ORIGINAL ARTICLE

BRONCHODILATOR AND ANTI-INFLAMMATORY ACTIVITIES OF ADHATODA SCHIMPERIANA

Ashenafi Assefa, BSc., Kelbessa Urga, MSc., Daniel Melaku, Mulugeta Guta,. BSc., Walelign Mekonnen,

ABSTRACT

BACKGROUND: ***Adhatoda schimperiana is a plant believed to have several therapeutic effects including anti-asthmatic properties. The objective of this study was to investigate the bronchodialatory, anti-inflammatory effects and toxicity of the hydromethanolic extract of leaves of this plant***.
METHODS: ***The isolated guinea-pig trachea pre-contracted with histamine and acetylcholine was used to study the relaxation of hydromethanolic extract of leaves Adhatoda shimperiana. Salbutamol and atropine were used as standards. The effect of the hydromethanol extract of leaves of Adhatoda schimperiana on carageenin-induced acute inflammation was evaluated by the rat hind paw edema method. Oral and interaperitoneal acute toxicity studies of the extract were performed on mice***.
RESULTS: ***The hydromethanolic extract of Adhatoda schimperiana inhibited contractions of guinea pig tracheal chains induced by acetylcholine and histamine with an EC_{50} of 4.66 mg/ml and 5.92 mg/ml, respectively. Salbutamol and atropine also showed similar concentration dependent relaxation of the tracheal chains pre-contracted with both acetylcholine and histamine. The inhibitory activity of atropine was lower than the extract and salbutamol. The extract exhibited a moderate degree of anti-inflammatory activity. The LD_{50} of the extract for oral acute toxicity study was found to be 1286.76 mg/kg with 95 % confidence limit of 1161.9 – 1418.0. The plant extract therefore presents a relatively low acute toxicity.***
CONCLUSION: ***The results of this study show anti-inflammatory activity and a relatively potent relaxant (bronchodialatory) effect of Adhatoda schimperiana on the tracheal chain of the guinea pig. These activities justify the traditional use of this plant in the treatment of bronchoconstrictive diseases. More detailed studies are required to investigate the mechanism of action, the toxicity and the therapeutic utility of Adhatoda schimperiana for further development towards a proper drug.***

KEY WORDS: ***Adhatoda schimperiana, anti-inflammatory, bronchodialator***

INTRODUCTION

Bronchial asthma is a disease characterized by increased responsiveness of the trachea, bronchi and bronchioles to 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 β_2 -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 β_2 receptor agonists, antimuscarinics, and anti-inflammatory therapy with corticosteroids and administration of oxygen if necessary (2).

A number of bronchodialatory compounds from plant origin are being used for the treatment of asthma. Typical examples of these compounds are theophilline and ephedrine. Many medicinal plants including A. shimperiana have been documented as remedy for the treatment of asthma in the Ethiopian traditional medicine (4). A. shimperiana (Hochst) Nees Acanthaceae, Synonym Justicia shimperiana (Nees in DC) locally known as Sensel or Simiza (Amharic), Dhumuga (Oromifa) is a popular folk medicine, extensively used in the treatment of respiratory diseases such as asthma (4, 5). The plant is also reported to be a remedy for malaria and Leishmaniasis and has demonstrated activity against HIV 1 and 2 subtypes (5-7). A. shimperiana is commonly found in villages and towns growing

Department of Drug Research, Ethiopia Health and Nutrition Research Institution, P.O. Box 1242 or 5654, Addis Ababa e-mail:ddr@ethionet.et, Fax: 0112754744 or 0112757722

on wastelands, as a hedge or as live fences around native homesteads (8). Despite the widespread use of A. shimperiana by the Ethiopian population for the treatment of respiratory diseases, there is little information about its pharmacological properties. As the isolated guinea-pig trachea is a well-accepted pharmacological model for the study of bronchospasmolytics, the effect of hydromethanolic extract of leaves A. shimperiana on guinea-pig respiratory smooth muscle was studied (9). The effect of the hydromethanol extract was also evaluated on carageenin-induced acute inflammation of the rat hind paw edema method and acute toxicity studies of the extract were investigated on mice.

MATERIALS AND METHODS

The leaves of A. schimperina were collected from Kebena, Addis Ababa, Ethiopia. Voucher specimens have been deposited at the Herbarium of Department Drug Research, Ethiopian Health and Nutrition Research Institute. Fresh dried leaves were macerated and extracted with methanol for 24 hours at room temperature. The extracts were filtered and dried under reduced pressure at a temperature not higher than 40°C. The material obtained was divided in aliquots, which were stored at 4^0C until further use.

Acetycholine bromide (Riedl-de Haen 64241), Acetylsalicylsaeure (Bayer AG A3-05-6), Histamin dihydrochloride (Fulka, 53300) and Salbutamol phosphate BP [(Sal/007/2003), Harmesh Chemicals, Khandelwal Lab. Ltd. Dadha] were used and the other chemicals were from Sigma Chemical Co., St. Louis, USA. Extracts were either directly dissolved in Krebs solution or in distilled water.

Male guinea pigs weighing 250-350 gram and bred in the animal house of the Ethiopian Health and Nutrition Research Institute were used. The guinea pigs were killed by sharp head blowing and cutting the neck blood vessel. The trachea was gently and rapidly dissected and cut transversely between the segments of cartilage so as to give a number of rings of tracheal muscle. All fat and connective tissue were removed from the trachea. Six tracheal rings were tied together with cotton string so as to form a chain, and were mounted in an organ bath containing 20 ml of Krebs solution (composition in mM: NaCl 120, KCl 4.77, $CaCl_2$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11) which was maintained at pH 7.4, 37°C bubbled with 5%CO_2 in oxygen (10,11). The tissues were allowed to equilibrate under a resting tension of 0.5 gram for 1 hour (12). The isotonic contractions were recorded on a slow moving smoked Kymograph paper using a frontal writing lever.

Tissues were contracted with acetylcholine (10^{-5}M) or histamine (10^{-5}M). Once the response to the contractile agents had reached a plateau (100% contraction), experimental tissues were incubated during 10 minutes with various doses of hydromethanolic extract of A. schimperiana, salbutamol, atropine, theophyline and ephedrine while control tissues were pre-incubated with vehicle (saline solution).

Isometric contractions were measured by model 7E Polygraph Recorder (Grass Instrument Co., Quincy, USA) using an FT-l0 sensor (CB-Sciences, Dover, NH, USA) connected and processed by an ATH-260 BioAmplifier. Responses to the test extract and standard drugs were expressed as percentages of the maximum relaxation of contracted tissues induced by acetylcholine or histamine. The EC_{50} value (the effective concentration causing 50% of maximum relaxation) was derived from the resulting dose-response curves. Each experimental group consisted of four to six tracheal preparations.

The effect of the hydromethanol extract of A.schimperiana on carageenan-induced acute inflammation was evaluated by the rat hind paw edema method (13). Male rats weighing 100-150 grams, divided into five groups (n=5) were pretreated with hydromethanol extract of A. schimperiana, standard drug, and distilled water (vehicle) 1 hour before receiving the injection of 0.5ml of 2% carageenan in normal saline solution into the sub-plantar area of the right hind paw. Control animals received an equivalent volume of acetylsalicylic acid (200mg/kg) and vehicle. The volume of the paw was determined with a plethysmometer (model 7140, Ugo Basile, Italy), at 1, 2, 3, and 4 hrs after carrageenan.

Oral and interaperitoneal acute toxicity studies of the extract were performed on mice (14). Albino mice of either sex weighing 20-25 grams were divided in to five groups (n=5 per group). Each group was treated with vehicle (distilled water) or various doses of the hydromethanol extract of A. schimperiana (30, 100, 300, 1000, 1500 mg/kg) intraperitoneally in single doses. Similar groups of mice were treated with vehicle or various doses of the hydromethanol extract of A. schimperiana orally in single dose. The animals were observed continuously for 6 hours with a gap of 30 minutes to determine various changes in the autonomic and behavioral responses. They were kept under further observation for a period of 48 hours for mortality (15).

All experiments were repeated at least three times. The results were expressed as means ± SEM and were analyzed by ANOVA. $P < 0.05$ was used as the significance level.

RESULTS

Acetylcholine and histamine induced a substantial contraction of the guinea pig tracheal chain at a concentration of 10^{-5} M and the tissue returned to the baseline after washout with Krebs solution.

The hydromethanol extract of A. schimperiana relaxed the contractions induced by acethylcholine and histamine in a dose dependent manner at concentrations of 0.8, 2, 4, 6 and 10 mg/ml giving an EC_{50} of 4.66 mg/ml and 5.92 mg/ml, respectively (Fig. 1 and 2). Salbutamol also showed a concentration dependent relaxation of the tracheal chains pre-contracted with both acetylcholine and histamine (Fig. 3 and 4). Similar dose-dependent relaxation was observed by atropine on tracheal chains pre-contracted by acetylcholine, the response is less potent compared with salbutamol (Fig. 5). Tracheal chains pre-contracted by acetylcholine were also observed to be inhibited by theophyline and ephedrine (data not shown).

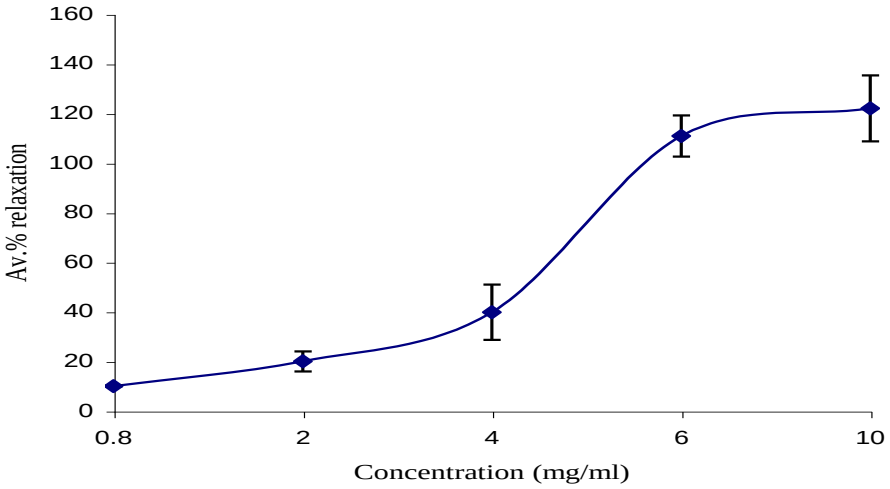

Fig.1. Effect of the extract on acetylcholine induced contraction of the isolated guinea pig tracheal chains. Symbols represent the mean ± S.E.M of n-3-5 preparations per group.

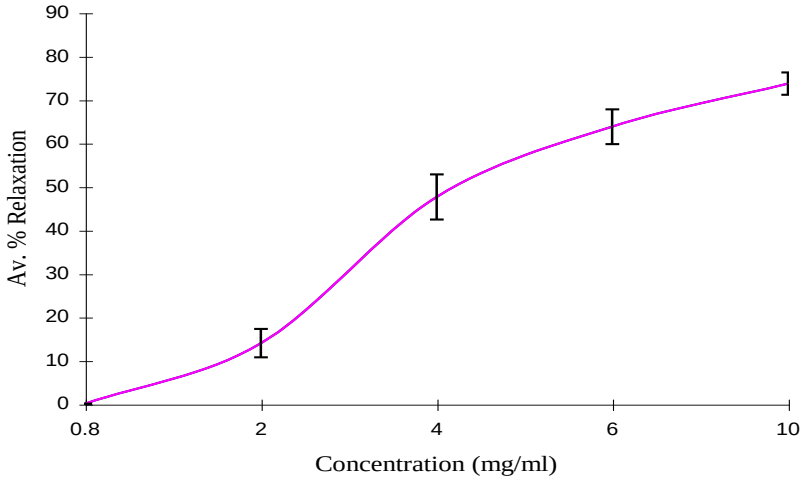

Fig.2. Effect of the extract on histamine induced contraction of the isolated guinea pig tracheal chains. Symbols represent the mean ± S.E.M of n-3-5 preparation per group.

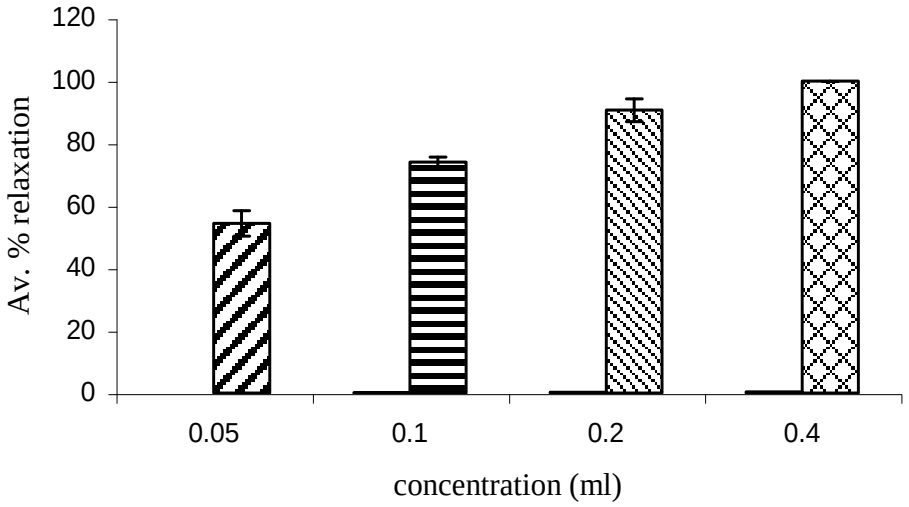

Fig.3. Concentration dependent relaxation effect of salbutamol on acetylcholine pre-contracted isolated guinea pig tracheal chains. Symbols represent the mean ± S.E.M of n-3-5 preparation per group.

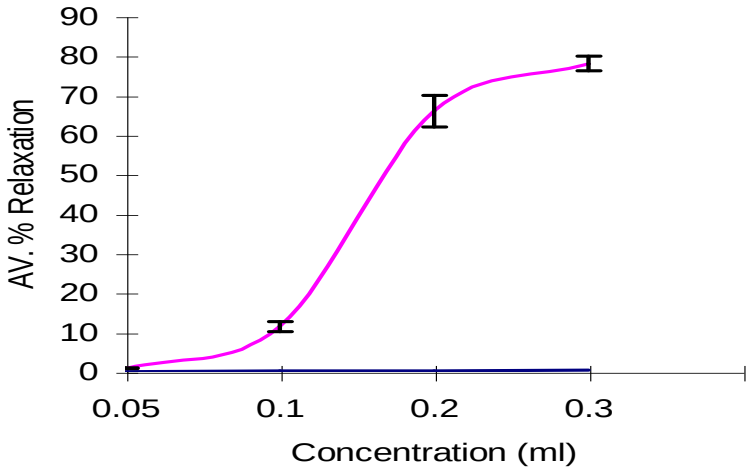

Fig.4. Concentration dependent relaxation effect of salbutamol on histamine pre-contracted isolated guinea pig tracheal chains. Symbols represent the mean ± S.E.M of n-3-5 preparation per group.

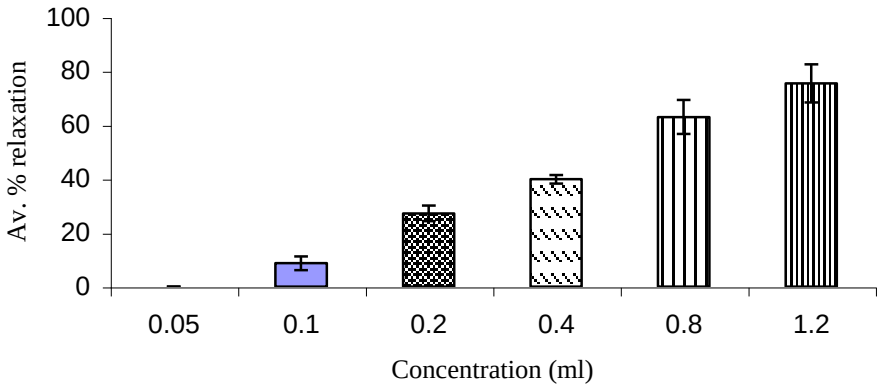

Fig.5. Concentration dependent relaxation effect of atropine on acetylcholine pre-contracted isolated guinea pig tracheal chains. Symbols represent the mean ± S.E.M of n 3-5 preparation per group.

Oral administration of the hydromethanol extract of A. shimperiana exhibited no visible sign of toxicity and mortality up to a dose of 1500 mg/kg bodyweight. However, a dose of 1750 mg/kg caused 100 % mortality when the extract was administered through the intraperitoneal route. Noticeable behavioral changes were observed after the lethal dose such as, depression (decrease in motor activity), decrease in respiratory rate, body tremor, lethargy, and pilocarpism. The acute median lethal dose (LD_{50}) of the extract was found to be 1286.76 mg/kg with 95 % confidence limit of 1161.9 – 1418.0. The plant extract therefore presents a rather low acute toxicity (Fig. 6).

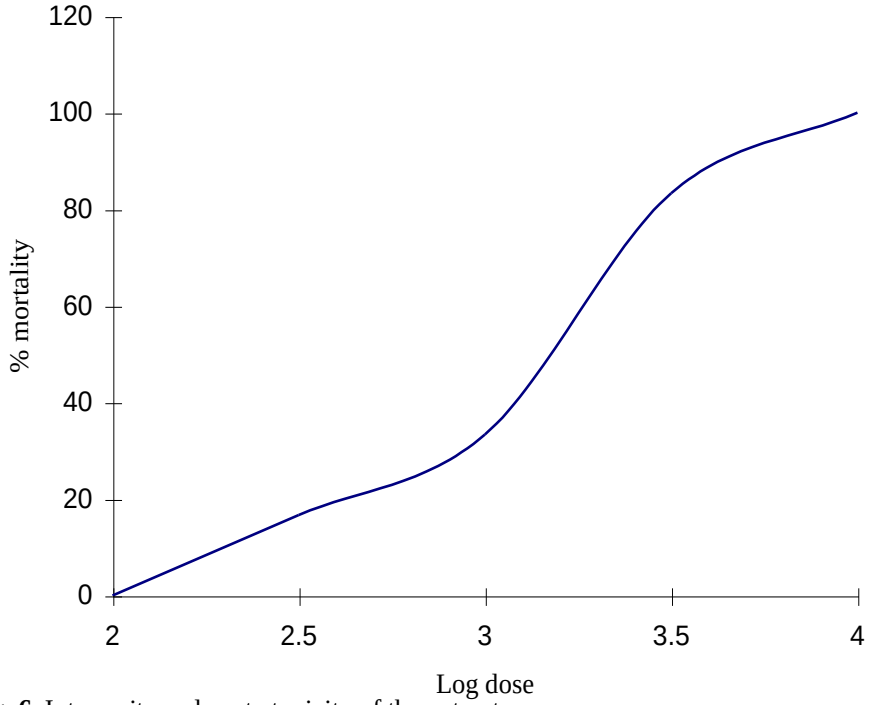

Fig. 6. Intraperitoneal acute toxicity of the extract

Anti-inflammatory activity was assessed in terms of decrease in the linear circumference of the treated rat paw relative to the value at zero time. Hydromethanolic extract of A. schimperiana showed a dose dependent anti-inflammatory activity at the three doses tested, 200mg/kg, 400mg/kg and 800mg/kg. The paw edema was inhibited by 12.16 %, 14.83 % and 16.66 % respectively, four hours after carageenan injection (Fig.7 which was statistically insignificant ($P > 0.05$). On the other hand Asprin at a dose of 200mg/kg showed a significant reduction ($P < 0.05$) compared to the control with 38 .01 % edema inhibition after four hours of carageenan injection (Fig.7).

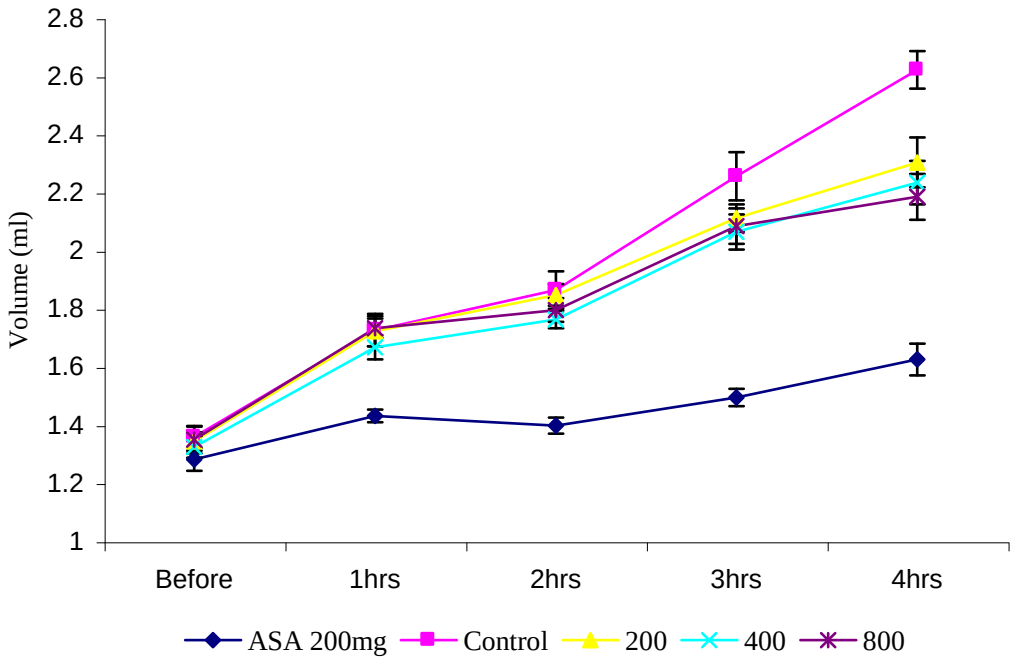

Fig 7. Dose dependent effect of the extract on carrageenan–induced paw edema in rats. Symbols represent the mean ± S.E.M of reading per group n-5.

DISCUSSION

The extract of A. schimperiana inhibited contractions induced by histamine and acetylcholine. These agents are implicated in various ways in the pathogenesis of asthma. Histamine is the most implicated mediator in bronchoconsitriction that accompany asthma (16).

Acetylcholine on its own can cause bronchoconstriction by activating different cholinergic fibers secondary to the stimulation of the sub-epithelial afferent fibers by inflammatory mediators such as histamine (17). The results of this study indicated that the hydromethanolic extract of the leaves of A. schimperiana relax significantly the tracheal muscle strips pre-contracted by acetylcholine similar to the antagonists, salbutamol and atropine. The extract probably acts by blocking the muscarinic receptors. Acetylcholine binds with the muscarinic receptors for its agonistic action in smooth muscle, where as antagonists compete with acetylcholine for a common binding site on muscarinic receptor of guinea-pig ileum preparation (18). Moreover, the results show that the effect of the extract is reversible since it does not modify the effect of acetylcholine tested after washing the extract from the preparation indicating the reversibility of its receptor binding.

The ability of the hydromethanolic extract of A. schimperiana to inhibit the contractions induced by the bronchoconstrictors, acethycoline and histamine suggests a possible role in the treatment of asthma. The inhibition of histamine-precontracted trachea by the hydromethanolic extract of A. schimperiana indicates the involvement of β_2-agonists on the relaxation of the tissue. From these findings it can be assumed that more than one mechanism of actions could be

involved in the relaxation of pre-contracted tissue by the hydromethanol extract of A. schimperiana. Furthermore, the relaxation of histamine pre-contracted trachea by the A. schimperiana indicates their potency in ameliorating established asthma. Airway responsiveness in asthma is attributed in part to changes in autonomic regulation particularly increased parasympathetic activity (19).

Airway obstruction/bronchoconstriction or airway hyperresponsiveness in asthma are believed to be a direct consequence of airway wall inflammation (20,21). The hydromethanolic extract of A. schimperiana exhibited a moderate degree of anti-inflammatory activity. The component(s) inhibiting the contractions induced by acetylcholine and histamine seem to be highly polar in nature since they were extracted by water-methanol, a very potent polar medium.

Mechanisms that possibly underlie this anti-inflammatory activity include inhibition of the actions of inflammatory mediators such as histamine, effect on adernocorticoid hormone and immunosuppression. Inhibition of immunosuppression also incorporates inhibition of the activity of proinflammatory mediators. This proposed mechanism is consistent with previous findings that anti-inflammatory plant principles have shown to act through control of adrenocorticoid hormone and immunosuppression, respectively (22,23).

Inherent anti-inflammatory activity is a desirable property of a putative anti-asthmatic agent, since asthma is a complex chronic inflammatory disease of the airways. The fact that the hydromethanol extract of A. schimperiana showed the anti-inflammatory activity may justify the local extracting method of macerating the leaves in local beer which is mainly ethanol.

Sequel to the above, it is apparent that the mechanism of anti-asthmatic property of the leaf extracts of A. schimperiana is multifaceted: inhibition of spasmogens and immunosuppression may be involved. The bioactive component(s) responsible for the observed activities is not precisely known but it may be one or more of the phytochemical constituents established to be present in the leaf extracts. The potential application of other Adhatoda species for treatment of asthma has been reported; Adhatoda vasica is used for the treatment of asthma in Indian Ayurveda (the plant contains the alkaloids, vasicine, vasicinol and vasicinone, which illicit bronchodialating effect) (24,25).

In the acute toxicity study of the methanol extract of leaves of A. schimperiana no deaths and no other signs of toxicity was observed at up to the highest dose tested (1500 mg/kg bodyweight) when administered orally. Since the extract had shown to be pharmacologically active, one can conclude that the active substances(s) presents rather low acute toxicity. Taking into account that the ED_{50} value and that no deaths were observed up to 750 mg/kg body weight, the safety margin (ALD/ED_{50}) seems to be wide (>276.13 and 217.35) for the extract. Besides the fact that ALD values following intraperitoneal administration were high, where human exposure to hydromethanolic extracts of A. schimperiana leaves is very unlikely to occur.

The results of this study showed anti-inflammatory activity, a relatively potent relaxant (bronchodialatory) effect of A.schimperiana on the tracheal chain of the guinea pig and low toxicity. These activities justify the traditional use of this plant in the treatment of bronchoconstrictive diseases. Detailed investigation of the efficacy, isolation of the active ingridient and further toxicity study may warrant the development of the plant extract in to proper drug.

REFERENCES

1. Neal M.J. Medical pharmacology at a glance. 3rd ed., UK, 2001:pp28
2. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of Solanum Xanthocarpum and Solanum trilobatum in bronchial asthma. *J Ethnopharmacol*, 1999; 66:205-10.
3. Soares de Moura R, Costa SS, Jansen JM, Silva CA, et al. Bronchodialaor activity of Mikania glomerata Sprengel on human bronchi and guinea-pig trachea. *J Pharm Pharmacol*, 2002;54: 249-256.
4. Abebe D, Debella A, Kelbessa U. Medicinal Plants and Other useful plants of Ethiopia. Camerapix Publishers International, Nairobi, Kenya, 2003: 103
5. Fichtl R, Adi A. Honeybee flora of Ethiopia. Edwards S, Kelbessa E (eds). Margraf Verlag: Weikerheim, Germany, 1994
6. Jansen PCM. Spices, condiments and medicinal plants in Ethiopia: Their taxonomy and agriculture significance. Center for Agricultural Publishing and Documentation: Wageningen, The Netherlands, 1981
7. Asres K, Bucar F, Kartnig T, Witvronw M, Panneconque C, De Clercq E. Antiviral Activity Against Human Immunodeficiency Virus Type 1(HIV-1)

and Type 2(HIV-2) of Ethnobotanically Selected Ethiopian Medicinal Plants. Phytoth. Res. 2001; 15: 62-9.

8. Getahun A. Some common medicinal and Poisonous plants used in Ethiopian folk medicine. Addis Ababa University Ethiopia, 1976.
9. Gilani AH. Bronchodilator, spasmolitic and calcium anthagonist activities of Nigella Sativa seeds (kalonji): a traditional herbal product with multiple medicinal use. *J Pak Med Assoc*, 2001; 51:115-20.
10. Mans DRA, Doelsie J, Mohan S, et al. Spasmolitic effect of a Solanum melongena leaf extract on guinea pig tracheal chains and its possible mechanism(s). *J Ethnopharmacol*, 2004; 95:329-33.
11. Hore SK, Ram Ottalwar, Koley KM, Pathak AK. Possible involvement of colinergic and adrenergic mechanisms in changing contractility of guinea pig ileum by Ipomoea Carnea. *J Ethnopharmacol*, 2000; 71:253-59.
12. Dar A, Channa S. Calcium antagonistic activity of Bacopa monniera on vascular and intestinal smooth muscles of rabbit and guinea pig. *J Ethnopharmacol*, 1999; 66: 167-174.
13. Okoli CO, Akah PA. A pilot evaluation of the anti-inflammatory activity of Culcasia scanders a traditional antirheumatic agent. *J Altern Complement Med*, 2000;6:423-427.
14. Kennedy GL, Ferenz RL, Burgess BA. Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD_{50}. *J Appl Toxicol*, 1986; 6:145-48.
15. Shankar K, Pathak NKR, Trividi VP, Chansuria JPN and Pandey VB. An evaluation of toxicity of Taxus baccata Linn (Talispatra) in experimental animals. *J Ethnopharmacol*, 2002; 79: 69-73.
16. Summers R, Sigler R, Shelhamer JH, Kaliner M. Effects of infused histamine on asthmatic and normal subjects: comparison of skin test responses. *J Allergy Clin Immuno*,1981; 67:456-464.
17. Flenley DC. Respiratory Medicine, 2nd ed., Bailliere Tindall, UK, 1990: pp.134-161.
18. Brown JH, Taylor P. Muscarinic receptor agonists and antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon, RW, Gilman AG.(Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed. McGraw-Hill Publication, New yourk, 1996: pp.141-60.
19. Rang HP, Dale MM. Textbook of Pharmacology,1st ed. Longman Group UK Ltd., 1988: p.302.
20. Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature*, 1999; 402:12-17.
21. Prasad MR, Baliekar RH, Rao AR.Recent perspectives in the design of antiasthmatic agents. *Pharmazie*, 2000; 55:475-482.
22. Barik BR, Bhowmik T, Dey AK, et al. Pemnazole, an isoxazole alkaloid of Premna integrifolia and Gmelina arbora with anti-inflammatory activity. *Fitoterapia*, 1992; 53:295-299.
23. Singh S, Bani S, Singh GB, Gupta BD, Banerjee SK, Singh B. Anti-inflammatory activity of Lupol. *Fitoterapia*, 1997; 68:9-16.
24. Dhuley JN. Antitussive effect of Adhatoda Vasica extract on mechanical or chemical stimulation-induced coughing in animals. *J Ethnopharmacol*, 1999; 67: 361-365.
25. Amin AH, Mehta BR. Bronchodialotor alkaloids (Vascinone) from adhatoda vasica. *Nature*, 1959; 184:1317.