



EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF DRIED WHOLE PLANT EXTRACT OF LEUCAS ASPERA USING VARIOUS EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Asthma is a chronic inflammatory disorder of the airways. Limitations of currently available therapies and associated side effects have created an urge to search new and better treatment options. Thus, present study was designed to evaluate the anti-asthmatic activity of *L. aspera* using various experimental animal models. Methanolic extract of dried whole plant of *L. aspera* was prepared and used in the dose of 100 mg/kg; (p.o.). Various *in-vivo* models like histamine induced bronchospasm in guinea pigs, passive paw anaphylaxis in rats and milk induced eosinophilia mice and *in vitro* model like mesenteric mast cell degranulation by egg albumin and inhibition of histamine and acetylcholine induced contraction in guinea pig tracheal chain and ileum preparations were used for evaluating anti-asthmatic activity of the drug. Methanolic extract of the drug showed a significant bronchodilatory and anti-histaminic, anti-inflammatory, mast cell stabilization, and anti-cholinergic activity in histamine induced bronchospasm, passive paw anaphylaxis, degranulation of mesenteric mast cell and histamine and acetylcholine induced contraction in guinea pig tracheal chain and ileum preparations models respectively. However, significant anti-allergic effect was not observed in milk induced eosinophilia. Thus, methanolic extract of dried whole plant of *Leucas aspera* have significant antiasthmatic activity.

Key words: Antiasthmatic activity, *Leucas aspera*, Bronchospasm, Paw anaphylaxis, Mast cell stabilization, Ketotifen.

INTRODUCTION

Asthma comes from a Greek word meaning 'panting' or 'breathless'. It is a disease of the bronchial tubes that typically presents with wheezing, shortness of breath coughing, particularly in children (Holgate ST, 2008). Asthma is an allergic reaction triggering inflammation and narrowing of the airways, causing spasm and difficulty in breathing (Donno DM et al., 2000).

Asthma is a chronic lung disorder that occurs commonly in both children and adults in economically developed as well as developing countries. It is increasing in prevalence and severity especially in allergic patients (CDC, 2011). Asthma prevalence, (the percentage of

people who have ever been diagnosed with asthma and still have asthma) increased from 7.3% in 2001 to 8.4% in 2010. In 2010, an estimated 25.7 million people had asthma, 18.7 million adults aged 18 and over, and 7.0 million children aged 0–17 years (Akinbami LJ, 2012).

Asthma is characterized by airway inflammatory cells, including eosinophils, macrophages, mast cells, epithelial cells and activated lymphocytes that release various cytokines, adhesion molecules and other mediators. Inflammation results in an acute, sub-acute or chronic process that alters airway tone, modulates vascular permeability, activates neurons, increases secretion of mucus, and alters airway structure reversibly or permanently (EPR-3, 2007).

The currently available treatment for asthma most medications work by relaxing bronchospasm (bronchodilators) or reducing inflammation (corticosteroids). Though these available treatment are not efficient for treating asthma completely as they have

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many toxic and side effects (Goodman and Gillman, 2006).

Ayurveda suggests that the herbal plants have comparatively less toxic values and are more efficacious. They also have fewer chances of side effects and complications to patients as compare to available synthetic drug treatments. The Materia Medica of India suggests that the herbal drug *Leucas aspera* have anti-inflammatory and antihistaminic activity (Srinivas K et al., 2000; Vikrant Arya et al., 2011). It had effects on histamine induced bronchospasm and inflammation, mast cell degranulation and inflammatory cells like leucocytes and eosinophils. These parameters was helpful in evaluating antiasthmatic activity of *Leucas aspera* using various experimental animals like guinea pig, wistar rat and swiss mice (Khare CP, 2007).

MATERIALS AND METHODS

Animals

Healthy Wister rat weighing 200-250 gm, guinea pig weighing 350-500 gm and mice weighing 25-30 gm were used for the study. The animals were housed in under well-controlled conditions of temperature ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$) and 12h/12h light-dark cycle. Animals had received standard pellet diet (Pranav agro, Baroda) and drinking water *ad libitum*. Animals were divided into different groups for different models. The protocol of the experiment was approved by Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No.-RKCP/COL/RP/12/22).

Plant material extraction and authentication

Fresh whole plant of *Leucas aspera* was dried in the sun light. Dried whole plant were grinded in the mixer grinder and the powder was weighed. 200 gm of dried powder was taken and defatted with petroleum ether. Defatted material was extracted with methanol in soxhlet apparatus below 50°C . Extraction was carried out for 18 – 20 hours. Then plant material was removed from soxhlet apparatus and dried it till methanol gets evaporated. The methanolic extract was kept in refrigerator till use. The whole plant of *Leucas aspera* was authenticated by Department of Botany, Christ College, Rajkot (Das et al., 2011).

Preliminary phytochemical screening

ALKALOIDS: Mayer's Test: Formation of a yellow cream precipitate indicated the presence of alkaloids. Wagner's Test- Formation of brown or reddish brown precipitate indicated the presence of alkaloids. Dragendorff's Test- Formation of red precipitate indicated the presence of alkaloids. Hager's Test- Formation of

yellow colored precipitate indicated the presence of alkaloids.

FLAVANOIDS: Shinoda Test- Pink red or red coloration of the solution indicated the presence of flavonoids in the drug.

TRITERPENOIDS: Libermann-Burchard test- Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of triterpenoids (Kokate C K, 2004).

TREATMENT PROTOCOL ANTI-ASTHMATIC MODELS

In-vivo models

- 1) Histamine induced bronchospasm in conscious guinea pig.
- 2) Passive paw anaphylaxis in rat.
- 3) Milk induced leucocytosis and eosinophilia in mice.

In-vitro models

- 4) Degranulation of rat mesenteric mast cells.
- 5) Histamine and Acetylcholine induced contractions on isolated guinea pig tracheal chain and ileum preparation.

IN-VIVO MODELS

1) Histamine induced bronchospasm in conscious guinea pig.

Symptom like asphyctic convulsion resembling bronchial asthma can be induced by inhalation of histamine or other bronchospasmogen in guinea pig. The occurrence of these symptoms can be delayed by bronchodilator drugs.

Procedure

Animals with nearly same preconvulsion time were selected and randomly divided into four groups of six animals each.

Group 1: Normal control - Distilled water

Group 2: Asthmatic control- 0.5% Histamine HCl aerosol.

Group 3: Standard treatment - 0.5% Histamine HCl aerosol with Mepyramine (8 mg/kg, p.o.)

Group 4: Test treatment - 0.5% Histamine HCl aerosol with *L.aspera* Methanolic extract (100 mg/kg, p.o.)

The experimental animals were kept in a closed chamber and exposed to an aerosol of 0.5% histamine dihydrochloride and preconvulsion time was measured. Two hours after the above drug treatment, animals were exposed to histamine aerosol and pre-convulsion time was noted. As soon as dyspnea occurs, it leads to the appearance of convulsion. Animals were removed from the chamber and placed in fresh air to recover (Vogel HG, 2008; Armitage AK et al., 1961; Sheth UK et al., 1972).

Parameter

Symptoms like increased breathing frequency,

forced inspiration and asphyctic convulsion was observed.

The time taken to observe PCT (Preconvulsion time- time of aerosol exposure to the onset of dyspnoea.) was noted at day 0 and day 15.

Preconvulsion time (PCT) on day 0 (T_1) and day 15 (T_2)

% Increase in (PCT) = $1 - T_1/T_2 * 100$.

Where, T_1 = PCT on day 0,

T_2 = PCT on day 15.

2) Passive paw anaphylaxis in rat

Anti allergic model involves Passive immunization with serum containing IgE antibody and second antigen exposure cause immediate hypersensitivity in form of paw edema.

Procedure:

Preparation of anti serum: The albino wistar rats of either sex were injected intraperitoneally with 0.2 ml, 10% egg albumin and 0.2 ml of bordetella pertusis vaccine on day 1, 3 and 5. Twenty one days after the first immunization, blood was collected from retro orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C . The experimental animals were divided into six groups, six animals in each group. The drugs were administered once daily for seven days.

Group 1: Normal control - Distilled water

Group 2: Asthmatic control - 0.2 ml 10% egg albumin

Group 3: Standard treatment - 0.2 ml 10% egg albumin with Ketotifen (1mg/kg, p.o.)

Group 4: Test treatment - 0.2 ml 10% egg albumin with *L.aspera* Methanolic extract (100 mg/kg, p.o.)

Passive paw anaphylaxis: Two hours after the last dose of drug administration, rats were passively sensitized to the left hind paw with 0.1 ml of the undiluted serum. The contra lateral paw received an equal volume of saline. 24 hours after sensitization, the rats were challenged in the left hind paw with 10 μg of egg albumin in 0.1 ml of saline (Vogel HG, 2008; Yue dai et al., 1997; Jignesh patel, 2009).

Parameter

Hind paw volume was measured before and after 30 minute by volume displacement method using mercury column plethysmometer.

3) Milk induced leucocytosis and eosinophilia in mice

Leukocyte recruited during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein which promote ongoing inflammation. The eosinophils are the most characteristic inflammatory cells in the sub-mucosal and epithelial layers. The involvement of eosinophil in

bronchial mucosa, in which allergic inflammation occurs, is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion. Eosinophil secretes mediators such as eosinophil cationic protein, tumor necrosis factor, eosinophil-derived neurotoxin, and prostaglandin, which results in epithelial shedding, bronchoconstriction, and promotion of inflammation in respiratory tract often allergic.

Procedure

Mice were divided into three groups of 6 animals each. Blood samples were collected from retro orbital plexus under light ether anesthesia and following treatment was given.

Group 1: Normal control - Distilled water

Group 2: Asthmatic control - Boiled, cooled milk (4 mg/kg, s.c.)

Group 3: Treatment - Boiled, cooled milk (4 mg/kg, s.c.) with *L. aspera* methanolic extract (100 mg/kg).

Treatment drug was administered 1 hour before milk injection. Total leucocyte and eosinophil count was performed in each group before and after 24 hours after milk injection. Total and differential count was performed in high power light microscope (Vogel HG, 2008; Taur DJ et al., 2007).

Parameter

Total leucocyte and eosinophil count before and 24 hour after milk injection were measured.

IN- VITRO MODELS

4) Degranulation of rat mesenteric mast cells

Degranulation of rat peritoneal mast cells can be induced in vitro by different stimuli egg albumin, sodium cromoglycate and compound 48/80. This involves microscopic examination of rat mesenteric mast cells.

Procedure

Adult male albino wistar rats were sacrificed and pieces of mesentry with connecting lobes of fat and blood vessels were rapidly dissected out and placed in Ringer Locke solution. All the petri dishes were incubated for 30 minutes as per following schedule of treatment.

Petri dish no.1: Normal control - Distilled water

Petri dish no.2: Asthmatic control - 0.1 ml of 1% w/v egg albumin.

Petri dish no.3: Standard treatment - 0.1 ml of 1% w/v egg albumin with Ketotifen (20 $\mu\text{g}/\text{ml}$)

Petri dish no.4: Test treatment - 0.1 ml of 1% w/v egg albumin with *L. aspera* methanolic extract (100 $\mu\text{g}/\text{ml}$).

Then mesenteries were transferred to other Petri dishes containing 0.1 ml of 1% w/v solution of egg albumin for 20 minutes separately. Then all these mesenteries were transferred in 4% formaldehyde containing 0.1% toluidine blue dye and kept a side for 20

minutes. After staining and fixation of mast cells, mesentric pieces were transferred through acetone and xylene two times and mounted on slides. Six pieces of each mesentry were used for each concentration of drug. Each piece was observed under high power light microscope (Vogel HG, 2008; Norton S, 1954).

Parameter

% protection of mast cell from total of at least 100 mast cells was counted.

% protection of mast cells = total mast cells – degranulated mast cells.

5) Histamine and Acetylcholine induced contractions on isolated guinea pig tracheal chain and guinea pig ileum preparation

To detect antispasmodic and bronchodilator activity, the test compound was first added alone and washed out. The response of spasmogens like Histamine and Acetylcholine were taken and at the height of contraction and the treatment drug was added. It causes relaxation in dose dependant manner.

Procedure

a) Isolated guinea pig tracheal chain preparation

Guinea pigs were stunned by a sharp blow on the head and sacrificed by cutting neck blood vessels. The trachea was rapidly dissected free of surrounding tissues and placed in Petri dishes containing oxygenated Krebs' solution (NaCl - 114.0mM; CaCl₂ - 2.5 mM; KCl - 4.7 mM; glucose - 11.7 mM; NaHCO₃ - 25 mM; MgCl₂ - 1.2 mM; KH₂PO₄ - 1.2 mM). Trachea was sectioned into 12 rings of about the same width and connected by means of short loops of silk thread. Tracheal chains were suspended in organ tubes filled with 20 ml of krebs' solution and equilibrated under a uniform tension of 500 mg. The bathing solution was bubbled with 95% O₂ and 5% CO₂ at 37 ± 1⁰C. The tissues were equilibrated for a period of 30 minutes. The PSS in organ bath was changed every 10 minutes. The responses to histamine and acetylcholine were recorded using student physiograph (Bio Devices) using isotonic transducer. The effect of extract of treatment drug and its interaction with contractile response was recorded (Vogel HG, 2008; Kulkarni SK, 2005).

b) Isolated guinea pig ileum preparation

Guinea pigs were sacrificed using cervical dislocation method and ileum was quickly dissected out and cut into pieces. Ileum piece was suspended in organ bath containing 20 ml of Tyrode's solution (NaCl - 137.0mM; CaCl₂ – 1.8 mM; KCl – 2.7 mM; glucose – 15.55 mM; NaHCO₃ – 11.9 mM; MgCl₂ - 1 mM; NaH₂PO₄ – 0.4 mM) and was maintained at 37 ± 1⁰C under basal tension of 500 mg. The bathing solution was

bubbled with 95% O₂ and 5% CO₂. The tissues were equilibrated for a period of 30 minutes. The PSS in organ bath was changed every 10 minutes. The responses to histamine and acetylcholine were recorded using student physiograph (Bio Devices) using isotonic transducer. The effect of extract of treatment drug and its interaction with contractile response was recorded (Vogel HG, 2008; Kulkarni SK 2005).

Evaluation

The Inhibition of contraction produced by spasmogens i.e., Histamine and Acetylcholine was inhibited by test drug MELA.

Parameter

% Inhibition of acetylcholine and histamine induced contraction was measured.

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P value of less than 0.001 was considered significant.

RESULTS

IN -VIVO MODELS

1) Histamine induced bronchospasm in conscious guinea pig

When the animals were exposed to the aerosol of 0.5% histamine, there was a bronchospasm seen in form of Pre-Convulsion Dyspnoea (PCD) at day 0. Pre-treatment with the standard drug Mepyramine (8mg/kg, p.o.) and *L. aspera* methanolic extract (100 mg/kg, p.o.) given 2 hour before aerosol exposure to guinea pigs significantly increased Preconvulsion dyspnoea Time (PCT) (p < 0.001) at day 15. (Table 1) No convulsion was found in normal group.

2) Passive paw anaphylaxis in rat

Administration of Egg albumin into the hind paw of rats (passively sensitized with serum containing IgE against egg albumin) produced a rapidly developing edema observed 24 hour after serum injection and the paw attaining normality in 6 hours. The influence of drugs on paw edema induced during passive paw anaphylaxis was evaluated.

Treatment with *L. aspera* (100 mg/kg, p.o.) and standard drug Ketotifen (1 mg/kg, p.o.) produced significantly less edema (p<0.001) at 24 hour as compared to model control animals. Treatment with the standard drug Ketotifen (1mg/kg, p.o.) also produced significantly less (p<0.001) edema as compared to the model control animals. (Table 2)

3) Milk induced leucocytosis and eosinophilia in mice

Subcutaneous administration of boiled and cooled milk (4 mg/kg) into the Swiss albino mice acts as antigen and produced allergic response in mice. The total leucocyte and eosinophil count was increased 24 hour after milk injection in control and methanolic extract of *L. aspera* (100mg/mg) where as normal group respectively had no effect. The total leucocyte and eosinophil count in control group was significantly higher as compared to methanolic extract of *L. aspera* group ($p < 0.001$) (Table 3 and Table 4).

IN- VITRO MODELS

4) Degranulation of rat mesenteric mast cells

The petri dish containing mesenteries were incubated for 30 minutes and treated with egg albumin. The mesenteric pieces stained and fixed with toluidine blue dye and degranulated mast cells were counted. % Protection of mast cell from total of at least 100 mast cells was counted. The control group showed degranulation of mast cell while groups treated with MELA (100 µg/ml) and Ketotifen (20µg/ml) significantly ($P < 0.001$) protect degranulation of mast cells. (Table 5)

5) Histamine and Acetylcholine induced contractions on isolated guinea pig tracheal chain and ileum

preparation

Histamine (30µg/ml) and Acetylcholine (20µg/ml) were added in organ bath and dose dependent contractions were recorded. The modified physiological salt solution containing *L. aspera* methanolic extract (100µg/ml) significantly ($P < 0.001$) inhibited contractile effect of Histamine (46.8±0.96) and Acetylcholine (34.25±0.79) as compared to control group (62.2±0.72) and (63.05±0.27) respectively. There was a right side shift of dose response curve of Histamine and Acetylcholine in the presence of methanol extract of *L. aspera*.

6) Histamine and Acetylcholine induced contractions on isolated guinea pig ileum preparation

Histamine (10µg/ml) and Acetylcholine (10µg/ml) were added in organ bath and dose dependent contractions were recorded. The modified physiological salt solution containing *L. aspera* methanolic extract (100µg/ml) significantly ($P < 0.001$) inhibited contractile effect of Histamine (39.2±0.38) and Acetylcholine (50.97±0.56) as compared to control group (60.4±0.75) and (65.02±0.87) respectively. There was a right side shift of dose response curve of Histamine and Acetylcholine in the presence of methanol extract of *L. aspera*.

Table 1. Effect of methanolic extract of *Leucas aspera* on histamine induced bronchospasm in conscious guinea pigs

| Treatment Groups | % Increase in PCT (sec) |
|---------------------|-------------------------|
| Disease control | 4.16±0.83 |
| Mepyramine (8mg/kg) | 58.5±0.22 * |
| MELA (100mg/kg) | 47.83±0.47 * |

*Significantly different from disease control group. ($p < 0.001$)

DC- Disease control

MELA- Methanolic Extract of *Leucas aspera*,

PCT- Preconvulsion Time

The value were expressed as Mean ± SEM (n=6).

Table 2. Effect of methanolic extract of *Leucas aspera* on passive paw anaphylaxis in rats

| Treatment Groups | Difference in hind paw volume (ml) |
|--------------------|------------------------------------|
| Disease control | 0.55± 0.0079 |
| Ketotifen (1mg/kg) | 0.25±0.0042 * |
| MELA (100mg/kg) | 0.34±0.0055 * |

*Significantly different from disease control group. ($p < 0.001$).

DC- Disease control

MELA- Methanolic Extract of *Leucas aspera*.

The value were expressed as Mean ± SEM (n=6).

Table 3. Effect of methanolic extract of *Leucas aspera* on milk induced leucocytosis in mice

| Treatment Groups | Difference in total leucocyte count (cu/mm) |
|------------------|---|
| Disease control | 44.83± 15.78 |
| Milk (4 mg/kg) | 4533±56.18 * |
| MELA (100mg/kg) | 3530±44.74 * |

*Significantly different from disease control group. ($p < 0.001$).

DC- Disease control

MELA- Methanolic Extract of *Leucas aspera* (100 mg/kg).

The value were expressed as Mean \pm SEM (n=6).

Table 4. Effect of methanolic extract of *Leucas aspera* on milk induced eosinophilia in mice

| Treatment Groups | Difference in eosinophil count (cu/mm) |
|------------------|--|
| Normal control | 31.00 \pm 1.24 |
| Milk (4mg/kg) | 154.33 \pm 1.26 * |
| MELA (100mg/kg) | 98.83 \pm 1.25 * |

*Significantly different from disease control group. (p < 0.001).

DC- Disease control

MELA- Methanolic Extract of *Leucas aspera* (100 mg/kg).

The value were expressed as Mean \pm SEM (n=6).

Table 5. Effect of methanolic extract of *Leucas aspera* on degranulation of rat mesenteric mast cells

| Treatment Groups | % of Disrupted mast cells |
|---------------------------|---------------------------|
| Disease control | 76.5 \pm 0.68 |
| Ketotifen (20 μ g/ml) | 36.83 \pm 0.78 |
| MELA (100 μ g/ml) | 55.39 \pm 0.85 * |

*Significantly different from disease control group. (p < 0.001)

DC- Disease control

MELA- Methanolic Extract of *Leucas aspera* (100 mg/kg).

The values were expressed as Mean \pm SEM. (n=6)

DISCUSSION

Asthma is a chronic respiratory disease affecting a large proportion of population throughout the world. Bronchial provocation with allergen induces a prompt early phase immunoglobulin E (IgE)-mediated decrease in bronchial airflow (forced expiratory volume in 1 second) followed by a late-phase IgE-mediated reaction with a decrease in bronchial airflow for 4-8 hours. Initially asthma is characterized by the presence of increased numbers of various inflammatory mediators that are eosinophils, neutrophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretions, and mucus. The cross-linkage of IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation.

The currently used drugs for the treatment of this 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. Muscle tremor and hypokalemia are major adverse effects of β_2 . Theophylline has narrow therapeutic index and requires monitoring of drug. Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and psychosis.

Thus, the better anti-asthmatic drug needs to be explored. The plant *Leucas aspera* under this study belongs to Labiatae family which has chemical constituents such as alkaloids, flavanoids, triterpenoids

which are supposed to have anti-asthmatic activity of *L. aspera*. *L.aspera* is also reported to have antihistaminic activity and anti-inflammatory activity in adjuvant arthritis and Ayurveda suggests the role of *L. aspera* in bronchial asthma. Thus, in present study, antiasthmatic activity of methanolic extract of whole plant was evaluated. Different *in-vivo* and *in-vitro* models were used to demonstrate antiasthmatic activity.

Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine aerosol 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. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and convulsions as observed in our disease control group. However, treatment with standard and methanolic extract of *L. aspera* significantly increased PCT suggesting that rug has a good bronchodilatory and anti-histaminic activity.

Inflammation is responsible for infiltration and activation of many inflammatory cells and release of mediators like serotonin, histamine and prostaglandins etc. The methanolic extract of *L. aspera* possesses significantly more anti-inflammatory activity against control animals and less anti-inflammatory activity compared to standard drug Ketotifen in passive paw anaphylaxis model. Similar results were also observed in our study in DC group by inducing paw anaphylaxis. However, treatment with standard and test drugs

significantly prevented the passive paw anaphylaxis induced edema.

Antiasthmatic activity using milk induced eosinophilia and leucocytosis model in mice involves release of various types of mediators in pathology. It was reported that subcutaneous administration of milk produces a marked increase in the leukocytes and eosinophils count after 24 hour.

Leucocytes during asthmatic inflammation release the inflammatory mediators like cytokines, secondary to adaptogen type I hypersensitivity reactions. Hence, prevention of it by MELA indicates it anti-allergic activity.

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated mast cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. However, mast cell degranulation observed in disease control group was significantly prevented by administration of egg albumin by standard Ketotifen fumarate and MELA. Thus, it indicates that MELA has significant mast cell stabilizing activity.

The Bronchial asthma is characterized by increased airway reactivity to spasmogens. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks. Beta adrenergic agonists promote bronchodilation by direct stimulation of beta adrenergic receptors in the airway smooth muscle, that lead to relaxation of bronchial smooth muscle by rapid decrease in airway resistance in vivo. Specific β_2 agonists like salbutamol, salmeterol etc. are used since long for symptomatic relief in asthma.

The methanolic extract of *L. aspera* shows inhibition of contractile response produced by spasmogens like histamine and acetylcholine on guinea pig tracheal chain and guinea pig ileum. This suggests that MELA has significant spasmolytic effect by inhibiting both histaminic and muscarinic receptors.

Drugs effective in the asthma are mostly steroidal in nature. Phytochemical profile of the plant reveals the presence of alkaloids, flavanoids, steroidal nucleus in form of triterpenoids and various saponin glycosides. The antiasthmatic activity showed by the plant may be because of these chemical moieties. However this claim demands for further research and the studies to isolate and characterize the active principles responsible for the anti-asthmatic activity.

CONCLUSION

Various chemical and physical tests confirmed the presence of alkaloids, flavanoids, steroid, triterpenoids and saponins in the whole plant of *Leucas aspera* L. In histamine induced bronchospasm in conscious guinea

histamine, and major basic protein, which promote the ongoing inflammation. An abnormal increase in peripheral eosinophil to more than 4% of total leucocytes count is termed as eosinophilia. In asthmatic patient there is increase in eosinophil count. The disease control group mice treated with boiled or cooled milk (4mg/kg, s.c.) similarly showed increase in leucocytes and eosinophil while mice treated with methanolic extract of *L. aspera* showed significant difference in leucocytes and eosinophil count. Increase in leucocytes and eosinophil in pigs, PCT was increased in methanolic extract of *L. aspera* as compared to control group. Methanolic extract of *Leucas aspera* significantly raised the PCT in histamine induced bronchospasm. Thus, anti-histaminic activity and bronchodilating activity was obtained. It also prevented paw edema in passive paw anaphylaxis model. The egg albumin induced paw edema leads to inflammation in hind paw of rat. The differences in hind paw volume after egg albumin injection was decreased in rats treated with methanolic extract of *L. aspera*. It reveals that the plant having anti-inflammatory activity. Milk induced leucocytosis and eosinophilia was not significantly decreased that much in mice pre-treated with methanolic extract of *L. aspera* before and 24 hour after milk injection. The drug treatment also significantly prevented the disruption of mast cells and release of several mediators. Acetylcholine and histamine induced contractions in guinea pig ileum and tracheal chain preparation were also significantly inhibited by administration of methanolic extract of *L. aspera*.

Thus, methanolic extract of *Leucas aspera* shows significant anti-histaminic, bronchodilatory, anti-inflammatory, mast cell stabilizing, anti-allergic and anti-spasmodic activity in various anti-asthmatic models. All over we can say that methanolic extract of *Leucas aspera* has significant anti-asthmatic activity.

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