

**BRONCHODILATORY AND RESPIRATORY DISTRESS
PROTECTIVE EFFECT OF FRACTIONS ISOLATED
FROM *ADHATODA SCHIMPERIANA* IN GUINEA-PIGS**

Zelalem Petros^{1*}, Eyasu Makonnen¹, Asfaw Debella²

¹Department of Pharmacology, Faculty of Medicine, Addis Ababa University, P.O. Box 9086, Addis Ababa, Ethiopia, e-mail:zelalemgbc@yahoo.com, eyasumakonnen@yahoo.com

²Department of Drug Research, Ethiopian Health and Nutrition Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia, e-mail:asfawdebella@yahoo.com

*corresponding author

Summary

Adhatoda schimperiana has been used in Ethiopian traditional medicine as a remedy for bronchial asthma and cough. In the present study, bronchodilatory and respiratory distress protective effect of solvent fractions of hydro-alcoholic extract of the leaves of the plant were evaluated on guinea-pigs. The chloroform fraction of the crude extract showed a statistically significant ($p < 0.05$) dose dependent trachea relaxant activity and respiratory distress protective effect. These results suggest that the alkaloid-rich fraction of the crude extract might be responsible for the claimed anti-asthmatic effect of the plant.

Key words: *Adhatoda schimperiana*; Bronchial asthma; Bronchodilator; Guinea-pig; Alkaloid

Introduction

Among several non-infectious respiratory disorders affecting human being, bronchial asthma is the most common chronic disease that can impede breathing (1). Bronchial asthma is an airway inflammatory disease characterized by bronchial hyper-responsiveness, intermittent and reversible airway obstruction that leads to recurrent episodes of cough, wheezing, shortness of breath and chest tightness. It is thought that up to 10% of adults and 20% of children are affected globally (2). The etiology of bronchial asthma appears to have genetic and environmental components (3).

The standard of care in managing bronchial asthma is avoidance of exposure to allergens and non-specific exacerbating factors such as cigarette smoke, cold air, vigorous exercise and sensitizing agents (4). Currently available drugs for management of bronchial asthma are of two general categories: drugs that inhibit smooth muscle contraction and anti-inflammatory agents. Despite availability of wide range of drugs, the relief offered is mainly symptomatic and not curative. Moreover, their effects are short lived and the side effects are also quite disturbing. Thus, there is a need to have more effective and safe pharmacological agents that could interfere with the pathogenesis of bronchial asthma. One of these potential sources of therapeutic agents could be traditionally used plants.

Adhatoda schimperiana (Family: Acanthaceae) is a fast growing plant abundant in the highlands of Ethiopia and some other countries of East Africa (5). The plant is an erect shrub up to 4m high and usually much branched from the base. The leaves are simple, opposite and ovate in outline. The decoction of the dried leaves of the plant mixed with local beer ('Tela') is taken as a remedy for bronchial asthma (6). The crude hydro-alcoholic extract of the leaves of the plant was found to have bronchodilatory effect (7). The aim of the current study was, therefore, to evaluate bronchodilator and respiratory distress protective effects of solvent fractions of the crude hydro-alcoholic extract, and to carry out acute toxicity test on the most potent fraction.

Materials and Methods

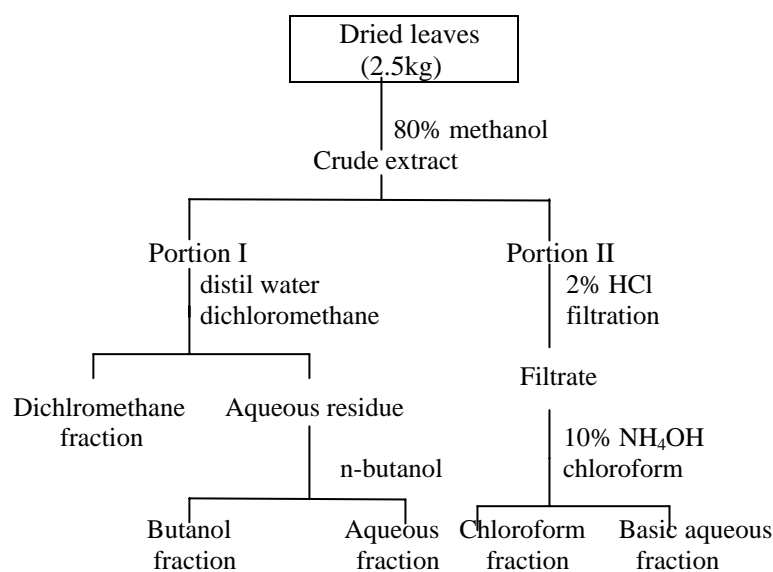
Collection of plant material

Adhatoda schimperiana leaves were collected in February 2006 around Semin Mazegaja, Addis Ababa, Ethiopia. The leaves were identified and voucher specimen (No AS-2035) was deposited in the herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa.

Extraction and solvent fractionation

Dried powdered leaves (2.5kg) of the plant were extracted with 5L of 80% (v/v) methanol by percolation. The extract was filtered and concentrated using rota vapor. The crude extract was then divided into two equal portions. The first portion was suspended in 200ml distilled water and partitioned with dichloromethane. The aqueous residue was further partitioned with n-butanol. The second portion was suspended in 2% HCl (500ml) stirred for an hour and filtered. Alkalinization of the filtrate with 10% NH_4OH (to pH 10) was followed by repeated extraction with chloroform. Fractionation of the crude extract is shown in fig 1.

Fig. 1 Solvent fractionation of the crude extract of *A. schimperiana*



The yield of the crude extract was 650g, 26% of the dried leaves. The solvent fractions were dichloromethane with the yield of (41g, 13%), n-butanol (38g, 12%), aqueous (222g, 68%), chloroform (25.5g, 8%), and basic-aqueous (257g, 79%). Phytochemical screening indicated the presence of alkaloids in the chloroform fraction.

Animals

Guinea pigs (400-600g) for *in-vitro* and *in-vivo* tests and Swiss albino mice (20-30g) for acute toxicity test were used. They were kept in an animal house under room temperature of about 24°C. Prior to the experiment, the animals were acclimatized to the test environment for an hour and randomly assigned to control, test and standard groups.

Chemicals

Methanol, dichloromethane, chloroform and n-butanol (Riedel-de Haen, Germany), salts for physiological solution (Labort chem., India), tween 80, bismuth subnitrate and potassium iodide (Mayer and Baker lab., England), histamine dihydrogen phosphate (Sigma-Aldrich, Germany) and diphenhydramine hydrochloride (Loba chem., India) were used in the experiment.

Pharmacological evaluation

I. Testing relaxant effect on isolated guinea-pig trachea

Guinea pigs were killed by a blow at the back neck against a table edge and sacrificed. The trachea was rapidly removed and kept in Kreb's solution (gm/l): NaCl (6.8), MgSO₄·7H₂O (0.25), CaCl₂ (0.28), KCl (0.35), NaHCO₃ (2.1), KH₂PO₄ (0.16) and glucose (2.0) (8). The trachea was then cut transversely between segments so as to give five rings. A cotton thread was tied to the cartilages to form a tracheal chain. The chain was suspended in a 25 ml thermo-regulated organ bath containing Kreb's solution, maintained at 37°C and supplied with air. One end of the tracheal chain was attached to a tissue holder at the base of the organ bath and the other end to a recording device.

The suspended trachea chain was allowed to equilibrate for at least an hour. During equilibration, the bath was supplied with fresh Kreb's solution every 15minutes (8). Then cumulative concentration-response to histamine (10^{-6} - 10^{-2})M in absence and presence of 400 μ g/ml concentration of the solvent fractions were recorded. The tissue was exposed to the fractions for 10 minutes before addition of histamine. The responses were recorded with Grass recorder model 7E polygraph with force-displacement transducer equipped with time and event marker. The chart speed was 5mm/minute.

Tracheal-relaxant effect of each fraction was observed from the reduction of contractile effect of histamine. In presence of each fraction, the percentage maximum response to histamine and concentration of histamine that produces half maximum response were compared to those of the control and diphenhydramine.

II. Testing respiratory distress protective effect in guinea pigs

Guinea pigs were assigned into three groups. The test groups were pre-treated intraperitoneally with the fractions, the standard group with diphenhydramine and the control group with the vehicle (3% tween 80). After an hour, all the groups were injected with histamine intraperitoneally and pre-respiratory distress time was recorded. Then the percent protection against respiratory distress was calculated (9) as follows:

$$\text{Protection (\%)} = [1 - (T_1/T_2)] \times 100$$

Where T_1 - pre-respiratory distress time in the control group

T_2 - pre-respiratory distress time in the pre-treated group

III. Acute toxicity test in mice

Swiss albino mice (ten mice per group) weighing 20-30g were fasted overnight. Five dose levels: 2.1g/kg, 3.0g/kg, 4.5g/kg, 6.8g/kg and 10.2g/kg were used as determined from pilot study. The dose suspended in 3% tween 80 was given orally as a single dose. The mice were observed continuously for 2hrs, occasionally in 4hrs, and overnight mortality was recorded. Oral median lethal dose was determined using probit analysis (10).

Statistical analysis

The results of the experiment were expressed as mean \pm standard error of the mean. For group comparison, analysis of variance followed by Tukey-Kramer multiple comparison test using SPSS version 13.0 was used. The difference among means considered to be statistically significant when p-value was less than 0.05.

Results

Effect of fractions on isolated guinea pig trachea

As shown in table 1, the maximum response to histamine (effect at 10^{-2} M) in presence of 400 μ g/ml concentration of the chloroform fraction and the crude extract were significantly lower than that of the control ($p < 0.05$). With the same concentration, the maximum responses to histamine in the presence of the other fractions were not statistically significant. The maximum response to histamine in presence of the chloroform fraction was significantly lower than that of the crude extract and the other fractions ($p < 0.05$).

Table 1. Maximum response to histamine in presence of the crude extract and the solvent fractions (values as $M \pm SEM$, $n=6$)

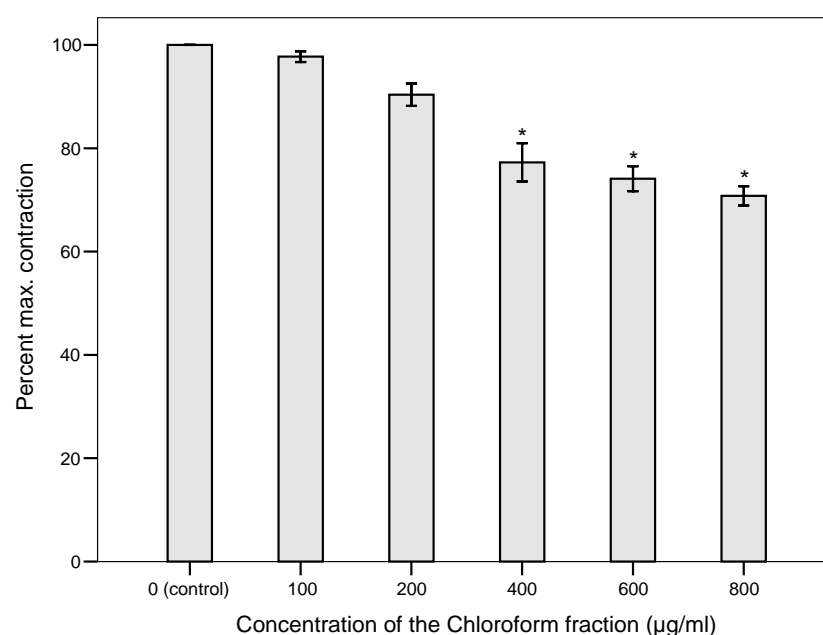
Vehicle/fraction	Maximum response (%)
Saline (control)	100.0 \pm 0.0
Crude extract, 400 μ g/ml	87.7 \pm 1.8 ^a
Dichloromethane fr., 400 μ g/ml	92.8 \pm 1.5
Butanol fr., 400 μ g/ml	95.6 \pm 2.2
Aqueous fr., 400 μ g/ml	90.9 \pm 2.3
Chloroform fr., 400 μ g/ml	77.2 \pm 3.7 ^{a, b}
Basic aqueous fr., 400 μ g/ml	95.0 \pm 1.4
Diphenhydramine (0.01 μ M)	58.6 \pm 3.2 ^{a, b}

a - $p < 0.05$ compared to the control (saline)

b - $p < 0.05$ compared to the other solvent fractions

The maximum response to histamine tends to decrease with an increase in concentration (100-800 μ g/ml) of the chloroform fraction (Fig 2). The maximum response in presence of 400 μ g/ml (77.27 ± 3.70), 600 μ g/ml (75.68 ± 3.14) and 800 μ g/ml (73.40 ± 2.08) concentrations were significantly lower than that of the control ($p < 0.05$). The response in the presence of 100 μ g/ml (97.73 ± 1.05) and 200 μ g/ml (90.37 ± 2.16) concentration, however, were not statistically significant.

Fig 2. Percent maximum contraction of histamine in the presence of various concentrations of chloroform fraction (n=6, * $p < 0.05$)



The concentration of histamine producing 50% of the maximum response (EC_{50}) in presence of the chloroform fraction was statistically significant ($p < 0.05$) as compared to that of the control and the crude extract (Table 2). About ten times higher concentration of histamine was needed to achieve the same half maximal response as in the absence of the chloroform fraction.

Table 2. EC₅₀ of histamine in presence of crude extract and chloroform fraction (values as EC₅₀ (95 % CI), n=6, * p<0.05)

Solution	EC ₅₀ (10 ⁻⁴ M)
Control (Saline solution)	0.69 (0.66 – 0.73)
Crude extract, 400 µg/ml	2.01 (1.90 – 2.10)*
Chloroform fraction, 400 µg/ml	6.40 (6.08 – 6.72)*
Diphenhydramine, 0.01 µM	46.55 (44.23 – 48.87)*

Effect of fractions on histamine induced respiratory distress

Respiratory distress protective effect of the chloroform fraction (600 mg/kg) was significantly (p<0.05) higher than that of the crude extract and the other fractions (Table 3). The protective effect of the chloroform fraction was, however, significantly less than (p<0.05) that of diphenhydramine (20 mg/kg).

Table 3. Pre-respiratory distress time and percent protection against histamine (9 mg/kg) induced respiratory distress in presence of 600 mg/kg dose of the fractions (values as M ± SEM, n=6)

Vehicle/fraction	Pre-respiratory distress time (sec)	Respiratory distress protection (%)
Tween 80 (control)	107 ± 4.8	-
Crude extract, 400µg/ml	364 ± 7.7	69.5 ± 0.7 ^b
Dichloromethane fr., 400µg/ml	131 ± 8.7	17.5 ± 3.6
Butanol fr., 400µg/ml	120 ± 7.3	10.4 ± 3.2
Aqueous fr., 400µg/ml	326 ± 17.7	66.8 ± 1.8
Chloroform fr., 400µg/ml	548 ± 35.7	80.2 ± 0.8 ^{a,b}
Basic aqueous fr., 400µg/ml	124 ± 6.5	13.3 ± 2.9
Diphenhydramine (20 mg/kg)	3283±257.1	96.6 ± 0.2 ^{a,b}

a - p<0.05 compared to the crude extract

b - p<0.05 compared to the other solvents fractions

Acute toxicity

During 24hrs of observation, mice which received lower doses of the chloroform fraction did not show any change in the general behaviour. At higher doses of the fraction piloerection, staggering, hypo-activity and hypnosis were observed. The median lethal dose was estimated to be 5.01g/kg.

Discussion

The leaves of *Adhatoda schimperiana* has been used traditionally to relieve respiratory disorders, such as bronchial asthma (6). In the present study, the crude hydroalcoholic extract of the leaves of the plant showed trachea relaxant and respiratory distress protective effect. This is in agreement with bronchodilatory effect of crude extract obtained by a previous study (7).

Our *in-vitro* study results showed trachea relaxant effect of the crude extract was higher than that of the control. With fractionation, the chloroform fraction was found to have better trachea relaxant effect than the crude extract. The reason for increased activity of the chloroform fraction could be due to increased concentration of active components. Fractionation could therefore, help isolate and identify active bronchodilatory compounds. With increasing the concentration of the chloroform fraction (100 to 800 µg/ml), more pronounced relaxant effect was observed indicating the effect is dose-dependent.

In the presence of the chloroform fraction, the maximum response to histamine was significantly reduced. It was not possible to recover the maximum response to histamine in spite of increased concentration hinting the antagonisms is non-competitive or irreversible competitive antagonistic effect at histamine H₁ receptors (11). The trachea relaxant effect might also be mediated through activation or inhibition of ion channels or other receptors. For example, calcium channel blockage might have contributed to the relaxant effect observed (12).

In the *in-vivo* study, the chloroform fraction showed better respiratory distress protective effect than the crude extract and the other fractions. This is in agreement with the *in-vitro* result where the chloroform fraction showed better trachea relaxant activity than the crude extract and the other fractions. The respiratory distress protective effect of the chloroform fraction might be due to inhibitory nature on the effect of histamine. The fact that other mediators like leukotrienes play more roles than histamine in the etiology of bronchial asthma could explain other possible mechanisms such as bronchodilatory effect (9).

A previous phytochemical study showed that the crude extract of *Adhatoda schimperiana* contains glycosides, steroids, terpenoids, alkaloids and saponins (7). Our study also revealed the presence of alkaloids in the chloroform fraction. From a study on a closely related Indian plant *Adhatoda vasica*, trachea relaxant alkaloids vasicine and vasicinone were isolated (13). Thus, the alkaloid constituents of *Adhatoda schimperiana* may be novel or similar to those of *Adhatoda vasica*.

In the acute toxicity test, hypo-activity was observed at moderate doses of the chloroform fraction, while hypnosis was pronounced at higher dose. The fraction seems less likely to be toxic when taken orally as demonstrated by higher median lethal dose.

Conclusion

In the present study, the chloroform fraction of the crude hydroalcoholic extract of the leaves of *Adhatoda schimperiana* was found to have dose dependent bronchodilatory and respiratory distress protective effect, and alkaloid components seems most likely responsible for the observed effects. Although the chloroform fraction inhibited the effect of histamine, it is beyond the scope of the present study to establish the exact mechanism(s) of bronchodilatory effect of the plant. The present study however, could hint the possible mechanism(s) of action. Further studies therefore, are needed to determine the mechanism(s) of action of the active components and to structurally elucidate them.

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