Antiasthmatic Activity of *Leptadenia reticulata* (Retz) Wt & Arn leaves

Jagdish Baheti and Sandip Awati

**Abstract**—The present investigation was aimed to investigate the possible anti-asthmatic activity of hydro alcoholic extract of leaves *Leptadenia reticulata* (Retz) Wight & Arn (LRLHE). The extract is evaluated for its anti-asthmatic activity in Guinea pig ileum, tracheal chain and rat ileum preparation, compound 48/80 induced mast cell degranulation, passive cutaneous anaphylaxis in rats and HPTLC analysis of isolated sapogenin fraction from the plant against β-sitosterol as standard marker. LRLHE exhibited a significant (P<0.05, P<0.01) anti-asthmatic activity with the doses of 100, 200 and 300 mg/kg b.w. in rats and significant (P<0.05, P<0.01) inhibition in histamine and acetylcholine induced contraction of smooth muscle preparations. The isolated sapogenin fraction from the plant showed presence of β-sitosterol. From results of this study we concluded that LRLHE has potential anti-asthmatic activity in various animal models and suggestive potential in the management of asthma.

**Keywords**— *Leptadenia reticulata* (Retz) Wight & Arn, Hydro alcoholic extract, Asthma, HPTLC.

I. INTRODUCTION

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this dreadful disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Hence numbers of drugs from indigenous plant sources have been explored for their anti-asthmatic and bronchodilator activities during the last three decades [1]. *Leptadenia reticulata* (Retz) Wight & Arn (Family-Asclepiadaceae) is a twiner found in Gujarat, Maharashtra, sub-Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India, ascending up to an altitude of 900m. The plant is referred to as ‘Jivanti’ in ayurvedic text and considered to be a Rasayana (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body [2]. Ethno medicinally the leaves and seeds are used in asthma and cough [3]. The major therapeutic claim is its galactogogue action, which has been proved in rats [4] along with the antimicrobial [5], anticarcinogenic [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, rheumatism [3,9]. β-sitosterol is one of the reported chemical constituent of plant [10] and it is a main phyto-sterol found in numerous plants including rice, wheat, corn, nut, peanut etc. It is structurally related to cholesterol [11]. β-sitosterol has recorded an amazing health benefits as an hepatoprotective12, antioxidant and antipyretic inflammatory disorders13 and rheumatoid arthritis colon cancer [14] benign prostatic hypertrophy [15,18] and breast cancer [16,17,19]. Preliminary phytochemical screening, TLC fingerprinting of *Leptadenia reticulata* (Retz) Wight & Arn leaves revealed the presence of β-sitosterol. Considering the wide therapeutic applications of β-sitosterol, as well as an alternative quantification technique of marker constituent was generated to ensure identity and quality of the selected plants. HPTLC method is a sensitive, specific and reproducible for the quantification of β-sitosterol from the plants [20].

II. MATERIAL AND METHODS

A. Plant material

The leaves of *Leptadenia reticulata* (Retz) Wight & Arn. were collected from western ghat region of Maharashtra during Aug 2010 and authenticated by Dr. T. Chakraborty Joint Director, at Botanical Survey of India (BSI), Pune, India and voucher specimen was deposited to the same (voucher no.SAWLEP1).

B. Preparation of extract

The shade dried leaves of *Leptadenia reticulata* (Retz) Wt & Arn. Powdered (40 size mesh) and around 500 gm of powder was subjected to extraction (soxhlet) with petroleum ether to defatt the powder. and the defatted powder was macerated with Hydro-alcoholic solvent (30:70). After the effective extraction, solvent were concentrated under rotary vacuum evaporator and extract was then weighed (yield 6.34% w/w). The obtained extracts were subjected to phytochemical investigation and pharmacological screening for its anti-asthmatic activity.

C. Phytochemical screening

The freshly prepared crude Hydro-alcoholic extract of *Leptadenia reticulata* (Retz) Wt & Arn. (LRLHE) was qualitatively tested for the presence of major
phytoconstituents. This was carried out by using standard procedure.

D. Drugs and Chemicals

All chemicals used in the present study were analytical grade and purchased from Merck specialties pvt. Ltd. Mumbai, India. Compound 48/80 and Egg albumin purchased from Sigma (USA) and Glaxo Laboratories, Mumbai, respectively.

Animals

Male wistar albino rats (180-200 g), Swiss albino mice (20-25 g) and Guinea pigs (350-400g) were obtained from the lacsmi biofarms animal Centre, Pune and kept in standard environmental conditions. They were fed with standard pellet diet and water ad libitum. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee (1197/c/o8/CPCSEA).

E. Acute toxicity study

Acute oral toxicity study was carried out as per guidelines set by Organization for Economic Co-operation and Development (OECD) [21] revised draft guidelines 425 received from CPCSEA.

F. Anti-asthmatic activity Isolated tissue experiments:

A. Guinea pig ileum and tracheal chain preparation

Guinea pigs were fasted for 24 hr and later were sacrificed and pieces of ileum as well as trachea were isolated. The tissues were quickly transferred to petridishes containing Tyrode solution and Kreb’s solution respectively. The tracheal chain was prepared and mounted in an organ bath maintained at 37°C and containing Kreb’s solution according to the method described by [22-23].

B. Rat ileum preparation

Albino rats were fasted overnight. The next day the animals were sacrificed and a small piece of ileum was isolated and mounted in an organ bath containing Tyrode solution maintained at 37°C. A basal tension of 500 mg was applied and the tissue was stabilized for 30 min. The tissue was then exposed to graded doses of acetylcholine and contractions were recorded as described by [23].

2.7.2 Compound 48/80 induced rat mesenteric mast cell degranulation

The pieces of rat mesentery were collected in petri dish containing Ringer Locke solution and then subjected to the different treatment schedules as described by [24-25]

2.7.3 Homologous Passive cutaneous anaphylaxis in rats

1. The homologous antiserum was prepared according to the method described by [22,25]. The rats were injected with 0.1 ml of egg albumin and 0.1 ml of Bordetella pertusis vaccines i.p. on 1st, 3rd and 5th day. After 21 days from the first day of the immunization, blood was collected from retro-orbital plexus under light ether anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C before use as described by Katayama et al, 1998 and The Evans blue content was measured in this 70% acetone colorimetrically at 612 nm. [22, 28].

2.7.4 Statistical analysis:

The results were expressed as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way analysis of variance (ANOVA), followed by Dunnett’s t-test and tested for significance using paired Student’s t-test where P<0.05 was considered statistically significant.

III. ISOLATION AND CHARACTERIZATION USING HPTLC ANALYSIS

A. Extraction and Fractionation

500 g defatted drug powder of Leptadenia reticulata Wt & Arn. leaves was hydrolyzed by using 200 ml of 4 N H2SO4 and kept overnight at room temperature, filtered and marc washed with distilled water. The dried marc was refluxed for 30 min using fresh 200 ml of 4 N H2SO4 to ensure complete hydrolysis of drug. The hydrolyzed drug powder was fed in a Soxhlet extractor and subjected to extraction with ethanol (95%) and crude sapogenins were collected (Peach et al, 1995). The crude sapogenins isolated by this method exhibited identical TLC profile for isolation of sapogenins from the drug. The chromatographic analysis of crude sapogenins were performed on silica gel-GF254 precoated plates (E Merck, Germany) using chloroform: glacial acetic acid: methanol: water (16:8:3:2, v/v) as mobile phase, four spots were visualized upon derivatization with anisaldehyde sulfuric acid reagent. HPTLC analysis of isolated Sapogenin fraction was characterized on Benzene: Methanol (9:1, v/v) as a mobile phase against β-sitosterol as standard marker.(26-27).

B. Chromatographic conditions

Stationary Phase: Pre-coated silica gel plates Merck 60 F254 (10 x 10, 0.2 mm thickness)

Experimental conditions: Temperature 25± 2°C

Relative humidity: 40%

Mobile Phase- Benzene: Methanol (9:1 v/v)

Spotting device: Linomat III Automatic sample spotter, CAMAG (Switzerland);

Development Mode: CAMAG twin trough chamber, CAMAG Densitometer: TLC Scanner III, CATS software, CAMAG.

A. Preparation of standard solution

The stock solution of β - sitosterol (1 mg/ml) was prepared in methanol. The stock solution was quantitatively transferred into a 10 ml volumetric flask to give solution of appropriate concentration range of β - sitosterol.

B. HPTLC analysis of the standard and isolated sapogenin fraction

The working standard of suitable concentration (100 - 500 ng/ml) was applied in triplicate on precoated silica gel 60 F254 HPTLC plates (E. Merck), of uniform thickness of
0.2mm. The plates were developed in a solvent system of Benzene: methanol (9:1 v/v) in CAMAG twin trough chamber up to a distance of 8 cm. After development, the plate was dried in air and sprayed by using anisaldehyde sulphuric acid reagent solution and subsequently heated at120°C for derivetization. These plates were scanned at 525nm absorbance/reflection mode using reflectance mode by CAMAG Scanner III and CATS software was used to analyze the plates. The peak areas were recorded (Mesar et al, 2010). The completely dried isolated sapogenin fractions were accurately weighed and solutions of (10 mg/ml) were prepared. Theses stock solutions were further diluted with methanol to get solutions of (1 mg/ml) 10 μl per spot of these solutions were applied on to a precoated silica gel 60 F254 HPTLC plates in triplicates. The plates were developed by ascending mode to a distance of 8cm and scanned at 525nm absorbance/reflection mode. The Rf value of standard β-sitosterol (0.52) was compared with the Rf value of isolated sapogenin fractions.

IV. RESULTS

A. Phytochemical screening:

Phytochemical screening of the plant extract revealed the presence of flavonoids, tannins, saponins, steroids, carbohydrates, and glycosides.

B. Acute toxicity study:

Single dose (250, 500, 2000 and 5000 mg kg⁻¹) of L. reticulata leaves hydro-alcoholic extract administered to albino mice showed no death up to 14 days study period. Hydro alcoholic extract of Leptadenia reticulata was found to be safe upto 5000 mg/kg p.o. given to mice. No any sign for behavioural as well as any physical changes were found.

C. Anti-asthmatic activity

A. Isolated tissue experiments: Guinea pig ileum preparation

Histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum. Pretreatment with hydro alcoholic extract of Leptadenia reticulata (0.8 mg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. (Fig.)

4.3.1.2 Guinea pig tracheal chain preparation

Histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum. Pretreatment with hydro alcoholic extract of Leptadenia reticulata (1.2 mg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. (Fig.)

4.3.1.3 Rat ileum preparation

Acetylcholine (10µg/ml) produced dose dependent contraction of rat ileum. Pretreatment with hydro alcoholic extract of Leptadenia reticulata (1 mg/ml) significantly inhibited (p<0.01) the contractile effect acetylcholine. (Fig.)

4.3.2 Compound 48/80 induced rat mesenteric mast cell degranulation

Compound 48/80 produced significant disruption of mast cells which was significantly inhibited in a dose dependant manner by pretreatment with the hydro alcoholic extract of Leptadenia reticulata in concentrations of 300µg/ml, 500µg/ml and700µg/ml resulted in significant reduction (p<0.01) in degranulation of mast cells when challenged with compound 48/80. The protection was comparable to the standard drug Disodium cromoglycate (10µg/ml) (Table )

4.3.3 H6omologous Passive cutaneous anaphylaxis in rats

The anti-ovalbumin antiserum treatment showed immediate hypersensitivity reactions results into dye leakage on the clipped dorsal skin. Pretreatment with hydro alcholic extract of Leptadenia reticulata showed significant protection (p<0.01) against immediate hypersensitivity and produced significantly lesser amount of dye leakage at a dose of 100 mg/kg, 200 mg/kg, 300 mg/kg and by Dexamethasone at a dose 5 mg/kg, when compared with the control group (Table 5. Isolation and characterization using HPTLC analysis)

The isolated sapogenin fraction was tested in many mobile phases for desired resolution. Benzene: methanol 9:1 v/v combination was giving best resolution. The both spots of β-sitosterol and isolated sapogenin fraction visualized by using anisaldehyde sulphuric acid reagent. The Rf value of the β-sitosterol (0.52) was compared with the spots of the isolated sapogenin fraction (0.50).

TABLE I

<table>
<thead>
<tr>
<th>Histamine (10 µg/ml) Dose (ml)</th>
<th>-ve log mol. conc. of Histamine</th>
<th>Control</th>
<th>LRLHE (0.8 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>6.04</td>
<td>30.79 ± 1.580</td>
<td>23.03± 1.869**</td>
</tr>
<tr>
<td>0.2</td>
<td>5.74</td>
<td>44.68 ± 4.240</td>
<td>33.37± 2.295**</td>
</tr>
<tr>
<td>0.4</td>
<td>5.44</td>
<td>59.89 ± 3.968</td>
<td>44.11± 3.898**</td>
</tr>
<tr>
<td>0.8</td>
<td>5.14</td>
<td>77.84 ± 2.143</td>
<td>64.30± 1.675**</td>
</tr>
<tr>
<td>1.6</td>
<td>4.84</td>
<td>88.35 ± 1.638</td>
<td>73.11 ± 1.067**</td>
</tr>
<tr>
<td>3.2</td>
<td>4.54</td>
<td>100 ± 2.450</td>
<td>79.17±4.870*</td>
</tr>
</tbody>
</table>

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student’s t-test, *p<0.05, **p<0.01 significantly different from control.

Fig. 1 Effect of LRLHE (0.8 mg/ml) on histamine induced contraction of guinea pig ileum preparation.
Fig. 2 Effect of LRLHE (1.2 mg/ml) on histamine induced contraction of guinea pig tracheal chain preparation.

TABLE II
ISOLATED TISSUE PREPARATION
EFFECT OF LRLHE (1.2 MG/ML) ON HISTAMINE INDUCED CONTRACTION OF GUINEA PIG TRACHEAL CHAIN PREPARATION
MAXIMUM CONTRACTION (%)

<table>
<thead>
<tr>
<th>Histamine (10 µg/ml)</th>
<th>Control</th>
<th>LRLHE (1.2 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (ml)</td>
<td>-ve log molar conc. of Histamine</td>
<td>Maximum contraction (%)</td>
</tr>
<tr>
<td>0.1</td>
<td>6.04</td>
<td>43.68 ± 2.711</td>
</tr>
<tr>
<td>0.2</td>
<td>5.74</td>
<td>49.85 ± 2.358</td>
</tr>
<tr>
<td>0.4</td>
<td>5.44</td>
<td>64.19 ± 3.156</td>
</tr>
<tr>
<td>0.8</td>
<td>5.14</td>
<td>82.00 ± 3.507</td>
</tr>
<tr>
<td>1.6</td>
<td>4.84</td>
<td>100 ± 2.320</td>
</tr>
</tbody>
</table>

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student’s t-test, *p<0.05, **p<0.01 significantly different from control.

TABLE III
ISOLATED TISSUE PREPARATION
EFFECT OF LRLHE (1 MG/ML) ON ACETYLCOLINE INDUCED.contraction of RAT ILEUM PREPARATION
MAXIMUM CONTRACTION (%)

<table>
<thead>
<tr>
<th>Histamine (10 µg/ml)</th>
<th>Control</th>
<th>LRLHE (1.2 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (ml)</td>
<td>-ve log molar conc. of Histamine</td>
<td>Maximum contraction (%)</td>
</tr>
<tr>
<td>0.1</td>
<td>6.15</td>
<td>38.14 ± 2.226</td>
</tr>
<tr>
<td>0.2</td>
<td>5.85</td>
<td>43.92 ± 1.914</td>
</tr>
<tr>
<td>0.4</td>
<td>5.55</td>
<td>55.98 ± 1.996</td>
</tr>
<tr>
<td>0.8</td>
<td>5.25</td>
<td>76.74 ± 1.730</td>
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<tr>
<td>1.6</td>
<td>4.95</td>
<td>100 ± 2.420</td>
</tr>
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</table>

n=6, Values are in Mean ±S.E.M. Statistical analysis done by using Student’s t-test, *p<0.05, **p<0.01 significantly different from control.

TABLE IV
ISOLATED TISSUE PREPARATION
EFFECT OF LRLHE ON COMPOUND 48/80 INDUCED RAT MESENTRIC MAST CELL DEGRANULATION
MAXIMUM CONTRACTION (%)

<table>
<thead>
<tr>
<th>Histamine (10 µg/ml)</th>
<th>Mast Cells%</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (ml)</td>
<td>Intact</td>
<td>Disrupted</td>
</tr>
<tr>
<td>Control</td>
<td>27.20 ±1.02</td>
<td>72.80 ±1.02</td>
</tr>
<tr>
<td>Standard</td>
<td>71.40 ± 1.36**</td>
<td>28.60 ± 1.36**</td>
</tr>
<tr>
<td>LRLHE 300</td>
<td>52.60 ± 1.03**</td>
<td>47.40 ± 1.03**</td>
</tr>
<tr>
<td>LRLHE 500</td>
<td>56.40 ± 1.12**</td>
<td>43.60 ± 1.12 **</td>
</tr>
<tr>
<td>LRLHE 700</td>
<td>64.60 ± 1.80**</td>
<td>35.40 ± 1.80**</td>
</tr>
</tbody>
</table>

n=6, values are expressed in Mean ±S.E.M. Statistical analysis done by ANOVA followed by Dunnett’s test *p<0.05,**p<0.01 compared to control group.

V. DISCUSSION

The development of bronchial asthma is related to immediate hypersensitivity reaction. With the ever growing interest in natural medicine many herbal plants has been reported to be used in treatment of patients with asthma (28) due to their less adverse reaction compared with synthetic drugs. The present study was undertaken for the evaluation of antiallergic activity of hydro alcoholic extract of Leptadenia reticulata. It seems to be promising plant for the treatment of bronchial asthma because of its reported hepatoprotective [8]and anticancer activity[13]. The result of the present study reveals significant protection against mast cell degranulation, anti anaphylactic and antispasmodic activity on various invivo and invitro experimental models.

Mast cell disruption is mediated by activation of IgE antibodies. Stabilization of mast cell membrane could be one of the possible mechanisms of LRLHE responsible for their effectiveness, probably by raising the cyclic Amp levels due to inhibition of the phosphodiesterase enzyme [29, 31]. On isolated tissue like guinea pig ileum and tracheal chain preparation substantiate the H1 antihistaminic and antimuscarnic activity of LRLHE. Histamine is the most implicated mediator in bronchoconstriction that accompany asthma [30]. The result of this study indicates a similar rightward shift in dose response curve of histamine in presence of LRLHE indicating antihistaminic activity [28-29]. Passive cutaneous anaphylaxis is an anti-IgE antibody induced invivo model of anaphylaxis in local allergic reaction in which mast cells play an important role. Pretreatment with antiovalbumin antisera is reported to produce inflammation and wheals, mediators like leukotriens, prostaglandin, platelet activating factor and cytokines are reported to be responsible for such
inflammatory response. In our study penetration of the dye into the skin area of rat, where antiovalbumin antisera were injected is an indicator of amount of dye leakage in control animals was significantly higher, resulting development of inflammation due to antigen-antibody reaction. The leakage of dye was significantly less in animals treated with LRLHE. This can be partly due to inhibition of Leukotriens synthesis [31]. Passive paw of anaphylaxis is another invivo model for IgE mediated to immediate hypersensitivity reaction. In this model a prominent inhibitory effect of LRLHE is suggestive of its antianaphylactic activity. The antiasthmatic activity of \textit{Leptadenia reticulata} Retz. Wt & Arn. can be attributed to its antihistaminic (H$_1$-antagonist), antiallergic, mast cell stabilizing, anti-inflammatory activity suggestive of its potential in prophylaxis and management of asthma. The results of phytochemical, chromatographical investigation had led to the conclusion that the isolated compound from the \textit{Leptadenia reticulata} leaves may be saponin derivative (B-sitosterol).

VI. CONCLUSION

From the results of this study we concluded that LRLHE has potential antiasthmatic activity in animal models and supports its traditional claim. The antiasthmatic activity is probably may be due to presence of flavonoids, saponins, further detail study is in progress to isolate and characterize the chemical compounds in the plant which are responsible for biological activity.

REFERENCES

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