



EVALUATION OF THE ANTI-ASTHMATIC ACTIVITY OF AZIMA TETRACANTHA LAM LEAVES IN ANIMAL MODEL

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ABSTRACT

Asthma is a chronic disease that affects approximately 300 million people worldwide. Although wide range of drug is available, the relief provided by them is mainly symptomatic and short lived. Moreover, the side effects of these drugs are also quite disturbing. Hence, a continuous search is on going to identify effective and safe remedies to treat bronchial asthma. Ayurveda is a great Indian tradition and have an important role in discovery of new medicines. There are many natural herbs that can be used for asthma, treatment. Azima Tetracantha Lam is traditionally used to treat asthma. Although these plants possess diverse pharmacological actions, the scientific data on anti-asthmatic actions of this plant has got little attention. An attempt has been made to evaluate antiasthmatic activity of Azima Tetracantha Lam of aqueous and alcoholic extracts of medicinal plant. Different groups of guinea pigs (350-500 g) of either sex were subjected to acetylcholine (n = 5) and histamine-induced (n = 10) airway constriction. AQEAT and ALEAT (200 and 300 mg/kg) showed potent bronchodilator activity on histamine-and acetylcholine-induced airway constriction in guinea pigs. These extract has shown the sub-effective dose produced at 100, 200 and 300 mg/kg in all the models. The LD50 of the Aqueous and Alcoholic extracts at a dose of 100, 200 and 300 mg/kg are found to be 2262.7 and 1131.4 by oral route. These LD50 values fall within the practically non-toxic range. The present study for the first time indicates anti-asthmatic, acute, sub-acute studies of Azima Tetracantha Lam medicinal plants, confirming their traditional claims. The anti-asthmatic action of Azima Tetracantha Lam could be due to the inhibition of the synthesis, release or action of histamine and acetylcholine effect.

Key Words: Antiasthmatic, Azima Tetracantha Lam, Histamine-induced model and Acetylcholine induced model.

INTRODUCTION

Asthma is a disease of the lung's airways. It affects 155 million individuals in the world. Its Prevalence and severity among children have increased significantly in the world over the past 40 year. Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world¹. The plant is referred to as 'Jivanti' in Ayurvedic text and considered to be Rasayana (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body³. Ethno medicinally the leaves and seeds are used in asthma and cough². The major therapeutic claim is its galactagogue action, which has been proved in rats along with the antimicrobial⁴, anticarcinogenic^{5,6} and hepatoprotective properties of plant^{7,8} in traditional system of medicine leaves of *L. reticulata* (Retz) Wight & Arn are mainly used for the treatment of cough, asthma, rheumatism⁹. Many asthma attacks are triggered by allergens, such as dust, mould spores, mites, animal hair or feathers but the onset may equally be caused by cold air, or it may be preceded by an infection such as a cold. Certainly, stress and more

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specifically acute anxiety are known to be the immediate trigger for many attacks, and this can sometimes give rise to a vicious circle of asthma - anxiety about the asthma - further attacks. Thus a wide range of etiological factors can be involved in this all too common problem ¹⁰.

A number of different groupings can be applied:

Extrinsic asthma - caused by allergic responses to house dust, animal fur, or various foods. Such causes 10-20% of adult asthma.

Intrinsic asthma - caused by genetics, structural problems, infections, pollutants and stress - both physiological and psychological. Such causes 30-50% of adult asthma. The symptoms of people with asthma differ greatly in frequency and degree. Some have an occasional episode that is mild and brief; otherwise they are symptom free. Others have mild coughing and wheezing much of the time, punctuated by severe exacerbation's of symptoms following exposure to known allergies, viral infections, and exercise or nonspecific irritants. A series of stages have been characterized for describing the severity of an acute asthma attack.

Medicinal Plants used in asthma: Asthma is a global problem. Many synthetic drugs are used to treat acute symptoms of asthma, but they are not completely safe for long term use. Hence search has been started once again to look back to traditional medicine which can be used to treat asthma.

Azima tetracantha (lam) is a low, spinouts, highly branched bush, woody below but with pale green, herbaceous, almost quadrangular young branches. The leaves are in opposite to sub-opposite, decussate pairs. They are shortly petiolate, about 2x4cm long, entire, elliptic, acute, sharply mucronate, rigid, pale green with an acute base. Usually, there are two laterally placed spines in the axil of a leaf. The spines which morphologically represent the first pair of leaves of the auxiliary shoot are about three cm long, more or less, triangular in cross section, very sharp and with an indurate apex. The plant is dioeciously. The flowers are borne in the axils of leaves. Generally, there is cymes of three flowers in the axil of a leaf which is the upper branches, especially of the male plants become greatly reduced or even completely suppressed.

MATERIALS AND METHODS

Collection, identification and authentication of plant materials

The leaves of *Azima Tetracantha Lam* were collected. The collected plant samples were deposited in the Department of Pharmacology, Suralabs, Dilsukhnagar, India for future reference.

Drugs and Chemicals

Histamine dihydrochloride, acetylcholine chloride, atropine sulfate, mepyramine meleate, carrageenan were purchased from Sigma (St. Louis, MO, USA). Histamine solution was freshly prepared in normal saline (NaCl, 8.5 g/l). All the other drugs were of analytical grade and they were dissolved in distilled water and desired concentration was prepared.

Extraction

Leaves of *Azima Tetracantha Lam* were washed with distilled water to remove dirt and soil, and shade dried. Routine pharmacognostic studies including macroscopic and microscopic observations were carried out to confirm the identity of the materials. The dried material of each plant was coarsely powdered (500 g) and defatted with aqueous (60-80°C), and then extracted separately with alcohol (95% v/v) in Soxhlet apparatus (Figure 4.5). The extracts were filtered, and concentrated by distilling off the solvents and evaporated to dryness using water bath to get crude ethanol extract. The yield values for aqueous extract of *Azima Tetracantha Lam* and alcoholic extract of *Azima Tetracantha Lam* were 20.48% and 10.96% w/w, respectively. Now these extracts were subjected to phytochemical screening and screened for possible pharmacological actions. For the pharmacological tests, the extracts were suspended in double distilled water containing carboxy methyl cellulose (CMC, 0.5% w/v).



Figure.1 Soxhlet apparatus for the extraction of different plant Extracts

Experimental animals

Antiasthmatic studies were conducted on guinea pigs (350-500 g) of either sex fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed in polypropylene cages (640 x 410 x 250 mm high) under standard conditions of temperature ($22 \pm 20^\circ\text{C}$), relative humidity ($60 \pm 5\%$). They were divided in groups of ten animals each. The saline fed group served as control and one group was treated with a standard drug.

Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum*, Animals receiving different doses of plant extracts were also observed for any alteration in their general behavior.

They were provided with standard rodent pellet diet (Amrut, Pranav Agro Industries Ltd, Baroda, Gujarat, India) and tap water *ad libitum* except the food was withdrawn 18–24 h before the experiment. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Habituation to animals

Guinea pigs were habituated to handling by holding them and injecting vehicle through oral/i.p. routes to minimize non-specific stress and to simulate the actual protocol conditions. Handling also consisted of weighing and restraining animals on platform for 1 min, and gently massaging on dorsal site as was done in the actual protocols. The same platform was used during drug administration. Moreover, the animals were familiarized with the diet in their home cage-environment and laboratory environments before subjecting them to the tests. All the experiments commenced 24 hours following the final habituation and were conducted according to the protocols mentioned below.

Acute toxicity test (Determination of LD50)

The acute toxicity tests (LD50) for the different plant extracts were determined according to the procedure described by Lorke (1983). The crude aqueous and alcoholic extracts were used for the test. Guinea pigs (300–350 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10, 100, and 1000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior. From the result of the above step, four different doses of 200, 400, 800, and 1600 mg/kg were chosen and administered (p.o.) respectively to four groups of one guinea pig per group. The treated animals were again monitored for 24 h. The LD50 was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

Preparation of plant extracts

Preparation of Aqueous Extract:

Fresh leaves of *Azima Tetracantha Lam* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to $80-100^\circ\text{C}$ for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Preparation of Alcoholic Extract:

Fresh leaves of *Azima Tetracantha Lam* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to $50-60^\circ\text{C}$ for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Phytochemical screening of aqueous and ethanolic extracts of *Azima Tetracantha Lam*

Aqueous and Alcoholic extracts were chemically tested to know the presence of different primary, and secondary metabolites present in them as per the following scheme:

Tests for sterol/steroids

Salkowski test

Few mg of the sample was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid and shaken. The development of red color in the chloroform layer indicates the presence of sterols/steroids.

Test for terpenoids

Liebermann–Burchard test

Few mg of the sample was dissolved in 1 ml of chloroform and few drops of acetic anhydride. Concentrated sulphuric acid was added by the side of the test tube. Production of purple color indicates the presence of triterpenoids and blue–green color indicates the presence of sterols.

Test for alkaloids

Few mg of the sample was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was then tested using following reagents:

Dragendorff's reagent

It is a solution of potassium bismuth iodide. It was prepared by dissolving bismuth nitrate (8 gm) in nitric acid (20 ml), and separately dissolving potassium iodide (27.2 gm) in water (50 ml), mixing the two solutions, and making up the volume to 100 ml. Above Dragendorff's reagent was sprayed on Whatman No. 1 filter paper then the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red color on the paper indicated the presence of alkaloids.

Mayer's Reagent

1.36 gm of mercuric chloride, and 3 gm of potassium iodide were dissolved in water to make 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream colored precipitate shows the presence of alkaloid.

Hager's reagent

It is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

Wagner's Reagent

It is a solution of potassium triiodide in water which was prepared by dissolving 1.3 gm iodine in a solution of potassium iodide (2 gm) in water to make 100 ml. Formation of brown precipitate after addition of this reagent in extract indicates the presence of alkaloids.

Test for tannins

Sample was taken separately in water, warmed, and filtered. Tests were carried out with the filtrate using following reagents:

Ferric chloride test

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue color shows the presence of tannins.

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate indicates the presence of tannins.

Test for flavonoids

Shinoda test

To the sample, 5 ml of 95% ethanol, few drops of HCl, and few magnesium turnings were added. The pink color shows the presence of flavonoids.

Test for carbohydrates

Molisch's test

About 0.1 gm of the sample was dissolved in 2 ml of water, and added 2-3 drops of 1% ethanolic solution of alpha naphthol, and then carefully poured 2 ml of concentrated sulphuric acid down the side of the test tube so that it forms a heavy layer at the bottom. A deep violet color is produced if carbohydrates are present.

Fehling's test

1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and boiled for 1 minute. Equal volume of sample was then added, heated in boiling water bath for 5-10 minutes. First a yellow then brick red color shows the presence of carbohydrates.

Benedict's test

Equal volumes of the reagent, and sample were added. Mixture was then boiled for five minutes. Depending on the reducing sugar present a range of colors develops.

Test for proteins

Biuret test

To the sample 4% NaOH, and few drops of 1% CuSO₄ were added. Violet or pink color indicates the presence of proteins.

Xanthoproteic test

Sample was mixed with 1 ml of concentrated sulphuric acid, formation of precipitate shows positive test.

Millon's test

3 ml of sample was mixed with Millon's reagent, formation of precipitate indicates the presence of proteins.

Test for saponins

Foam test

A few mg of the sample was shaken vigorously with water. Honeycomb like foam indicates the presence of saponins.

Heamolytic test

Sample was added to one drop of blood placed on glass slide. Hemolytic zone indicated the presence of saponins.

Test for anthraquinone glycosides

Borntrager's test

To 3 ml of the sample, dilute sulphuric acid was added, boiled, and flittered. To the filtrate equal volume of chloroform was added, and shaken. After separating the organic layer, ammonia was added. Turning pink of ammonical layer indicates the presence of said glycosides.

Test for cardiac glycosides

Keller-Kiliani test

The sample was dissolved in acetic acid containing trace amounts of ferric chloride. It was then transferred to the surface of concentrated sulphuric acid. A reddish brown ring will be formed at the junction, and the color slowly changes to blue.

Legal's test

A few drops of pyridine, and sodium nitroprusside were added to the sample, and made alkaline. A pink or red color is obtained.

Screening for antiasthamatic activity

Histamine-induced bronchospasm in guinea pigs

The guinea pigs fasted for 24 h were exposed to an atomised fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24 cm, made of perplex glass). Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia, and death. The time until signs of convulsion appeared is called pre-convulsion time (PCT/PCD). By observation experience was gained so that the preconvulsion time can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. In the present experiments the criterion used was time for onset of dyspnoea and percent protection was calculated. Those animals which developed typical histamine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in the histamine chamber. They were divided in groups of ten animals each. Mepyramine 8.0 mg/kg, i.p., and different doses of plant extracts or polyherbal formulations intraperitoneally were administered 30 min prior to exposure. Animals, which did not develop typical asthma within 6 min, were taken as protected.

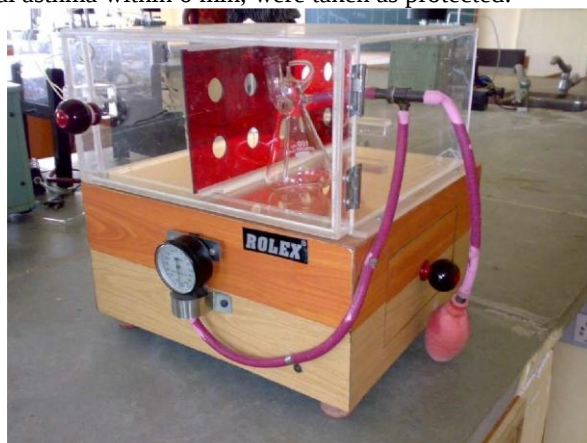


Figure.2 Histamine chamber for screening of anti-histamine and anticholinergic properties of drugs

Acetylcholine-induced bronchospasm in guinea pigs

The guinea pigs fasted for 24 h were exposed to an atomised fine mist of 0.5% acetylcholine aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24 cm, made of perplex glass) (Figure). Guinea pigs exposed to acetylcholine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia, and death. The time until signs of convulsion appeared is called pre-convulsion time (PCT/PCD). By observation experience was gained so that the preconvulsion time can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. In the present experiments the criterion used was time for onset of dyspnoea and percent protection was calculated. Those animals which developed typical acetylcholine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in the histamine chamber. They were divided in groups of ten animals each. Atropine sulphate 2 mg/kg, i.p., was taken as standard and different doses of plant extracts intraperitoneally were administered 30 min prior to exposure. Animals, which did not develop typical asthma within 6 min, were taken as protected.

Data analysis

The data are presented as mean $\pm$ S.E.M. Statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Dunnett's *t*-test or Chi-square test with *Yate's* correction factor. Differences were considered significant at $P < 0.05$.

Extraction

Dried leaf powder of Azima Tetracantha Lam 500 gm) were extracted with water and alcohol (95% w/v) to get concentrated aqueous and alcoholic extract. The yields of extracts were as follows: AQEAT (20.48% w/w), ALEAT (11.45% w/w).

Acute toxicity test (Determination of LD50)

The LD50 of the AQEAT and ALEAT was calculated to be 2262.7 and 1131.4mg/kg by oral route. During the acute toxicity study, animals were observed for gross behavioral and morphological changes (respiratory distress, immobility, convulsion, loss of righting reflex etc.). Based on the results of acute study, doses were then selected. The plant extracts did not produce any significant changes in the normal behavior of the animals, and no toxic symptoms were seen at the dose levels studied.

Phytochemical screening of different plant extracts

Table ----- depicts results of screening of different plant extracts for various phytochemical constituents. AQEAT showed the presence of alkaloids, proteins, anthraquinone glycosides, flavonoids, saponins, sterols, tannins, and carbohydrates. ALEAT was found to contain alkaloids, flavonoids, saponins, steroids, and tannins.

Table.1 Phytochemical tests

Compounds	Name Of The Test	Leaf Extracts	
		Aqueous	Alcoholic
Alkaloids	Dragendorff's test	+	-
	Mayer's test	-	+
	Wagner's test	-	-
	Borntrager's test	+	+
Anthraquinone	Modified Borntrager's test	-	-
	Molisch's test	-	+
Carbohydrates	Anthrone reagent test	+	+
	Benedict's reagent test	+	+
Cardiac Glycosides	Kellar Kilani test	-	-
	Potassium hydroxide test	-	-
Flavonoids	Shinoda test	+	-
	Ammonia test	-	+
Glycosides	Benodict's test	+	-
	Fehling's test	-	-
Phenols	Ferric chloride test	-	-
	Phosphomolybdic acid test	-	-
Phlobatannins	Hydrochloric acid test	-	-
	Xanthoprotein teswt	-	-
	Biurete test	-	-
Proteins	Million's reagent test	+	-
Quinines	Sodium hydroxide test	-	-
Reducing sugar	Benedict's reagent test	-	-
Resins	Turbidity test	+	+
Saponins	Foam test	+	+
Steroids/Terpenoids	Salkowski test	+	+
	Lieberman-Burchard test	-	-
Tannins	Bramer's test	+	+

Histamine-induced bronchospasm in guinea pigs

The results are summarised in Tables. Histamine aerosol produced bronchoconstriction in all control group animals. All control animals showed convulsion during the first 3 min of the experiment. The aqueous and alcoholic extracts were significantly protected animals ($P < 0.01$) from histamine-induced bronchoconstriction. Prior treatment of AQEAT (200, and 300 mg/kg, i.p.) offered significant protection to guinea-pigs against histamine aerosol-induced bronchospasm ($P < 0.01$). ALEAT at the doses 200, and 300 mg/kg, i.p. significantly protected guinea-pigs against histamine aerosol-induced bronchospasm ($P < 0.01$). Mepyramine (a standard anti-histaminic drug, 8 mg/kg, i.p.) significantly protected 90% of animals from asphyxia ($P < 0.001$). However,

prior treatment with AQEAT (100 mg/kg), and ALEAT (100 mg/kg) could not offer protection from the development of asphyxia produced by histamine aerosol.

Table.2 Effect of aqueous and alcoholic extracts of Azima Tetracantha Lam leaves on acetylcholine-aerosol-induced bronchospasm in guinea pigs

Treatment	Dose (mg/kg, i.p.)	Protection (%)
Control	Saline, 1ml/kg	-
Mepyramine	8	90
AQEAT	100	62
	200	83
	300	87
ALEAT	100	66
	200	82
	300	85

*Values are mean $\pm$ S.E.M. (n = 5); nsNot significant, **P < 0.01 vs. control; Dunnett's t-test after One-way ANOVA.

Acetylcholine-induced bronchospasm in guinea pigs

The results are summarized in Table. Acetylcholine aerosol produced bronchoconstriction in all animals of control group. The aqueous and alcoholic extracts were significantly protected animals (P < 0.01) from acetylcholine-induced bronchoconstriction. Prior treatment of AQEAT (200, and 300 mg/kg, i.p.), and ALEAT (200 and 300mg/kg, i.p.) has offered significant protection (P < 0.01) to guinea-pigs against acetylcholine aerosol-induced bronchospasm. AQEAT (100 mg/kg, i.p.), and ALEAT (100 mg/kg, i.p.) could not offer protection from the development of asphyxia produced by acetylcholine aerosol. Atropine sulfate (a standard anti-muscarinic drug, 2 mg/kg, i.p.) significantly prolonged pre-convulsion time to 460 ± 4.33 sec (P < 0.01) and protected animals from asphyxia.

DISCUSSION

In this study, an attempt has been made to evaluate antiasthmatic activity of Azima Tetracantha Lam in the experimental animals. The results of the study indicated that antiasthmatic effect has shown significant protection of Azima Tetracantha Lam against Histamine and Acetyl Choline induced in rodents.

The acute toxicity was determined by the method of Lorke (1983), which utilizes less number of mice and provides a 24-h LD50 value, which is adequate for most practical purposes. The LD50 values for AQEAT were found to be 2262.7 mg/kg, p.o. The LD50 values ALEAT were found to be 1131.4 mg/kg. These LD50 values fall within the practically non-toxic range (Loomis, 1978). Based on these results, doses of 100, 200, 300 mg/kg of different plant extracts were selected for various animal models. The antiasthmatic action was evaluated by sub-effective doses of Azima Tetracantha Lam extracts.

Histamine and acetylcholine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of bronchospasm induced by histamine and acetylcholine, respectively (Broadbent & Bain, 1964). The results of the present study showed that prior treatment of aqueous and alcoholic extracts of Azima Tetracantha Lam protected the animals to a significant extent from the development of asphyxia produced by both the spasmogens. This is indicative of antihistaminic and anticholinergic activities of the Azima Tetracantha Lam. Interestingly, the effect was significantly higher in both aqueous and alcoholic plant extracts when compared to control.

In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Moreover, the resemblance of pulmonary responses and anaphylactic sensitization to histamine challenge in both guinea pigs and humans made this species the model of choice. Although there is no perfect model of asthma which simulates asthmatic patients, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans (Popa et al., 1973; Agrawal et al., 1991). Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to α -adrenergic antagonists (Matsumoto et al., 1994). Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release.

The role of histamine and acetylcholine in asthma is well established (Nelson, 2003). In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (Bosquet et al., 2000). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like α -adrenergic agonists, xanthine derivatives, and anticholinergics are used as quick relief medications in such acute asthmatic attacks (Horwitz & Busse, 1995).

In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Mepyramine (8 mg/kg, i.p.) and atropine sulphate (2 mg/kg, i.p.)

were used as reference standard against histamine and acetylcholine-induced bronchospasm respectively (Shah & Parmar, 2003). The Azima Tetracantha Lam have shown significant bronchoprotection against both the types of spasmogens as compared to control. AQEAT and ALEAT showed significantly protection from histamine-induced bronchoconstriction and anticholinergic action in the acetylcholine-induced bronchoconstriction. Ayurveda has recommended a number of plants for the treatment of asthma and other allergic disorders and has been successful in controlling the disease as well (Zhimmiet & Tashkin, 2000). Large numbers of medicinal plant preparations have been reported to possess bronchodilatory effects; some of these include *Adhatoda vasica* (Amin & Mehta, 1959), *Benincasa hispida* (Kumar & Ramu, 2002), *Azima Tetracantha Lam* Tripathi & Das, 1977), *Cissampelos sympodialis* (Thomas et al., 1997), and *Sarcostemma brevistigma* (Saraf & Patwardhan, 1988). Phytoconstituents like alkaloids and flavonoids are attributed to possess bronchodilatory activity (Amin & Mehta, 1959; Saraf & Patwardhan, 1988). Qualitative phytochemical investigation of ethanolic extracts which are present as ingredients in the aqueous and alcoholic extracts of *Azima Tetracantha Lam*, have shown presence of these constituents. Therefore, the results of present investigation suggested that, leaves extracts have significant bronchodilatory activity.

SUMMARY

1. The fresh leaves of *Azima Tetracantha Lam* used for this project work were supplied.
2. The dried leaves of *Azima Tetracantha Lam* were successively extracted with alcohol and water. Percentage yield was calculated.
3. The dose was given for Guinea pigs is 100,200 and 300mg/kg.
4. Therapeutic dose of the extracts were calculated after carrying acute toxicity studies in Guinea pigs.
5. Extracts were tested for their anti-asthmatic activity in Guinea pigs.
 - By Histamine induced bronchospasm
 - Both aqueous and alcoholic extracts (200 and 300mg/kg) showed significant protection from the development of asphyxia produced by the spasmogens.
 - By Acetylcholine induced bronchospasm
 - Both aqueous and alcoholic extracts (200 and 300mg/kg) showed significant protection from the development of asphyxia produced by the spasmogens.

CONCLUSION

Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. The therapy of asthma usually employs steroids or disease modifying drugs. The long-term use of these, however, may not limit the disease progression. All of these drugs have side effects and the search for a novel anti-asthmatic drug continues. There is a need to identify effective and safe remedies to treat bronchial asthma. The herbal medicines are rich for the treatment of asthma, selected plant might prove to be the most potent for the treatment of asthma and the side effects of the allopathic treatment can be reduced. For this purpose, *Azima Tetracantha Lam* has been used as ayurvedic medicine to cure asthma and other disease.

The present study demonstrates the potent mast cell stabilizing activity, anti-histaminic activity, anti-cholinergic activity of leaves of *Azima Tetracantha Lam* medicinal plants in different models of asthma thereby indicating the possibility of developing a herbal drugs as cheaper, safer and potent anti-asthmatic therapeutic agent. The AQEAT and ALEAT have produced protective effect against asphyxia. The selected plant species have been used in the traditional systems of medicine for treating various ailments. Importantly, there was no scientific evidence for the anti-asthmatic activity of these plants.

The present study validates traditional claims of these plants. Although the results from this study are quite promising for the use of *Azima Tetracantha Lam* as a medicinal agent for asthma, several limitations exist in the current literature. Further studies are suggested to establish the antiasthmatic activity by conducting clinical trials and also to isolate and characterize the active principle/s responsible for the action.

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