16]. If the aqueous extract had a potassium channel opening effect, they would not have relaxant effect on tracheal chains contracted by KCl, while they could show relaxant effect when the tracheal chain was contracted by metacholine. In fact, the results from experimental group 2 and 3 may support this suggested effect for the aqueous extract. While KCl affect calcium channels [17] and with regard of bronchodilatory effect of calcium channel blockers [18,19], another explanation for these findings is the absence of a blocking effect of this extract on calcium channels. However, the significant relaxant effect of boiled extract in group 1 experiment may suggest the absence of any effect on potassium channels and/or a calcium channels blocking effect for this extract.

The results of this study confirmed those of Okwuasaba et al. published in 1986 and 1987 [4,5] and those of Parry et al. in 1988 [6], which indicated a relaxant effect of this plant on skeletal and smooth muscles, respectively. In fact, the still on-going study in our laboratory showed a relatively potent bronchodilatory effect of the boiled extract from this plant on asthmatic patients (unpublished data) that confirm the results of our present study. Additional studies in our laboratory also showed a potent antitussive effect for this plant. Therefore, as indicated in ancient Iranian medical books, this plant could have therapeutic effects on respiratory diseases. Obviously, more studies are needed to determine the different therapeutic effects, the effective substance(s), and the mechanism of action of portulaca oleracea.

The other possible mechanisms that contribute to the bronchodilatory effect of portulaca oleracea

include: stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC [20], methylxanthin activity [21], and inhibition of phosphodiesterase activity [22]. However, more studies are needed to determine the contributing role of these mechanisms to the relaxant (bronchodilatory) effect of portulaca oleracea extracts in the tracheal chains of guinea pigs as well as the effective substance(s) involved.

With regard to the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, portulaca oleracea might also has an anti-inflammatory effect, which will contribute to the therapeutic effect of this plant on asthma. In fact, the anti-inflammatory [7] and antioxidant effects of this plant have already been observed [8,9], but the potential effect of portulaca oleracea on airway inflammation associated with asthma requires further studies.

In summary, the results of this study indicated a relatively potent relaxant (bronchodilatory) effect of portulaca oleracea on tracheal chains of guinea pig, which was comparable with that of theophylline.

5. ACKNOWLEDGEMENT

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