Research Article

Evaluation of Anti-Histaminic and Bronchodilator Activity of Linga Mathirai (Formal Siddha Drug)

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ABSTRACT

'Asthma' is a Greek word which means 'breathless' or 'to breathe with open mouth'. Bronchial asthma is an important allergic disorder. Prevalence of asthma is between 100 and 150 million people around the globe and India has an estimated 15-20 million asthmatics. The cost of medication in India was estimated as US$ 30 per month. Aim of the study is to investigate the safety and efficacy of Linga Mathirai (LM) (Siddha Drug) for the treatment of Bronchial asthma. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where preconvulsive dyspnea was used as an end point following exposure to histamine aerosol. It was evaluated for antihistamine and bronchodilator activities and it administrated at the doses of 100, 200 and 400 mg/kg body weight. A dose response curve for histamine + LM is lower, when compared with histamine induced contraction ($p<0.05$) at moderate dose level. The LM at moderate dose level significantly prolonged the latent period of convulsions as compared to control following the exposure of histamine aerosol. The result of present study shows that Linga Mathirai significantly protected the Guinea pigs against histamine-induced bronchospasm. Significant increase in-between pre and post treatment time (**$P<0.01$). The present study reveals that the Linga Mathirai (LM) can be more effective in the treatment of Bronchial asthma.

Keywords: Linga Mathirai (LM), asthma, anti-histaminic activity, bronchodilator activity

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INTRODUCTION

'Asthma' is a Greek word which means 'breathless' or 'to breathe with open mouth' [1]. It is defined as 'a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night/early morning [2]. Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulins, mast cells, and various autacoids in the etiopathogenesis of allergic conditions [3, 4]. As a public health problem, asthma is an important allergic disorder. Prevalence of asthma is between 100 and 150 million people around the globe and India has an estimated 15-20 million asthmatics. This number is rising [5]. The total estimated burden of Asthma is an overall prevalence of 3% (30 million patients), and among adults over the age of 15, a median prevalence of 2.4% [6]. The cost of medication in India was estimated as US$ 30 per month [7]. In siddha system of medicine asthma is analogues with 'Swasa kasam'. Many medicinal plants and herbo-mineral preparations in siddha system used for the treatment of asthma (Swasa kasam) which have anti-inflammatory, immuno-modulatory, antihistaminic, smooth-muscle relaxants and allergic activity. In the hope that Linga Mathirai can be a better therapeutic agent for the treatment of asthma with less or no side effects we aimed at evaluating the potency in asthma management using animal models. The interventional drug Linga mathirai (LM) is quoted in the Kannusamiyam parambarai vaithyam, and the drug efficacy is not

known. The chosen drug is quoted for the purpose of Swasa kasam, which is an analogous with Bronchial asthma.

MATERIALS AND METHODS

PREPARATION OF LINGA MATHIRAI

The official siddha preparation Linga Mathirai has the ingredients of lingam **(Fig. 1)** (Red sulfide of mercury), Vengaram **(Fig. 2)** (Borax), Poondu **(Fig. 3)** (Garlic). Lingam and Vengaram are made in to fine powder and garlic is added and grinds it thoroughly into paste like and make into pill or Tablet form [8].

Figure-1

Figure-2

Figure-3

DRUGS AND STOCK SOLUTION

Drugs used were Histamine diphosphate (Sigma Chemical, USA) and Promethazine hydrochloride (Rhone – Poulenc, Mumbai). Histamine dihydrochloride was dissolved in distilled water and desired concentrations were prepared. The test drug Linga Mathirai concentration was 100microgram per ml prepared by suspending with 2% CMC and then the volume was adjusted to 10 ml with normal saline for making the concentration of 100 µg/ml.in distilled water.

Animals

Male albino guinea pig weighing 350–400 gm was kept in fasting condition 18 hours prior to commencement of experiment and given water ad libitum. It was housed under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle and then sacrificed by a blow to the head and exsanguinated as per CPCSEA (XIII/VELS/PCOL/15/2000/ CPCSEA/IAEC/08.08.2012) recommended guidelines.

In-vitro antihistaminic study

Guinea pig was sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. The Tyrode solution composition (pH 7.4) was (concentration in gm/lit.) NaCl 8.0, KCl 0.2, CaCl2 0.2, MgCl2 0.1, NaHCO3 1.0, NaH2PO4 0.05, and Glucose 1.0gm/liter. It was continuously aerated and maintained at 37 ± 0.5ºC The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, a dose response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle [9-11].

Bronchodilator Study

Animals were divided into four groups of six animals each. Each animal were served as its own control. Animals belonging to each group were subjected to a histamine aerosol (0.2% Histamine diphosphate in saline) using a glass nebulizer for 2 sec in an airtight Perspex chamber. Aerosolization of the solution was achieved via a compressed air line operating at a pressure of 8 Psi and a flow rate of 5ml/min. After exposure to the histamine aerosol, the animal showed signs of immediate immobilization and bouts of coughing. This was followed by shallow breathing symptoms, after which the animal collapsed, fell on its back and convulsed. The time taken by the animal to fall on its back after exposure to the aerosol was designated as the exposition time. The exposition time for each animal in all the four groups was noted [12, 13].

Once the animal fell on its back, it was immediately taken out of the chamber and exposed to fresh air where the animal returned back to normal. After 1 hour the animals in the first three groups were administered orally 100, 200 and 400

mg/kg p.o, of Linga Mathirai respectively. While the fourth group of animals received 300µg/kg of Promethazine by oral route. One hour later, the animals were reexposed to the aerosol and exposition time for each animal was noted. The difference in the exposition time before and after Linga Mathirai administration was taken as a measure of the protective effect. Percent protection afforded by the Linga Mathirai was calculated by the formula.

$$\text{Percentage Protection} = \frac{\text{Eta-Etb}}{\text{Etb}} \times 100$$

Where 'Eta' is the mean exposition time after treatment with extract and 'Etb' is the mean exposition time before treatment with extract.

Statistical Analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by test drug calculated. Percentage of ileum contraction was expressed as mean ± SEM. Results were analyzed using one-way analysis of variance (ANOVA). Probability value less than 0.05 were considered as significant

RESULTS AND DISCUSSION

No animals died during the acute toxicity test, nor were any adverse effects detected in animals treated with Linga Mathirai at 2000 mg/kg. This indicates that the drug is nearly nontoxic in mice up to an oral dose of 2.0 g/kg of body weight. One by twentieth, one tenth and one fifth of dose was selected from acute toxicity study and administered to animals.

Histamine when inhaled has been shown to induce bronchoconstriction by direct H1-receptor activation and also by a naturally mediated bronchoconstrictors effect via vagal reflexes results in preconvulsive dyspnea and it also may lead to the appearance of convulsions. In the present study of Linga Mathirai have been shown the significant increase in pre-convulsion time due to pre-treatment with Linga Mathirai at the dose of 100, 200 and 500mg/kg of body weight of guinea pigs, when the guinea pigs were exposed to histamine **(Table 1, Fig. 5)**.

The percentage protection of Linga Mathirai -100, 200, 500mg/kg is 27.43, 28.79, 29.83% and standard drug showed 50.31% respectively [**Tab-2, Fig-6**]. The guinea pigs exposed to histamine aerosol showed signs of progressive dyspnea leading to convulsions. The results presented here confirm the traditional claim that Linga Mathirai confers some protection on guinea-pigs against the effects of a histamine aerosol. The immediate protection is not statistically significant but later extended, however the protection is not prolonged. Under the conditions employed, Linga Mathirai was found to possess moderate antihistaminic activity **(Fig.-4).**

Figure 4: Anti Histaminic Activity of Linga Mathirai

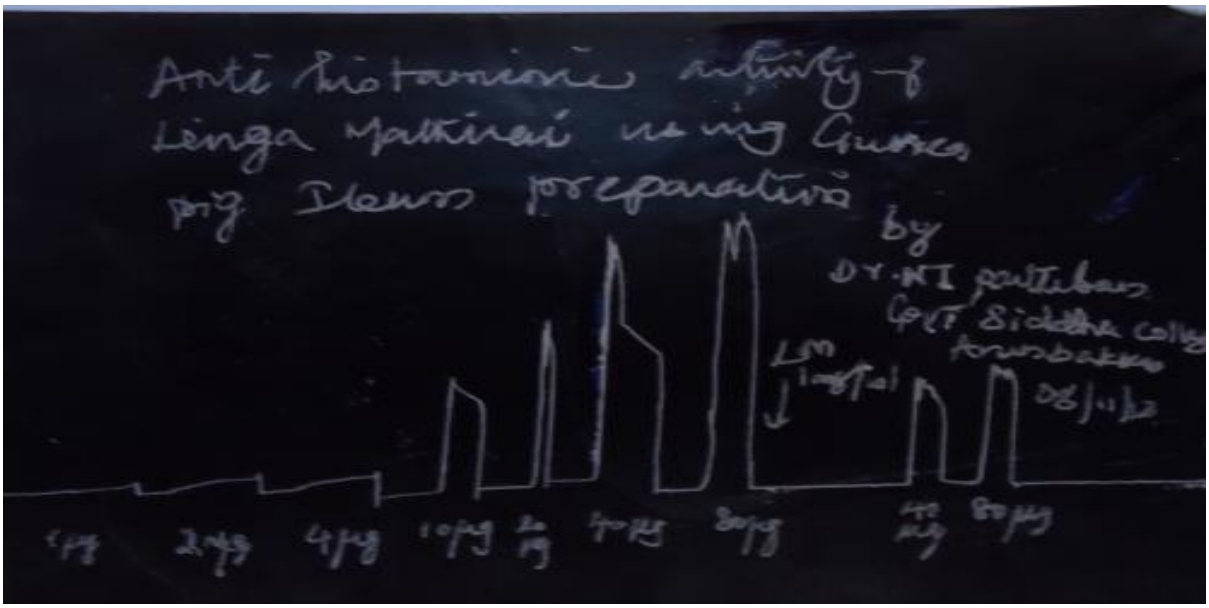

Table 1: Effect of Linga Mathirai on Isolated Guinea Pig Ileum Preparation

Sr. No	Dose of Histamine (µg/ml)	Percent of Maximum Response	
		Histamine alone	Histamine+Linga Mathirai (1mg/ml)
1	1	0.3±0.04	0.10±0.01
2	2	0.5±0.04	0.2±0.02
3	4	0.5±0.03	0.3±0.26
4	10	2.5±0.82	1.1±0.30
5	20	3.8±0.96	1.9±0.55
6	40	5.8±1.02	2.5±0.72
7	80	6.6±1.11	2.7±0.81

Values are expressed in mean ± SEM, *p< 0.05 compared with histamine induced contraction (45mm as 100%); n=3.

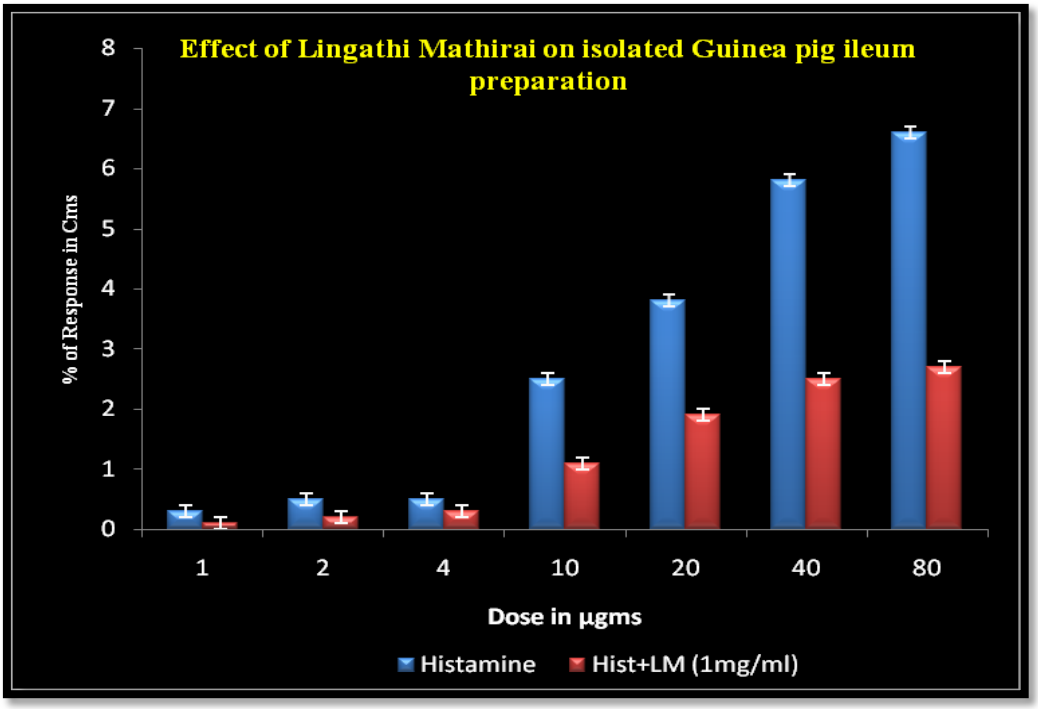

Figure 5: Effect of Lingathi Mathirai on isolated guinea pig ileum preparation

Table 2: Bronchodilator Effect of Linga Mathirai on Histamine Induced Bronchoconstriction

Treatment	Pre-Treatment Exposition in seconds	Post-Treatment Exposition in seconds	Percentage Protection
Linga Mathirai 100mg/kg. p.o.	88.02 ±3.65	121.30±3.72**	27.43%
Linga Mathirai 200mg/kg. p.o.	91.24±3.00	128.13±4.98**	28.79%
Linga Mathirai 400mg/kg. p.o.	95.11±3.45	135.56±5.45**	29.83%
Promethazine (300mg/kg, p.o)	89.15±3.15	179.44±6.10**	50.31%

N=6; Values are expressed as mean ± SEM; *Significant between pre and post treatment time (Student's –'t') **P<0.01.

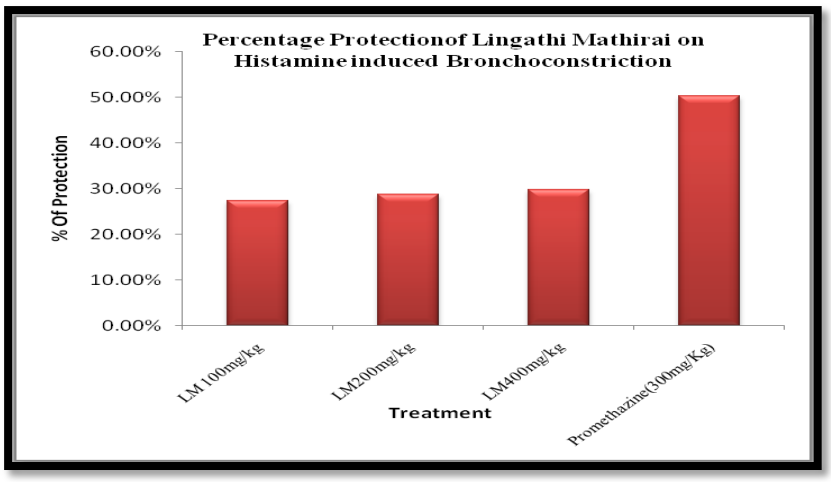

Figure 6: Percentage of histamine induced bronchoconstriction

CONCLUSION

The results of present study suggested that Linga Mathirai significantly protected the Guinea pigs against histamine-induced bronchospasm. The traditional use of Linga Mathirai is substantiated in the management of asthma. The Linga Mathirai at moderate dose level significantly prolonged the latent period of convulsions as compared to control following the exposure of histamine aerosol. The action started after 90minutes of drug administration. Thus, our findings suggest that Linga Mathirai possess significant antihistaminic activity.

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