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Research Article

EVALUATION OF ANTIASTHMATIC ACTIVITY OF METHANOLIC EXTRACT OF LUFFA ECHINATA ROXB. FRUIT IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Luffa echinata Roxb. (Curcurbitaceae) is a climber herb useful traditional in various disease treatment. The present study was designed to evaluate antiasthmatic effect of methanolic extract of Luffa echinata Roxb. fruit using various experimental animal models. Various invivo models like histamine induced bronchospasm in guinea pig, passive paw anaphylaxis model in rat, milk induced leukocytosis in mice and in vitro model clonidine induced mast cell degranulation were used to study antiasthmatic activity of the fruit extract. The methanolic extract of Luffa echinata fruit was administered in dose 50 mg/kg and 100 mg/kg dose (p.o.). The methanolic extract in both doses showed significant increase in PCT time and inhibition of paw oedema in histamine induced bronchospasm and passive paw anaphylaxis model respectively. Further the extracts (50 mg/kg and 100 mg/kg dose (p.o.) exhibited significant reduction in total leukocyte count in milk induced leukocytosis in mice as compared to disease control. In vitro clonidine induced mast cell degranulation model, the methanolic extract of Luffa echinata fruit produced significantly dose dependent protection against clonidine induced mast cell degranulation. These results suggest that methanolic extract of Luffa echinata Roxb. fruit possessed bronchodilatory, antiinflammatory and mast cell stabilizing effect. Therfore, we can conclude that Luffa echinata Roxb. fruit may have therapeutical value as antiasthmatic drug.

KEYWORDS: *Luffa echinata, Antiasthmatic activity, Mast cell stabilizing effect.*

INTRODUCTION

Asthma is a chronic inflammatory disease of airways. It mainly produces increasing in airway hyper responsiveness which causes episodic coughing, wheezing, chest tightness and breathlessness. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment [1].

In this inflammatory disease of airway many cells play important role like mast cell, macrophages, eosinophil, neutrophils and epithial cells. Asthma affects around 300 million peoples and it is responsible for 250000 deaths annually [2]. The pathogenesis of asthma involves both genetic and environmental factors [3]. For management of asthma currently various bronchodilator and antiasthmatic drugs are available. The systemic and inhalation corticosteroid agents are also quite often used for treatment of asthma. Although, there are wide range of allopathic medicines are available, they are given symptomatic relief [4]. These drugs are also not completely subside disease condition and associated with many side effects.

In traditional system of medicine, many traditional herbal medicine is reported to possess ability to prevent sustain inflammatory condition in Asthma.

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Luffa echinata Roxb. (*Curcurbitaceae*) is a spreading climber herb, extremely bitter taste, grows widely in Pakistan, India, Bangladesh and Northern Tropical Africa [5]. The fruit is traditionally used in the treatment of liver ailments, piles, rhinitis, epilepsy, diseases related to head, loos of appetite, liver disorders, ascites, poisoning, hiccough, worm infection, dysuria, amenorrhea, fever, in Skin diseases and for purification of abdomen [6]. In the form of infusion or decoction it is used in jaundice, in biliary and intestinal colic [7].

A number of compounds such as saponin, cucurbitacin-B, eletarin (cucurbitacin-E), eletarin-2-glucoside, isocucurbitacin B, β-sitosterol glucoside, chrysirol-7-glucoside, chrysirol7-epiglucoside, echinatol A, echinatol B, echinatin are present in *Luffa echinata* fruits [9]. Previous scientific study also reported that fruit shows antioxidant [9], antidepressant activity, anxiolytic activity, antiepileptic activity [10], hepotoprotective activity [11], antitumor activity [12].

The methonolic ectract of *Luffa echinata* Roxb. produced potential antiinflammatory and analgesic effect [13]. The fruits of *Luffa echinata* Roxb. plant is useful in cure of chronic bronchitis, lung complain and dropsy [5]. But there is lack of scientific investigation in this regard. Therefore, the present study is aimed to investigate antiasthmatic effect of the methanolic extract of fruits of *Luffa echinata* Roxb. in various experimental animal models.

MATERIALS AND METHODS

Collection of plant material and preparation of different extract:

The fresh fruits of *Luffa echinata* Roxb. were Collected from Rajkot region of of Gujarat, and was authenticated by a botanist of R.K. School of Science, R.K. University, Rajkot, Gujarat. Voucher specimen (SJTPC 73) was deposited in the department of pharmacognosy, S.J. Thakkar Pharmacy College, Rajkot. The fruits of plant were dried under shade and were grinded to powder (60# sieve) and stored into air tight container.

Extraction:

The dried powder of fruit (100 gm) was exhaustively extracted with 500 ml of methanol as solvent in soxhlet apparatus at controlled temperature (40°C) for 72 hours. Resulting solutions were filtered through Whattman filter paper (No.42). The methanolic extract of *Luffa echinata* fruit (MELE) was concentrated in a water bath at low temperature (40°C). The % yield of MELE was 6.4 % w/w.

Selection of Animals:

Duncan Harley guinea pigs (350-500 gm), wistar albino rats (150–200 gm) and swiss albino mice (25-30 gm) of either sex were selected for different animal models. All animals were kept at ambient temperature (22±1°C), relative humidity (55±5°%) and 12:12 hours light/dark cycle in the animal house of S. J. Thakkar Pharmacy college. Animals were free to access to standard pellet diet (Pranav Agro Ltd. India) and water *ad libitum*. All experimental protocols (No. SJT-72/2012) of pharmacological study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), S. J. Thakkar Pharmacy College, Rajkot and all experimental procedures were conducted according to guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Drug and chemicals:

All the chemicals used in the study were of analytical grade. Histamine dihydrochloride was obtained from Hi-media Pvt. Ltd (Mumbai, India). Bordetella pertussis vaccine was procured from Sigma Aldrich Pvt LTD (USA). Clonidine was obtained as gift sample from Alembic Pharma Pvt. Ltd. (Baroda, India), Dexamethasone and Ketotifen were obtained from Cadila Pharma Pvt. Ltd., Ahmadabad.

Acute toxicity study:

Acute toxicity study was performed as per OECD guideline 423. Overnight fasted female wistar rats randomly divided in two group control and test groups (n=3). Control group received 0.5 ml sodium carboxymethylcellulose (0.5% w/v SCMC) suspension orally and test group administered with single high dose of 2000 mg/kg body weight of crude extract of MELE. After the respective treatment, animals in each group were observed individually at least once during first 30 minutes after dosing, periodically during first 24 hours and then for a period of 14 days daily for any signs of toxicity or mortality. No sign of toxicity or mortality observed in MELE (2000 mg/kg) dose after completion of 14 days study.

Histamine induced bronchospasm in conscious guinea pigs: [14]

Harley strain guinea pigs of either sex weighing 350-500 gm were used and divided into four groups (each containing six animals) as following:

Group 1: (Normal Control) Administered vehicle (0.5% w/v Sodium Carboxy methyl cellulose (SCMC), 1 ml/kg, p.o.)
Group 2: (Standard treatment group) Treated with Ketotifen (1 mg/kg, p.o)
Group 3: (Test) Treated with MELE (50 mg/kg, p.o.)
Group 4: (Test) Treated with MELE (100 mg/kg, p.o.)

All test and standard drugs were dispersed in 0.5%w/v sodium CMC as vehicle.

The animals were kept in a histamine chamber (30×30×15cm) and exposed to an aerosol of 0.5 % w/v histamine dihydrochloride and pre-convulsion time (PCT) (The time of aerosol exposure to dyspnoea leading to appearance of convulsion) was noted. This time considered as base value. On the 15th days, two hours after the respective drug treatment, animals were exposed to histamine dihydrochloride aerosol and PCT was measured for each animal.

% Increase in PCT was calculated using following formula.

$$\% \text{ Increase in PCT} = [1-T_1/T_2] \times 100$$

Where, T_1= PCT on day 0, T_2= PCT on 15th day.

Passive paw anaphylaxis in rats: [15]

Preparation of anti-serum from rats:

The Wistar rats of either sex were injected intraperitoneally with 0.2 ml, 10% egg albumin and 2 ml of Bordetella pertussis vaccine on day 1, 3 and 5. Twenty one days after the first immunization, blood was collected from orbital plexus under light ether anesthesia. The collected blood was allowed to coagulate and serum was separated by centrifugation at 1500 rpm for 10 min. The separated serum was stored at -20 ºC untill it was used further.

Procedure:

Wistar rats of either sex weighing 150-200 gm were selected and randomly divided into four groups (six animals in each) and respective drug treatments were given once a daily as following:

Group 1: (Control) Treated with vehicle (0.5%w/v NaCMC)
Group 2: (Standard) Treated with Dexamethasone (0.27mg/kg, p.o.)
Group 3: (Test-1) Treated with MELE (50 mg/kg, p.o.)
Group 4: (Test-2) Treated with MELE (100 mg/kg, p.o.)

Test and standard drugs were dispersed in 0.5% w/v sodium CMC.

All treatment were given orally once daily for 7 days. 2 hours after last dose on 7th day rats were passively sensitized by subplanter administration to left hind paw with 0.1ml undiluted serum and after 24 hour of sensitization, rats were again challenged with 10 µg of egg-albumin in 0.1ml saline and hind paw volume was measured by plethysmometer for 4hour at interval of 1hour after 30 minutes of sensitization in each animal.

Milk induced leukocytosis in mice: [16]

Procedure:

Swiss albino mice of either sex weighing 25-30gm were divided into three groups. (n=6)

Group 1: (Normal Control) Treated with vehicle (0.5%w/v NaCMC) (1ml/kg, p.o.)
Group 2: (Diseases control) Treated with Boiled and cooled milk (4 mg/kg, s.c.)
Group 3: (Test-1) Treated with Boiled and cooled milk (4 mg/kg, s.c.) and MELE (50 mg/kg, p.o.)
Group 4: (Test-2) Treated with Boiled and cooled milk (4 mg/kg, s.c.) and MELE (100 mg/kg, p.o.)

Drug treatment was given 1 hour prior to milk injection each group and then milk (boiled and cooled, 4mg/kg) was injected subcutaneously in each group except control group. Total leukocyte was measured under high power microscopy before and after 24 hour milk injection in all group. Difference in Total leukocyte count before and 24 hours after milk administration were compared for animal of each group.

Clonidine induced mast cell degranulation (In vitro model): [17]

Normal saline containing 5 units/ml of heparin was injected in the peritoneal cavity of male wistar rats lightly anaesthetized with ether. After gentle abdominal massage, the peritoneal fluid was collected in centrifuge tubes. Peritoneal fluid of 4 - 5 rats was collected and centrifuged at 2,000 rpm for 5 min. Supernatant solution was discarded and the cells were washed twice with saline and resuspended in 1 ml of saline. 0.1 ml of the peritoneal cell suspension was transferred to 6 test tubes and all standard and test drug were added in following way

- Test tube no. 1 - Negative control
- Test tube no. 2 – Positive control
- Test tube no. 3 – 0.1 ml of 10 µg/ml Ketotifen in Saline
- Test tube no. 4 - 0.1 ml of 1000 µg/ml methanolic of *Luffa echinata* fruit in Saline
- Test tube no. 5 - 0.1ml of 1500 µg/ml methanolic extract of *Luffa echinata* fruit in Saline
- Test tube no. 6 - 0.1ml of 2000µg/ml methanolic extract of *Luffa echinata*. fruit in Saline

Each test tube was incubated for 15 min at 37°C and then Clonidine (0.1 ml, 80µg/ml) was added to each test tube. After again incubation for 10 minutes at 37°C, the mast cells were stained with 0.1% toluidine blue solution in distilled water in each test tube and resultant mixture was kept on slide and examined under the high power of light microscope.

Protection of the mast cells in the control group and the treatment groups was calculated by counting the number of degranulated mast cells from total 100 mast cells counted from different region of slide.

Statistical analysis:

All values were expressed as mean ± SEM (standard error mean). The statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's test when compared to Disease control or control respectively. Value of $p < 0.5$ was considered as significant. The statistical software Graphpad Prism (version 5.0) was used to perform all statistical analysis.

RESULTS

Histamine induced bronchospasm in conscious guinea pigs:

The animal treated with standard drug ketotifen (1 mg/kg, p.o.) , MELE (50 mg/kg p.o.) and MELE (100 mg/kg p.o.) were showed significant increase in PCT ($p < 0.001$) as compared to control group after 15 days respectively (Table 1).

Passive paw anaphylaxis in rats:

Animals treated with standard drug dexamethasone showed significant less paw oedema volume at 1,2,3,4 hour ($p < 0.001$). MELE (50 mg/g, p.o.) treatment revealed significant reduction in paw oedema volume at 4 hour interval ($p < 0.5$) only. However, MELE (100 mg /kg p.o.) treated group was also showed less paw oedema volume ($p < 0.001$) similar to standard treated group at 1,2,3 and 4 hour interval ($p < 0.001$) (Table. 2).

Milk induced leukocytosis in mice:

The disease control group showed significant high total leukocyte count as compared to normal control group after 24 hours of administration of milk. Treatment with MELE (50 mg/kg, p. o.) and MELE (100 mg/kg, p. o.) were exhibit significantly reduction in total leucocyte count as compared to disease control group ($p < 0.001$) (Table. 3).

Clonidine induced mast cell degranulation:

In this in vitro model, Positive control group showed significant (73.50 ± 2.81) ($p < 0.001$) mast cell degranulation as compared to negative control. Treatment with ketotifen (10 µg/ml) and test drug MELE (1500 µg/ml and 2000 µg/ml) significantly reduced mast cell degranulation ($p < 0.001$) (Table.4).

Table No. 1: Effect of Methanolic extract of *Luffa echinata* Roxb. fruit on Histamine induced bronchospasm in conscious guinea pigs

Group No.	Groups	% Increase in PCT
1	Control (Vehicle, 0.5%W/W, SCMC, p.o.)	4.26 ± 0.49
2	Standard Ketotifen (1 mg/kg,p.o.)	79.58 ± 0.83 ***
3	Test -1 MELE (50 mg/kg p.o.)	57.91 ± 1.6***
4	Test - 2 MELE (100 mg/kg, p.o.)	78.83 ± 0.63 ***

*All values represented as Mean ± SEM (n=6); ** p < 0. 01, *** p < 0.001, one-way ANOVA followed by Tukey's test to when compared to control group*

Table No. 2: Effect of Methanolic extract of *Luffa echinata* Roxb. fruit on Passive paw anaphylaxis in rats

Groups	Paw oedema volume (Vt-Vo) (ml)			
	1 hr	2 hr	3 hr	4 hr
Control	0.51±0.007	0.50±0.004	0.48±0.010	0.43± 0.02
Standard Dexamethasone (0.27 mg/kg, p.o.)	0.25±0.019***	0.24±0.030***	0.21±0.026***	0.18±0.018***
MELE (50 mg/kg, p.o.)	0.47±0.016 n.s.	0.45 ±0.017 n.s.	0.41± 0.014 n.s.	0.36± 0.015*
MELE (100 mg/kg, p.o.)	0.42±0.016***	0.39±0.018**	0.35±0.019***	0.29±0.017***

*All values represented as Mean ± SEM (n=6); n.s. No significant, * p < 0.5, ** p< 0. 01, *** p < 0.001, one-way ANOVA followed by Tukey's test to when compared to control group*

Table No. 3: Effect of Methanolic extract of *Luffa echinata* Roxb fruit on Milk induced leukocytosis in mice

Groups (n=6)	Total leukcocyte counts(cu/mm)		
	Before	Before	Before
Normal control	3265± 90.04	3726 ±168.15	461 ± 72.113
Disease control	3335±98.10	6560±198.00	3235±131.64###
MELE (50 mg/kg, p.o.)	3450±106.64	5617±102.19	2167± 80.27***
MELE (100 mg/kg, p.o.)	4133±133.33	5767±270.45	1634±66.66***

*All values represented as Mean ± SEM (n=6); *** p < 0.001, one-way ANOVA followed by Tukey's test to when compared to disease control group; ### p < 0.001, When compare to normal control group*

Table No. 4: Effect of Methanolic extract of *Luffa echinata* Roxb fruit on Clonidine induced mast cell degranulation invitro model

Sr. no.	Groups	% Mast Cell Degranulation	% Inhibition of Degranulation
1	Negative control	2.33 ± 0.61	--
2	Positive control	73.50 ± 2.81###	--
3	Ketotifen 10 µg/ml	27.5 ± 2.53***	72.5%
4	MELE 1000 µg/ml	65.16 ± 3.21	34.84%
5	MELE 1500 µg/ml	53.83 ± 2.38 ***	46.17%
6	MELE 2000 µg/ml	40.66 ± 3.30 ***	59.34%

*All values represented as Mean ± SEM (n=6); *** p < 0.001, one-way ANOVA followed by Tukey's test to when compared to positive control; ### p < 0.001, When compare to Negative control*

DISCUSSION

In the present study, we tried to find antiasthmatic potential of *Luffa Echinata* Roxb. fruit by using various animal models. Bronchial asthma is effect large number of people in the world. Exposure to spasmogen increases airway activity in asthmatic patients. Exposure of such allergen cause bronchial smooth muscle spasm and releases inflammatory mediators including histamine, luekotrienes, prostaglandins and tryptase. These leads to airway hyperresponsiveness, airway inflammation and severe bronchoconstriction.

In present primary investigation, the bronchodilator potential of methanolic extract of *Luffa echinata* Roxb. fruit (MELE) was evaluated by histamine induced bronchospasm model in guinea pig. Histamine is an inflammatory mediator. In the histamine induced bronchospasm model, histamine causes hypoxia which produced convulsion in guinea

pig. The histamine ingestion by air produces airway smooth muscle contraction, hypotension and capillary dilation. So histamine exposure to guinea pigs in control group showed severe bronchoconstriction and showed reduction in preconvulsion time (PCT) [18]. The methanolic extract of *Luffa echinata* Roxb. fruit (MELE) and standard drug were significant increase in PCT suggesting MELE has potent bronchodilator effect. The past study reported that MELE was produced reduction in carrageenan induced oedema in paw early phase may be due to release of histamine, acetylcholine and bradykinines [13]. This evidence suggest that MELE produced potent bronchodilator effect may be due to antihistaminic activity.

In allergy induce asthma, exposure to allergens activate T – lymphocytes in airway tissue. Activated T lymphocytes increase Ig E antibody production which leads to antigen- antibody reaction. This pathological reaction stimulates inflammatory mediator production and process anaphylactic reaction. This high level of Ig E causes mast cell degranulation in airway and lungs produces allergic asthma. The passive paw anaphylaxis induced oedema model, exposure to allergen revealed anaphylactic reaction in rat paw and causes oedema [15]. Dexamethasone like immune modulatory drugs inhibits the antigen-antibody (AG-AB) reaction and thus reduces the release of inflammatory mediators mediated anaphylactic reaction which showed by reduction in paw oedema in rats. MELE was also reduced paw oedema volume after 3 hours in rat. These results suggest that methanolic extract of *Luffa echinata* Roxb. fruit might be suppressed allergen induced inflammatory reaction in allergic asthma.

It is reported that after parenteral administration of milk, there is an increase in total leukocyte count. Leucocytes infiltration in asthmatic condition release the inflammatory mediators like cytokines, histamine, and major basic protein, which promote the ongoing inflammation [19]. In milk induced leukocytosis model, boiled milk showed increase in total leukocyte count. However, treatment with MELE (100mg/kg) reduced significantly total leukocyte count and thus decrease allergic asthma.

After allergen exposure, as immediate response, mast cell degranulation initiates release of inflammatory mediators and chemotactic factors. They start eosinophilia, late phase response and severe bronchoconstriction [20]. In the invitro model, clonidine induced mast cell degranulation significantly inhibited by MELE like standard mast cell stabilizer agent ketotifen. These results suggest that methanolic extract of *Luffa echinata* Roxb. fruit inhibits mast cell degranulation.

CONCLUSION

The results of our study provide evidence that methanolic extract of *Luffa echinata* Roxb. fruit produced powerful bronchodilation and reduced further inflammatory reaction by mast cell stabilizing potential. The methanolic extract of *Luffa echinata* Roxb. fruit also reduced leukocytosis and allergen induced anaphylactic hypersensitivity condition in asthmatic animal. Therefore, we can conclude that *Luffa echinata* Roxb. fruit possessed potent antiasthmatic activity. Further phytochemical investigation will be required to correlate antiasthmatic potential of *Luffa echinata* Roxb. fruit with important phytochemicals.

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