



Anti-asthmatic & Anti-allergic Activity of *Lagenaria siceraria* Mol. Standley

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Abstract

The present attempt was aimed to evaluate the Anti-asthmatic and anti allergic activity of the aqueous extract of *Lagenaria siceraria* leaf (LSA) using various animal models like histamine and acetylcholine induced bronchoconstriction in Guinea pigs, compound 48/80 induced mast cell degranulation in rats and paw edema in mice. LSA exhibited significant and dose dependent bronchodilatory activity at the doses of 150 and 300 mg/kg intraperitoneally (i.p.). LSA at the concentrations of 10, 20 and 30 µg/ml significantly inhibited compound 48/80 induced mast cell degranulation. The anti inflammatory activity was observed at the doses of 50, 75 and 100 mg/kg (i.p.) against compound 48/80 induced paw edema in rats. These results prove the traditional claim of the drug in treatment of asthmatic disorders.

Keywords: *Lagenaria siceraria* Mol. Standley, Bronchoconstriction, Compound 48/80, Mast cell stabilization, Paw edema.

1. Introduction

Asthma is a chronic lung disease characterised by increased responsiveness of trachea and bronchi to various stimuli and manifested by narrowing of airways. India alone has an estimated 15-20 million asthmatics [1]. In the United States alone, asthma affects almost 17 million people, and this is a 75% increase in the last 20 years. This means that about 1 out of every 20 adults and close to 1 out of 13 children today have asthma. An alarming fact is that since 1980, asthma in children under age 5 has risen remarkably [2].

Allergy is one of the common diseases that affect mankind with diverse manifestations. The

prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the public [3]. A large number of plants are used traditionally in Ayurveda and other systems of medicines for bronchial disorders.

Lagenaria siceraria is a climbing perennial plant cultivated as a vegetable in tropical countries like India, Thailand and Japan. This plant is known as Louki or Dudhi in Hindi. *Lagenaria siceraria* leaves are used traditionally for cough, bronchitis, asthma, fever, inflammation, skin diseases, jaundice, headache, baldness and as purgative and emetic [4, 5]. Leaves are reported

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to contain cucurbitacins B, D and E, steroidal saponinins and amino acids [5,6]. In the present study we have investigated the anti asthmatic and anti allergic activities of the aqueous extract of *Lagenaria siceraria* leaf (LSA) using various animal models.

2. Materials and methods

2.1. Preparation of extract

Leaves of *Lagenaria siceraria* were collected from Nirma Herbal Wealth, Nirma University, Ahmedabad, Gujarat, India in the month August, 2010 and the sample was identified and authenticated by Dr. B.L. Punjani, Ethnobotanist, P.G. Centre in Botany, Smt. S.M. Panchal Science College, Talod, Gujarat, India. The voucher specimen (IPS/PCOG/MPH10-11/505) has been preserved at Dept. of Pharmacognosy, Institute of Pharmacy, Nirma University for future reference. The aqueous extract of the leaves was freshly prepared from 500 gm of dried leaves using hot infusion method, which yielded 25.20 % (w/w) of dry extract.

2.2. Animals

Guinea pigs (300-400g), wistar rats (200-250 g) and albino mice, Kasuali strain, of either sex were procured from Torrent Research Centre, Ahmedabad. Animals were housed in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature $25 \pm 2^\circ\text{C}$, relative humidity $50 \pm 5\%$, and photo-schedule (12 h light and 12 h dark). Animals had free access to food and water. Institutional Animal Ethics Committee approved the experimental protocol (IPS/PCOG/MPH10-11/2015).

The pharmacological work was carried out as per norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Drugs and chemicals

Histamine Hydrochloride (S.D. Fine Chem. Ltd.,

Mumbai), Compound 48/80 (M.P. Biomedicals, France), Acetylcholine Chloride (Central Drug House, New Delhi) and Aminophylline (Torrent Pharma, Baddi) were used in present studies.

2.4. Histamine and Acetylcholine aerosol induced bronchoconstriction in Guinea pigs

Albino Guinea pigs were divided into 4 groups (n=6), Control group received normal saline i.p., the group LSA-150 and LSA-300 received 150 and 300 mg/kg freshly prepared aqueous extract of *Lagenaria siceraria* leaf i.p., respectively and standard group received Aminophylline (25 mg/kg, i.p.). Experimental bronchospasm was induced in Guinea pigs by exposing them to 2% histamine and 4% acetylcholine aerosol at a constant pressure using histamine chamber. The Guinea pigs showed pre convulsion dyspnea (PCD). The animals were then taken out of the chamber and placed into fresh air for recovery. At the interval of two days the same group of animals were administered with drug extract and standard aminophylline respectively 45 minutes prior to histamine and acetylcholine aerosol and pre convulsive time was measured. The % protection offered by extracts and standard drug was calculated using following formula [7, 8].

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

Where, T_1 is a mean of control PCD time and T_2 is a PCD time after drug treatment

2.5. Compound 48/80 induced paw edema in mice

Albino mice of either sex were divided into 5 groups (n=6). Compound 48/80 was administered subcutaneously in the plantar region of the right hind paw and the left hind paw received an equal volume of saline. The hind paw thickness was measured using a pocket thickness gauge (Mitutoyo Mfg. Co. Japan) after 30 minutes. The difference between left & right paw thickness was calculated. Freshly prepared aqueous extract of LSA at the

doses of 50, 75 and 100 mg/kg and ketotifen, 1 mg/kg i.p. was administered 30 min prior to injection of compound 48/80 [9].

2.6. Compound 48/80 induced mast cell degranulation studies

Albino rats were killed by exsanguinations. The piece of small intestine along with mesentery of rat was excised and connecting lobes of fat and blood vessels were rapidly dissected out. The pieces of mesenteries were spread in petri-dish containing Ringer Locke physiological salt solution. Drug extract at different concentrations or standard ketotifen incubated

mesenteries were challenged with 1µg/ml of compound 48/80 for 10 min. Pieces of mesentery were stained with 0.1% toluidine blue solution containing 4% formaldehyde for 20-30 min and then treated with acetone and xylene and mounted on slides. The % of degranulated mast cells were counted under microscope and the protection offered by aqueous extract of drug was calculated [12].

2.7. Statistics

All values were expressed as mean ± S.E.M. The data were analyzed by using one-way ANOVA followed by Dunnet's test.

Table 1: Effect of Aqueous extract of *L. siceraria* on histamine and acetylcholine induced bronchospasm in Guinea pigs.

Treatment	Dose (mg/kg)	Pre-convulsive time (sec)		% Protection	
		Histamine	Acetylcholine	Histamine	Acetylcholine
Control		68.40 ± 3.12	85.20 ± 2.03	-	-
LSA	150	91.33 ± 2.55	111.66 ± 2.73	25.02 ± 5.10	22.19 ± 3.60
LSA	300	129.66 ± 3.69	165.66 ± 2.23	45.96 ± 3.91	48.37 ± 0.82
Aminophylline	25	114.53 ± 4.50	182.40 ± 4.49	40.60 ± 2.78	53.10 ± 2.05

All the values were expressed as mean ± SEM. $P < 0.001$ vs. control.

Table 2: The effect of Aqueous extract of *L. siceraria* on compound 48/80 induced paw edema in mice.

Treatment	Dose (mg/kg)	Foot Pad Thickness(mm)	Percent Inhibition
Control	-	48.76 ± 4.63	-
Ketotifen(Std.)	1	8.26 ± 1.35	83.06 ± 2.78
LSA	50	32.6 ± 3.09	33.14 ± 2.33
LSA	75	24.8 ± 2.88	49.14 ± 1.45
LSA	100	15.35 ± 1.94	68.51 ± 2.13

All the values are expressed as mean±SEM. $P < 0.001$ vs. control.

Table 3: The effect of Aqueous extract of *L. siceraria* on compound 48/80 induced mast cell degranulation in rats.

Treatment	Conc ($\mu\text{g/ml}$)	% Degranulation	% Protection
Control (Compound 48/80)	1	67.63 ± 0.14	-
Ketotifen	10	16.66 ± 1.81	80.83 ± 0.52
LSA 10	10	33.66 ± 0.81	61.21 ± 0.39
LSA 20	20	24.83 ± 0.75	71.39 ± 0.77
LSA 30	30	17.33 ± 1.50	80.02 ± 1.50

All the values are expressed as mean \pm SEM. $P < 0.001$ vs. control.

3. Results

3.1. Histamine and Acetylcholine aerosol induced bronchoconstriction in Guinea pigs

Freshly prepared aqueous extract of *L. siceraria* significantly prolonged the latent period of convulsions at the dose of 150 and 300 mg/kg and showed protection of $25.02 \pm 5.10\%$ and $45.96 \pm 3.91\%$ after histamine aerosol and $22.19 \pm 3.60\%$ and $48.37 \pm 0.82\%$ after acetylcholine aerosol compared to aminophylline which offered maximum protection of $40.60 \pm 2.78\%$ and $53.10 \pm 2.05\%$ against histamine and acetylcholine respectively (Table 1).

3.2. Compound 48/80 induced paw edema in mice

Freshly prepared aqueous extract of *L. siceraria* produced dose dependent inhibition of edema at the doses of 50, 75 and 100 mg/kg producing inhibition of 33.14%, 49.14% and 68.51% respectively in mice compared to ketotifen which showed $83.06 \pm 2.78\%$ inhibition (Table 2).

3.3. Compound 48/80 induced mast cell degranulation studies

Aqueous extract of *L. siceraria* showed significant protection against degranulation at concentrations of 10, 20 and 30 $\mu\text{g/ml}$ produced percent protection of 61.22%, 71.40%, and 80.03% respectively compared to ketotifen which showed percent protection of $80.83 \pm$

0.52. The effect of aqueous extract at the concentration of 30 $\mu\text{g/ml}$ showed effect comparable to ketotifen (Table 3).

4. Discussion

Among various diseases or disorders, asthma is a serious disorder effecting large population of the world. Severity of the problem of asthma can be judged from the fact that mortality due to asthma is much more in number compared to day-to-day deaths due to other diseases. The management of asthma involves use of broncho-dilators for treatment of acute symptoms and using anti-inflammatory drugs for treating chronic inflammation-induced exacerbations [13]. Asthma is a chronic lung disease characterised by increased responsiveness of trachea and bronchi to various stimuli and manifested by narrowing of airways due to release of mediators like histamine and acetylcholine. Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in Guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. Bronchodilators can delay the occurrence of these symptoms [14]. Thus the results confirm the bronchodilatory properties of the plant.

Compound 48/80 was also used to induce inflammation in mice paw. Freshly prepared aqueous extract of *L. siceraria* (100 mg/kg) inhibited edema up to 49.14 % compared to ketotifen which showed 68.51% inhibition indicating its anti inflammatory activity against a non immunological stimulus.

Mast cells contain basophil granules loaded with active substances like histamine which when released can cause vascular and other tissue reactions like those characteristic of inflammatory process [15]. Mast cell degranulation can be evoked by the compound 48/80, which is a mast cell degranulator and has been used as a direct and convenient reagent to study the mechanism of anaphylaxis [12]. The pharmacological agents like ketotifen are known

to increase the cAMP levels, relax airway smooth muscles and stabilize the mast cell membrane preventing its degranulation. Aqueous extract of *L. siceraria* at the dose of 30 µg/ml has more activity than the standard, indicating its prominent mast cell stabilizing activity. Thus, the present investigations proved the usefulness of the leaves of *L. siceraria* in traditional medicine for the treatment of asthmatic disorders.

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