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(Research article)

Relaxant effects of adhatoda vasica on guinea pig Tracheal chains and its possible mechanisms

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ABSTRACT

Adhatoda vasica is a plant believed to have several therapeutic effects, including anti-asthmatic properties. The objective of this study was to investigate the relaxant effects of macerated and soxhlet extracts of leaves of this plant on tracheal chains of Guinea pigs were evaluated. The relaxant effects of 4 cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 W/V) in comparison with saline as negative control and 4 cumulative concentrations of theophylline (0.25, 0.5, 0.75, and 1.0 mM) as positive controls were examined on precontracted tracheal chains of two groups of 6 Guinea pig by 60 mM KCl (group 1, N=6) and 10 µM methacholine the non-incubated tissues (group 2, N = 6) and tissues incubated with 1 µM propranolol (group 3, N = 4)[24]. Decrease in contractile tone of tracheal chains was considered as the relaxant effect. The isolated guinea-pig trachea pre-contracted with KCl, methacholine and tissues incubated with propranolol were used to study the relaxation of macerated and soxhlet extracts of leaves Adhatoda vasica. In the group, 1 experiments only the last two higher concentrations of theophylline and soxhlet extract showed significant relaxant effect compared to that of saline ($p<0.001$ for both concentrations), which were significantly greater than those of macerated extracts ($p<0.001$ for all cases). The effects of two higher concentrations of theophylline in this group were significantly greater than those of macerated and soxhlet extracts ($P < 0.01$). And in group 2 and 3 experiments, both macerated and soxhlet extracts showed concentration-dependent relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$ for both extracts). There were no significant differences when comparing the relaxant effects of macerated and soxhlet extracts with those of theophylline in the group 2 experiments. The relaxant effects of macerated, and soxhlet extracts in group 1 were significantly lower than those of groups 2 and 3. These results showed a potent relaxant effect of Adhatoda vasica on tracheal chains of Guinea pigs, which were lower than theophylline at concentrations used.

Keywords: Adhatoda vasica, Bronchodilatory Guinea pig. Trachea

INTRODUCTION

Bronchial asthma is a disease characterized by increased responsiveness of the trachea, bronchi and bronchioles to be various stimuli and is manifested by wide-spread narrowing of the airways in allergic asthma, bronchoconstriction and bronchial secretion are the results of an immediate hypersensitivity reaction [1]. Bronchial asthma is one of the most disabling diseases, affecting nearly 7-10% of world population [2]. Bronchoconstriction plays a very important role on the physiopathology of asthma and compounds that relax respiratory smooth muscles such as β2 -agonists and cholinergic antagonists are usually used in symptomatic treatments of the disease [3]. Bronchodialotors help to stop asthma attacks after they have started or can help prevent recurrent attacks. The clinical management of acute asthma is with bronchodialators like β2 receptor agonists, antimuscarinics, and anti-inflammatory therapy with corticosteroids and administration of oxygen if necessary [2]. Many medicinal plants, including,

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Adhatoda vasica have been documented as the remedy for the treatment of asthma in the Ethiopian traditional medicine [4] [5]. Adhatoda vasica is a well-known plant drug in Ayurvedic and Unani medicine. Adhatoda leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders [6]. The medicinal properties of Adhatoda vasica, called vasa or Vasaka in Sanskrit have been known in India and several other countries for thousands of years. Adhatoda vasica and some of the reported activities of the plant include anti-diabetic [8], anti-implantation [9], anti-phlogistic, anti-allergic [10], anti-tussive [11], anti-ulcer [12], antioxidant, anti-genotoxicity. [13], anti-tubercular [14], abortifacient [15], radioprotective [16], bronchodilator [17], hepatoprotective [18]. Pahwa et.al, [19] studied the chronic toxicity of some compounds of Adhatoda vasica. The patents consisting Adhatoda vasica are used for treating asthma (US 6746694), allergy (US 6746694), malnutrition, chronic cough, cold (US 7247322). Adhatoda Adhatoda vasica Leaves, 10-30 cm long and 3-10 cm broad, lanceolate to ovate-lanceolate, slightly acuminate, base tapering, petiolate; petioles 2-8 cm long, exstipulate, glabrescent, 8-10 pairs of lateral vein bearing few hairs; dried leaves dull brown above, light greyish brown below; odour, characteristic; taste, bitter[7]. The therapeutic effect of the plant on asthma could be due to relaxant effect on airway smooth muscles leading to dilation of the airways. In the present study, the relaxant effect of macerated and soxhlet extracts of the leaves of Adhatoda vasica and its possible mechanism(s) on Guinea pig tracheal chains were examined.

MATERIALS AND METHODS

Plant and extracts

Adhatoda vasica was collected from the Vonaigarh Forest Sundergadh Orissa, and identified by taxonomist of botanical department of Vinoba Bhave University Hazaribagh Jharkhand. The extract was prepared by taking fifty grams of the chopped, dried plant leaves were extracted with 300 ml distilled water by soxhlet apparatus. For the preparation of the macerated extract, the same amount of plant was macerated with 300 ml distilled water (on a shaker) for 48 hr. The solvent of both extracts were then removed under reduced pressure at 50oC, and distilled water were added to residues in such a way that plant ingredient concentration in the final soxhlet extracts were 10% W/W.

Tissue preparations

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). The cartilages of all rings were then cut open opposite to the trachealis muscle, and sutured together to form a tracheal chain [20]. Tissue was then suspended in a 10 ml organ bath (Pinnacle Biomedical Research Institute(PBRI) Syamala Hills Bhopal (M.P.) India) 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 kept at 37°C under the stream of 95% O_2 and 5% CO_2 gases. 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 [22].

Protocols

The relaxant effects of four cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 g/100 ml), four cumulative concentrations of theophylline anhydrous (Shreeji Pharma International, India) (0.25, 0.5, 0.75, and 1.0 mM) as positive control, and saline as negative control were examined. For preparation of different concentrations in the case of macerated and soxhlet extracts, 0.25 ml of 10% W/V of the concentrated extracts and in the case of theophylline, 0.25 ml of 10 mM solutions were added to the organ bath. The consecutive volumes were added to organ bath at 5 min intervals. In each experiment, the effect of four cumulative volumes of each extracts, four cumulative volumes of theophylline, or saline on contracted tracheal smooth muscle were determined after exposure of 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 [21] [23].

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

1. On tracheal chains contracted by 60 mM KCl (group 1experiments N = 6).
2. 2. on non-incubated tracheal chains contracted by 10 µM methacholine hydrochloride (Shreeji Pharma International, India) (group 2 experiments N = 6).
3. on incubated tracheal chains with 1 µM propranolol hydrochloride (Shreeji Pharma International, India) 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 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 [22]. In all experiments responses were recorded on a kymograph (Pinnacle Biomedical Research Institute (PBRI) Syamala Hills Bhopal (M.P.) India) and were measured after fixation.

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

RESULTS

Relaxant (bronchodilator) effect

In group 1 experiments only, different volumes of theophylline and soxhlet 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 soxhlet extract were significantly higher than those of macerated extract in this group (TABLE 1). In groups 2 and 3 both extracts from Adhatoda vasica 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 macerated extract and two lower concentrations of soxhlet extract and theophylline did not show significant relaxant effects (TABLE 2 AND 3). There were no significant differences in the effect of the different concentrations between two extracts in groups 2 and 3 (TABLE 2 AND 3). 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, 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 macerated extract in group1 ($P < 0.05$ to $P < 0.001$) (TABLE 4). In addition, the effects of the different concentrations of soxhlet extract in this group were greater than those of macerated extracts, which were statistically significant ($p<0.05$), (Table 1 and 2). However, the effects of two higher concentrations of macerated extract and only the highest concentration of soxhlet extract in group 1 were significantly lower than those of theophylline ($p<0.01$ to $p<0.001$), (Table 1).

TABLES/FIGURES

TABLE:-1 Relaxant effect of two different extracts from Adhatoda vasica in comparison with negative control (Saline) and Positive control (Theophylline) in group I experiment (Contracted Tracheal chains with 60 mM Kcl)

DIFFERENT CONCENTRATION	SALINE	MACERATED EXTRACT	SOXHLET EXTRACT	THEOPHYLLINE
0.25	0	0.0 ± 0.0 NS, ns, nS	0.0 ± 0.0 NS, ns	0.50 ± 2.15 NS
0.5	0	0.0 ± 0.0 NS, ns, nS	2.93 ± 2.93 NS, ns	13.65 ± 6.15 NS
0.75	0	0.0 ± 0.0 NS, +++, nS	12.41 ± 6.24 NS, ++	49.00 ± 5.77 ***
1	0	1.54 ± 1.06 NS, +++,	31.55 ± 12.10 *, +++	85.83 ± 6.39 ***

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.

TABLE: 2. Relaxant effect of two different extracts from Adhatoda Vasica in comparison with negative control (Saline) and Positive control (Theophylline) in Group 2 experiments (Contracted tracheal chains by 10μM methacholine).

DIFFERENT CONCENTRATION	SALINE	MACERATED EXTRACT	SOXHLET EXTRACT	THEOPHYLLINE
0.25	0	8.94 ± 4.76 NS, ns	13.31 ± 5.80 NS, ns, nS	1.86 ± 7.98 NS
0.5	0	20.51 ± 17.94 NS, ns	29.59 ± 11.44 *, ns, nS	11.97 ± 3.47 NS
0.75	0	32.31 ± 13.51 ***, ns	36.91±7.27 ***, ns, nS	39.56 ± 6.35 *
1	0	44.94 ± 12.31 ***, ns	57.83 ± 6.10 ***, ns, nS	76.99 ± 6.74 ***

NOTE: For abbreviations see Table I.

TABLE: 3. Relaxant effect of two different extracts from Adhatoda vasica in comparison with negative control (saline) in Group 3 experiments (Incubated preparation with 1 μM propranolol contracted tracheal chains by 10 μM methacholine)

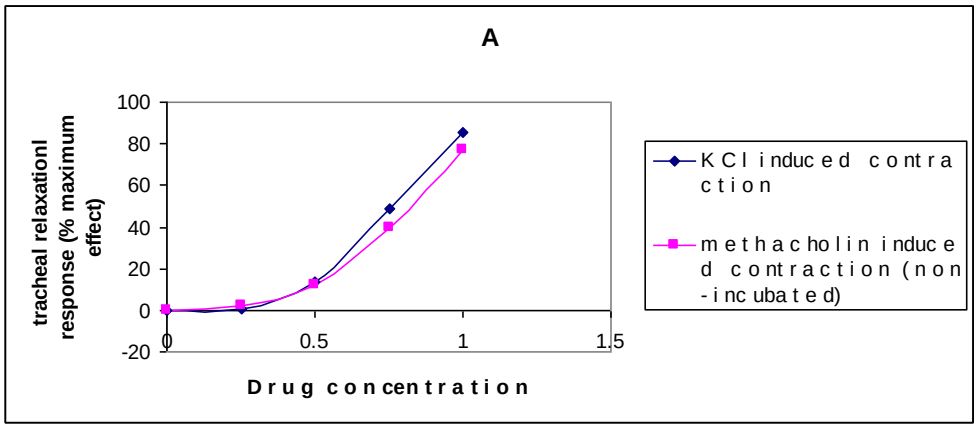
DIFFERENT CONCENTRATION	SALINE	MACERATED EXTRACT	SOXHLET EXTRACT
0.25	0	25.37 ± 14.05 NS, nS	27.26 ± 14.07 NS
0.5	0	69.10 ± 16.67 **, nS	64.19 ± 10.33 **
0.75	0	84.47 ± 12.69 ***, nS	81.70 ± 10.59 ***
1	0	88.54 ± 10.31 ***, nS	93.57 ± 6.61 ***

NOTE: For abbreviations see Table I.

TABLE: 4 Correlation (r) between the relaxant effects of two different extracts from Adhatoda vasica and theophylline with concentration in three groups of experiments.

DIFFERENT SUBSTANCES	GROUP 1	GROUP 2	GROUP 3
SOXHLET EXTRACT	0.621 ***	0.720 ***	0.720 ***
MACERATED EXTRACT	0.321 NS	0.675 ***	0.711 ***
THEOPHY-LLINE	0.939 ***	0.940 ***	-

FIGURE: 1 Concentration response curves of the relaxant effects of Theophylline (A).



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 (♦ 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.

FIGURE: 2. Concentration response curves of the relaxant effects of Macerated extract (B)

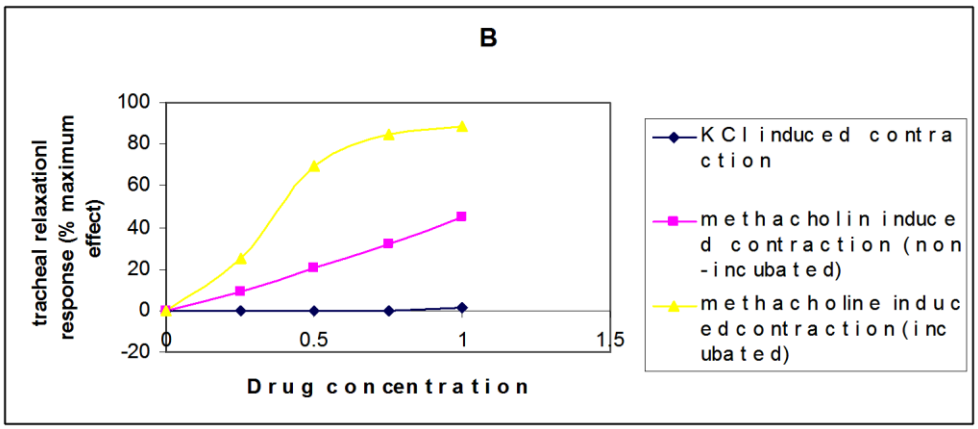
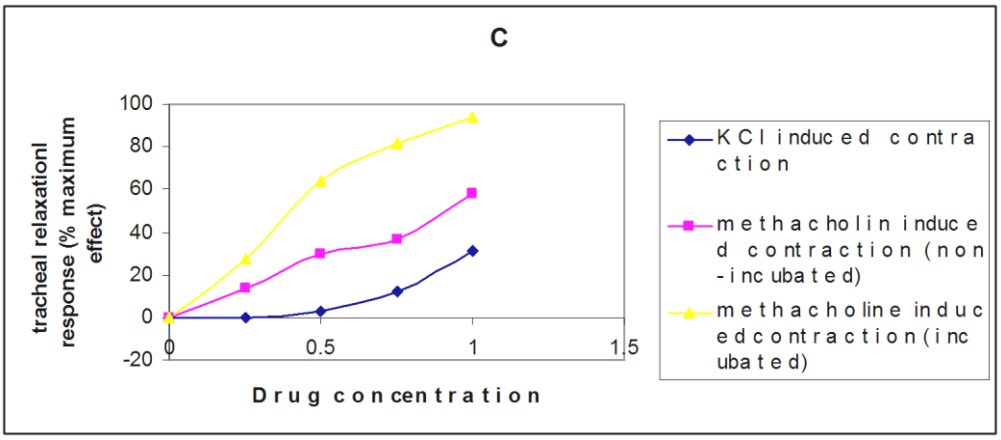


FIGURE 3. Concentration response curves of the relaxant effects of Soxhlet extract (c)



DISCUSSION

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

The relaxant effect of different extracts from Adhatoda vasica on tracheal chains of Guinea pigs might be produced due to several different mechanisms, including stimulation of β-adrenergic receptors [27], inhibition of histamine H1 receptors [28]. To evaluate the contribution of β-adrenergic stimulatory and/or H1 histamine blocking effect of soxhlet and macerated extracts from this plant on their bronchodilatory effects, the effects of these extracts on β-adrenergic and H1 histamine receptors inhibited by propranolol was re-examined in the group 3 experiments. The relaxant effects of most concentrations of both extracts from Adhatoda vasica obtained in the group 3 experiments were non-significantly greater than those of group 2. The relaxant effect of both extracts and theophylline was concentration dependent. There were positive correlations between increasing the concentrations of the soxhlet extracts and the relaxant effects in all three experimental groups. The absence of obvious relaxant effect of macerated extract from Adhatoda vasica 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 [27]. 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 methacholine. In fact, the results from experimental group 2 and 3 may support this suggested effect for the macerated extract. The KCl affect calcium channels [28] and with regard of bronchodilator effect of calcium channel blockers [29] [30] another explanation for these findings is the absence of a blocking effect of this extract on calcium channels. However, the significant relaxant effect of soxhlet extract in the group 1 experiment may suggest the absence of any effect on potassium channels and/or calcium channel blocking effect for this extract. The effects of different concentrations of soxhlet extract in both groups of experiments were greater than those of macerated extract. This may suggest that the effective substances in soxhlet extract are higher than that of macerated extract. The other possible mechanisms for bronchodilatory effect of Adhatoda vasica include: stimulation of β-adrenergic receptors [24], blocking of histamine H1 receptors [25], anticholinergic activity [26]. However, contributions of these mechanisms to bronchodilatory effect of extracts of Adhatoda vasica on tracheal chains of Guinea pigs (s) should be clarified in further studies. In addition, Adhatoda vasica might also have an anti-inflammatory effect, which will contribute to the therapeutic effect of this plant on asthma. While anti-inflammatory [17] and anti-oxidant [18] effects of this plant have been reported, the effect of, Adhatoda vasica on airway inflammation, which is present in asthma should be investigated in further studies.

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