

# Evaluation anti-asthmatic activity of hydroalcoholic extract of *Luffa cylindrica* leaves

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**ABSTRACT:** Asthma is an allergic chronic inflammatory disorder of the lung presented clinically in the form bronchial obstruction due to hyper-responsive bronchial wall inflammation and bronchial smooth muscle constriction. Conventional anti-asthmatic drugs are associated with many adverse effects as well as non-compliance and non-adherence to complicated drug regimens. Thus, to inhibit these adverse effects and to improve patient compliance, there is an unmet medical need for complementary therapies for asthma. Ayurveda has recommended a number of drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders. *Luffa cylindrica* Linn has been traditionally claimed to be used in treatment of bronchitis, bronchial asthma, skin diseases, rheumatoid arthritis, fever, etc. Considering phytochemical profile & traditional claims of *Luffa cylindrica*, the present study was designed to study anti-asthmatic activity of hydroalcoholic extract of *Luffa cylindrica* leaves (HAELC) using histamine induced contraction of goat tracheal chain, Clonidine induced catalepsy, Milk induced eosinophilia, Passive paw anaphylaxis and OVA induced airway inflammation. The total phenolic content, total flavonoid content and total alkaloid content was measured by colorimetric assay. In the present study, pretreatment with HAECL (250, 500 and 1000 mg/kg p.o.) significantly inhibited clonidine induced catalepsy, decreased milk induced eosinophilia, inhibited passive paw anaphylaxis and decreased number of eosinophil and macrophage count in the BALF in OVA induced airway inflammation. Moreover, histopathological analysis revealed that HAECL treatment suppressed the infiltration of inflammatory cells and the hyperplasia of goblet cells. Thus, the results confirmed anti-asthmatic potential of HAECL. From the results of present investigation, it can be concluded that HAECL possess significant anti-asthmatic activity which may be ascribed to its anti-allergic, bronchodilating, anti-histaminic, adaptogenic, and anti-inflammatory potential, confirming the traditional claim about *Luffa cylindrica*.

**KEYWORDS:** *Luffa cylindrica*; anti-asthmatic; anti-allergic; total phenolic content; adaptogenic.

## 1. INTRODUCTION

Asthma is an allergic chronic inflammatory disorder of the lung characterized by T-helper cell type 2 (Th2) lymphocyte mediated immune response and presented clinically in the form of sporadic or obstinate bronchial obstruction due to hyper-responsive bronchial wall inflammation and bronchial smooth muscle constriction [1]. According to WHO guesstimates around 235 million people are currently affected by asthma, children being more affected. Thus, asthma appears to be crucial public health illnesses causing death in developing as well as developed countries increasing widespread burden to affected patients and their families, if remained unchecked and untreated [2].

Currently drugs used for treatment of asthma include anti-inflammatory drugs (inhaled corticosteroids),  $\beta_2$  agonists, anticholinergics, methylxanthines, mast cell stabilizers, leukotriene antagonists, antiimmunoglobulin E (anti-IgE) antibody, to be administered for long duration which, triggers various adverse effects, like muscle tremors, restlessness, hypotension, hyperglycaemia, tachycardia, flushing, convulsions, mood changes and adrenal crisis. Also, unreasonable usage of these drugs is a chief hindrance in treatment of asthma. Principally, the irrational use of inhaled corticosteroids in children is known to disturb bone growth, and use of long acting  $\beta_2$ -adrenergic agonist alone increases morbidity. Additionally, complicated treatment regimens, poor inhalation technique and late results lead to non-compliance and non-adherence to these current anti-asthmatic drug therapies. Thus, to inhibit these adverse effects and to improve patient compliance, there is an unmet medical need for complementary therapies for asthma [3]. Ayurveda,

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an ancient system of Indian medicine, has recommended a number of drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders [4].

*Luffa cylindrica* Linn (Common name: sponge gourd, Family: Cucurbitaceae) is used in treatment of bronchitis, bronchial asthma, skin diseases, rheumatoid arthritis, fever, backache, internal bleeding, syphilis, leprosy, chest pains as well as hemorrhoids. It is known to possess carminative, laxative, emollient, expectorant and galactagogue activity [5]. The main phytoconstituents previously isolated from the plant (fruits and seeds) includes Cucurbitacins (A, B, C, D and E),  $\alpha$ -Elaterin, Amarin, Sapogenins, Epicucurbitacin, Saponins, Aminoacid, (N-ethyl asparginase, N-Hydroxy methyl asparginase), Bioflavonoids, (luteolin iroplumbagin), Flavonoids- apigenin, fatty acids (stearic acid linoleic acid) and Vitamins. Previous studies have reported hepatoprotective, anaesthetic, antiinflammatory, anthelmintic, antimicrobial, anticancer and enzyme inhibitor activity of *Luffa cylindrica* [6].

Zhou et al (2017) has reported various health benefits of apigenin viz. cytotoxic action against cancer cells, inhibition of atherogenesis, and thereby hypertension, cardiac hypertrophy, ischemia/reperfusion-induced heart injury, and autoimmune myocarditis, prevention of asthma, bleomycin-induced pulmonary fibrosis, abnormal behavior, and neural cell apoptosis. These health benefits of apigenin are suggestive of its probable therapeutic agent [7]. Considering phytochemical profile & traditional claims of *Luffa cylindrica*, the present study was designed to study anti-asthmatic activity of hydroalcoholic extract of *Luffa cylindrica* leaves.

## 2. RESULTS

### 2.1. Qualitative and quantitative phytochemical estimation

The qualitative phytochemical screening of HAELC exhibited occurrence of pharmacologically active phytoconstituents like alkaloids, flavonoids, glycosides, saponins, proteins and tannins. The results of quantitative estimation of total phenolic, flavonoid and alkaloid content of HAELC showed presence of significant amounts (Table 1).

**Table 1.** Total phenolic, flavonoid and alkaloid content of HAELC.

| Extract | Total Phenolic<br>(mg GAE/100 gm of<br>dry weight) | Total flavonoid<br>(mg CE/100gm<br>of dry weight) | Total alkaloid<br>(mg AE/100gm<br>of dry weight) |
|---------|--|---|--|
| HAELC   | 41.07  | 44.22   | 62.5   |

### 2.2. Anti-asthmatic activity

#### 2.2.1. Histamine induced contraction of goat tracheal chain

Histamine (30  $\mu$ g/ml) was added in organ bath and dose dependent contractions were recorded in the concentration range of 3-96  $\mu$ g. The modified physiological salt solution containing HAELC (500  $\mu$ g/ml) significantly ( $P < 0.01$ ) inhibited contractile effect of Histamine. There was a right side shift of dose response curve of histamine in the presence of HAELC (500  $\mu$ g/ml). The observations were given table 2.

**Table 2.** Effect of HAELC on histamine induced contraction of goat tracheal chain.

| Sr. No. | Dose of Histamine (30 $\mu$ g/ml) | Conc. in $\mu$ g | Log Dose | % Maximum Response |                  |
|---------|-----------------------------------|------------------|----------|--------------------|------------------|
|         |                                   |                  |          | Control            | HAELC            |
| 1       | 0.1                               | 3                | 0.477    | 43 $\pm$ 1.38      | 29.8 $\pm$ 0.66* |
| 2       | 0.2                               | 6                | 0.778    | 62 $\pm$ 2.30      | 48 $\pm$ 1.28*   |
| 3       | 0.4                               | 12               | 1.146    | 79.6 $\pm$ 1.36    | 62.2 $\pm$ 1.47* |
| 4       | 0.8                               | 24               | 1.380    | 90 $\pm$ 1.42      | 70.2 $\pm$ 1.53* |
| 5       | 1.6                               | 48               | 1.681    | 100 $\pm$ 0        | 81.6 $\pm$ 1.06* |
| 6       | 3.2                               | 96               | 1.982    | 100 $\pm$ 0        | 85.3 $\pm$ 2.08* |

n=3, values are expressed as mean  $\pm$  SEM

\* $P < 0.01$  compared to control group (Student t test)

### 2.2.2. Clonidine induced catalepsy

In present study, clonidine (1 mg/kg, s.c.) produced significant catalepsy in mice from 30 min which remained till 150 min. Chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited ( $P < 0.01$ ) duration of clonidine induced catalepsy compared to vehicle treated group. There was significant inhibition ( $P < 0.01$ ) of clonidine induced catalepsy in the animals pretreated with HAELC (250, 500 and 1000 mg/kg, p.o.) as shown by significant reduction in duration of catalepsy as compared to vehicle treated group. The observations were given in table 3.

**Table 3.** Effect of HAELC on clonidine induced catalepsy.

| Group | Treatment (mg/ kg,p.o.) | Duration of catalepsy (sec) at |         |          |          |          |         |
|-------|-------------------------|--------------------------------|---------|----------|----------|----------|---------|
|       |                         | 0 min                          | 30 min  | 60 min   | 90 min   | 120 min  | 150 min |
| I     | Vehicle (10 ml/kg)      | 0.00 ±                         | 78.16 ± | 145.33 ± | 206.66±  | 234.83 ± | 216 ±   |
|       |                         | 0.00                           | 4.18    | 5.62     | 7.55     | 4.90     | 10.21   |
| II    | Chlorpheniramine (10)   | 0.00 ±                         | 28.33±  | 59.83 ±  | 80.66 ±  | 102.33 ± | 90.33 ± |
|       |                         | 0.00                           | 3.89**  | 2.04**   | 2.66**   | 3.07**   | 3.18**  |
| III   | HAELC (250)             | 0.00 ±                         | 62.33 ± | 88.16±   | 122.66 ± | 157±     | 125 ±   |
|       |                         | 0.00                           | 4.81ns  | 8.73**   | 8.86**   | 7.55**   | 4.78**  |
| IV    | HAELC (500)             | 0.00 ±                         | 59.33 ± | 73.5 ±   | 113 ±    | 134.66 ± | 115 ±   |
|       |                         | 0.00                           | 4.99*   | 5.05**   | 7.61**   | 4.76**   | 2.36**  |
| V     | HAELC (1000)            | 0.00 ±                         | 27 ±    | 65.00 ±  | 93.66 ±  | 114.33 ± | 101±    |
|       |                         | 0.00                           | 3.85**  | 4.17**   | 2.29**   | 3.44**   | 1.80**  |

n=6, Values expressed as mean ± SEM

\*\* $P < 0.01$ , compared to vehicle treated group (ANOVA followed by Dunnett's test)

### 2.2.3. Milk induced eosinophilia

Subcutaneous injection of milk (4 ml/kg, s. c.) produced a significant ( $P < 0.01$ ) increase in the eosinophil count in group II (Neg control) compared to control group. Groups III, IV, and V pretreated with HAELC (250, 500 and 1000 mg/kg, p.o.) showed significant ( $P < 0.01$ ) decrease in milk-induced increase in the eosinophil count (eosinophilia). The observations were given in table 4.

**Table 4.** Effect of HAELC on Milk induced eosinophilia.

| Group | Treatment (mg/kg, p.o) | No. of Eosinophil |
|-------|------------------------|-------------------|
| I     | Control (10 ml/kg)     | 4.16 ± 0.47       |
| II    | Negative Control       | 9.16 ± 0.47**     |
|       | Milk (4 ml/kg, s.c.)   |                   |
| III   | HAELC (250)            | 5.83 ± 0.40       |
| IV    | HAELC (500)            | 2.16 ± 0.47*      |
| V     | HAELC (1000)           | 1.5 ± 0.42**      |

n=6, values are expressed as mean ± SEM; \*\* $P < 0.01$ , negative control compared to control group. (Student t test); \* $P < 0.01$  compared to control group. (ANOVA is followed by Dunnett's test)

### 2.2.4. Passive paw anaphylaxis

In control groups, egg albumin increased the paw volume in the sensitized animals, which was measured up to the time period of 3 hrs. Pretreatment with HAELC (250, 500 and 1000 mg/kg, p.o.) significantly reduced ( $P < 0.01$ ) the paw volume at 0.5, 1, 2 and 3 hr time interval compared to control group. The observations were given in table 5.

**Table 5.** Effect of HAELC on passive paw anaphylaxis in rat.

| Group | Treatment (mg/kg,p.o) | Paw Edema Volume (Percent inhibition) |                       |                       |                       |
|-------|-----------------------|---------------------------------------|-----------------------|-----------------------|-----------------------|
|       |                       | 0.5 hr                                | 1 hr                  | 2 hr                  | 3 hr                  |
| I     | Control (10 ml/kg)    | 2.44 ± 0.15                           | 2.35 ± 0.16           | 2.27 ± 0.16           | 2.13 ± 0.13           |
| II    | Dexamethasone (0.27)  | 1.14 ± 0.05** (53.88)                 | 1.01 ± 0.04** (57.03) | 0.94 ± 0.05** (58.60) | 0.88 ± 0.06** (58.69) |
| III   | HAELC (250)           | 1.97 ± 0.03 (19.27)                   | 1.62 ± 0.10** (31.36) | 1.49 ± 0.11** (34.37) | 1.43 ± 0.11** (32.87) |
| IV    | HAELC (500)           | 1.71 ± 0.05** (29.92)                 | 1.65 ± 0.05** (30.09) | 1.55 ± 0.05** (31.72) | 1.37 ± 0.05** (35.69) |
| V     | HAELC (1000)          | 1.43 ± 0.07** (41.4)                  | 1.31 ± 0.08** (44.5)  | 1.20 ± 0.07** (47.14) | 1.11 ± 0.07** (47.89) |

n=6, values are expressed as mean ± SEM

\*\*P < 0.01, compared with control group (ANOVA followed by Dunnett's test).

### 2.2.5. OVA induced airway inflammation

Montelukast (10 mg/kg) significantly reduced the total number of cells (P<0.01) in BAL compared with the untreated group of OVA sensitized rat. Treatment with HAELC (250, 500 and 1000 mg/kg) during the challenges significantly reduced the number of total cells in the BAL. Similar results were obtained for differential cell count. The observations were given in table 6.

**Table 6.** Effect of HAELC on OVA induced airway inflammation in rat.

| Group | Treatment (mg/kg,p.o)             | Neutrophils    | Lymphocytes    | Eosinophils  | Monocyte     | Macrophage   | Epithelial cell | Total cell count | OVERALL PATHOLOGICAL GRADE (LESION SCORE) |
|-------|-----------------------------------|----------------|----------------|--------------|--------------|--------------|-----------------|------------------|---|
| I     | Control (10ml/kg,p.o)             | 20 ± 0.7       | 31.2 ± 0.58    | 8.4 ± 0.24   | 8.4 ± 0.24   | 5.4 ± 0.24   | 5.4 ± 0.24      | 2043 ± 5.38**    | NAD                                       |
| II    | Neg. control Ovalbumin (20µg,i.p) | 39.6 ± 0.50    | 53.2 ± 0.37    | 9.6 ± 0.24   | 7 ± 0.31     | 10.8 ± 0.73  | 7.6 ± 0.24      | 10609 ± 6.78     | Moderate(+++)                             |
| III   | Montelukast (10)                  | 30.4 ± 0.50**  | 34.4 ± 0.87**  | 5.4 ± 0.24** | 9.6 ± 0.24** | 9.8 ± 0.20** | 6 ± 0.54**      | 4507 ± 5.38**    | Minimal(+)                                |
| IV    | HAELC (250)                       | 37 ± 0.70*     | 47.2 ± 1.11**  | 8 ± 0.54*    | 8 ± 0.31     | 6 ± 0.54**   | 8 ± 0.54ns      | 8350 ± 133.7**   | Mild (++)                                 |
| V     | HAELC (500)                       | 33.6 ± 0.24**  | 41 ± 0.70**    | 8 ± 0.44*    | 8 ± 0.31     | 8 ± 0.54**   | 8 ± 0.44**      | 6626 ± 85.18**   | Mild (++)                                 |
| VI    | HAELC (1000)                      | 34.02 ± 0.80** | 37.06 ± 0.50** | 6 ± 0.54**   | 8.8 ± 0.58** | 8 ± 0.44**   | 6.4 ± 0.40**    | 5602 ± 94.36**   | Minimal (+)                               |

Note : Overall Grade score as- NAD =No Abnormality Detected,

Minimal changes (+), Mild changes (++) , Moderate changes (+++) , Severe changes (++++).

Focal and minimal changes may not be significant for alteration of functional capacity of the organ

n=6, values are expressed as mean ± SEM; \*\*P<0.01,\*P<0.05 compared to Negative control group. (ANOVA is followed by Dunnett's test)

### 2.2.6. Histopathological study of lung

Histological analysis of the lungs from non-sensitized vehicle treated group showed normal lung histology. In contrast, histological sections of lung tissue in ovalbumin sensitized rats exhibited airway inflammation, infiltration of eosinophils, lymphocytes and sub mucosal edema of the lungs, bronchoconstriction shown as lumen plugging by mucus and cells. Treatment with HAELC (250, 500 and 1000 mg/kg) decreased infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation and bronchoconstriction which leads to normal lumen size. The observations were given in figure 1.

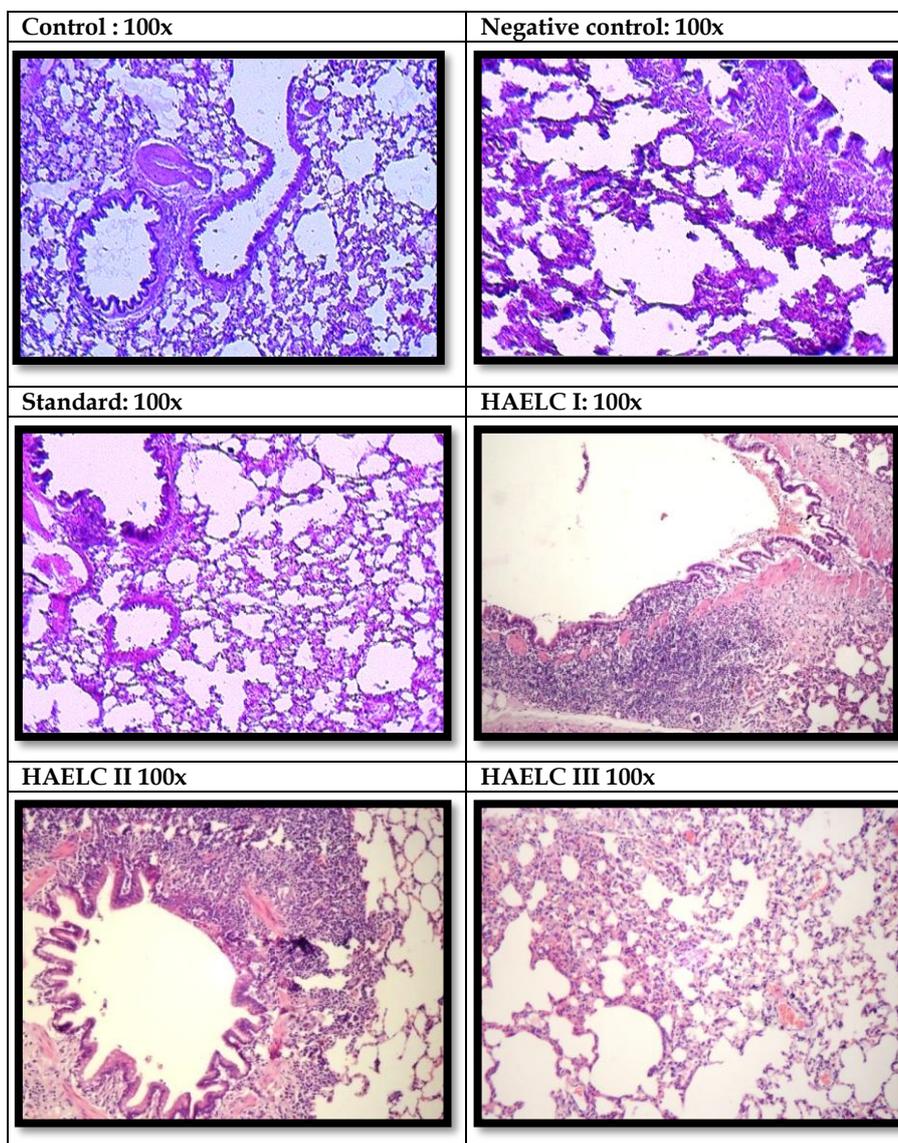


Figure 1. Effect of HAELC on histopathology of lung tissue.

### 3. DISCUSSION

Bronchial asthma is a multifactorial disease with involvement of many different chemical mediators triggering multiple biochemical reactions. Hence, it is really challenging to treat asthma with single drug. Thus, the present study was planned to evaluate the actions of *Luffa cylindrica* on various aspects of asthma like bronchoconstriction, eosinophilia, stress, mast cell degranulation and allergy associated with inflammation using various in-vitro and in-vivo animal models like isolated goat tracheal chain, milk-induced eosinophilia, passive paw anaphylaxis, Clonidine induced catalepsy and OVA induced airway inflammation. Bronchial Asthma is a chronic inflammatory respiratory disorder characterized by bronchoconstriction, bronchial hyperresponsiveness, an underlying bronchial inflammation and oedema with inflammatory cell infiltration and airway remodeling [17].

Histamine and acetyl choline are well-known chemical mediator that has been thought to have a critical role in the asthma pathophysiology. Histamine through interaction with H<sub>1</sub> histamine receptors and acetyl choline via M<sub>1</sub> muscarinic receptors contributes to bronchoconstriction, increased mucus secretion, inflammation and airway remodeling in bronchial asthma [18, 19]. In the present study, HAELC (500 µg/ml) significantly inhibited the histamine (30 mcg/ml) induced contractions on isolated goat tracheal chain preparation. This revealed that HAELC possess antihistaminic activity which may be contributing for its bronchodilator activity useful in asthma.

Further to confirm antihistaminic potential, effect of HAELC was studied on clonidine induced catalepsy which is increased histamine mediated behavior and which is inhibited by H<sub>1</sub> receptor antagonists but not by H<sub>2</sub> receptor antagonist [20]. Pretreatment with HAELC (250, 500 and 1000 mg/kg i.p.) significantly reduced duration of clonidine induced catalepsy. The maximum inhibitory effect was observed at the dose of 1000 mg/kg. This model further confirmed anti-histaminic potential of HAELC mediated through H<sub>1</sub> receptor antagonistic action. Thus, it can be concluded that HAELC possess H<sub>1</sub> receptor antagonistic action which may be responsible for its bronchodilator and therefor antiasthamtic activity.

Airway inflammatory response in asthma is dominated by eosinophil involvement contributing mainly to airways remodeling and the hyperresponsiveness, which is the constant damaging factor in asthma [21]. Eosinophilia in asthma is a physiological stress response which can be reduced by administration of an antistress or adaptogenic drug. In the present study, it has been found that, treatment with HAELC (250, 500 and 1000 mg/kg, i.p.) significantly decreased milk induced eosinophilia. Thus it can be stated that HAELC possess adaptogenic and anti-stress potential responsible for its antiasthmatic activity.

Allergens are being known as most important trigger of asthma [22]. It is recognized that exposure to an allergen initiates allergic immune response in asthma through generation of IgE antibodies by B cells against allergen. These IgE antibodies combines with high-affinity FcεR1 receptor on the surface of mast cells and basophils. Exposure to same allergen in future activates effector cells through FcεR1, which leads to release of mediators such as histamine, as well as brings into play numerous inflammatory cells causing bronchoconstriction, increased airway hyperresponsiveness, and mucus production [23, 24]. This has been scientifically studied using passive paw anaphylaxis (paw edema) [25] and OVA induced airway inflammation [23, 26, 27]. It was observed that pretreatment with HAELC (250, 500 and 1000 mg/kg, p.o) caused dose dependent reduction of paw volume in passive paw anaphylaxis model. In the OVA-induced model of allergic asthma, HAELC resulted in a significant decrease in the number of eosinophils and macrophage counts in the BALF. Moreover, histopathological analysis revealed that HAELC treatment suppressed the infiltration of inflammatory cells and the hyperplasia of goblet cells. Thus, the results confirmed antiallergic and antiinflammatory potential of HAELC and justified the use of the HAELC in the treatment of asthma and bronchitis as claimed in ancient ayurvedic system of medicine.

#### 4. CONCLUSION

From the results of present investigation, it can be concluded that HAELC possess significant anti-asthmatic activity which may be ascribed to anti-allergic, bronchodilating and anti-histaminic, adaptogenic, and anti-inflammatory potential, confirming the traditional claim about *Luffa cylindrica*. These actions may be due the synergistic action of flavonoids, alkaloids and other phenolic constituents present in *Luffa cylindrica* leaves extract. Further, thorough investigation about active phytoconstituent responsible for its anti-asthmatic activity and clinical efficacy in asthma patients needs to be explored.

#### 5. MATERIALS AND METHODS

##### 5.1. Plant material

The fresh leaves of *Luffa cylindrica* were collected from local region in Pune and authenticated by Dr. J. Jayanthi, Botanical survey of India, Western regional centre, Koregaon Road; Pune-411001. The plant sample of the same was placed in Botanical Survey of India (BSI), Pune. (Voucher Specimen No: PULUC4)

##### 5.2. Preparation of extract

About 1000 g of dry powder was defatted with Petroleum ether. The Petroleum ether extract was filtered through Watmann filter paper. The remaining marc was further extracted with ethyl alcohol and water (hydro-alcoholic 50:50) by using cold maceration for up to 72 hour with intermittent stirring. The hydro-alcoholic extract (HAELC) was filtered, concentrated under reduced pressure to a semisolid mass. The final obtained extract was weighed; percentage yield was calculated (% yield = 35.9 w/w) and stored in a cool place [8].

### 5.3. Qualitative Preliminary phytochemical screening

The preliminary phytochemical screening was carried out for presence of various phyto constituents in the extracts as per standard protocol [9].

### 5.4. Quantitative estimation

The total phenolic content was determined by using Folin -Ciocalteu assay. Total flavonoid content was measured by the aluminum chloride colorimetric assay [10]. Total alkaloid content was measured by bromocresol green colorimetric assay [11].

### 5.5. Drugs and chemicals

Histamine diphosphate (Sigma Aldrich, USA.), Clonidine (Unichem, India.), Dexamethasone (Cadila Healthcare Ltd., India.) Chlorpheniramine maleate (Research Lab Fine Chem. Industries, India), Montelukast (Dr. Reddy's laboratory) and Ethanol (S.D. Fine Chem. Limited, Mumbai, Maharashtra, India) of highest quality were used.

### 5.6. Animals

Swiss Albino mice weighing 22-25g of both sex and Sprague dawley rat weighing 150-200gm were obtained from Haffkine Institute, Mumbai, India. The animals were maintained under standard laboratory conditions at temperature  $24 \pm 2^\circ\text{C}$  and relative humidity (30-70%) with a 12:12 hr light: dark cycle throughout all the experiment. The animals were fed with standard pellet diet and free access of water. Isolated adult Goat trachea was acquired from local slaughter house instantaneously after sacrificing animal. All the procedures in this protocol were conducted as per Institutional Animal Ethics Committee (IAEC) guidelines.

### 5.7. Dose selection

Previous studies have reported that Hydroalcoholic extract of *Luffa cylindrica* leaves (HAELC) upto 5000 mg/kg dose (p.o) did not showed any signs and symptoms of any toxicity in the animals. Hence as per OECD guideline (423), the dose selected for *in vivo* models viz. clonidine induced catalepsy, milk induced eosinophilia, passive paw anaphylaxis, and OVA induced airway inflammation was 250, 500 and 1000 mg/kg (p.o.) [8, 12]. For *in vitro* model of histamine induced contraction of goat tracheal chain, dose response curve of histamine (30  $\mu\text{g}/\text{ml}$ ) was recorded in presence of HAELC (500  $\mu\text{g}/\text{ml}$ ) [13].

### 5.8. Anti-asthmatic activity

#### 5.8.1. Histamine induced contraction of goat tracheal chain

Isolated adult goat trachea was collected in ice-cold oxygenated kreb's solution, from local slaughter house instantaneously after sacrificing animal. Goat trachea was cut into individual rings and tied together to form a tracheal chain. This goat tracheal chain was mounted in organ bath containing Kreb's solution (NaCl-6.9 g/l, KCl-0.35 g/l, CaCl<sub>2</sub>-0.28 g/l, MgSO<sub>4</sub>-0.28 g/l, NaHCO<sub>3</sub>-2.1 g/l, KH<sub>2</sub>PO<sub>4</sub>-0.16 g/l, Glucose-1.0 g/l) maintained at  $37 \pm 0.5^\circ\text{C}$  with uninterrupted aeration (1 bubble/sec.). The isolated goat tracheal chain was allowed to equilibrate in Kreb's solution for 45 min. using a load of 400 mg. A dose response curve of histamine (30  $\mu\text{g}/\text{ml}$ ) was recorded in triplicate by maintaining 15 min time cycle. Then, the dose response curve of histamine (30  $\mu\text{g}/\text{ml}$ ) was recorded in presence of HAELC (500  $\mu\text{g}/\text{ml}$ ) [13].

#### 5.8.2. Clonidine induced catalepsy

Bar test as described by Kumar et al (2009) was used to study the effect of test drug extract on Clonidine induced catalepsy. Mice were divided into five groups, six animals in each group and were treated as follows:

- Group I served (Control): Vehicle (10 ml/kg, p.o.) + Clonidine (1 mg/kg, s.c.)
- Group II (Standard): Chlorpheniramine maleate (10 mg/kg, i.p.) + Clonidine (1 mg/kg, s.c.)
- Group III: HAELC (250 mg/kg p.o.) + Clonidine (1 mg/kg, s.c.)
- Group IV: HAELC (500 mg/kg p.o.) + Clonidine (1 mg/kg, s.c.)
- Group V: HAELC (1000 mg/kg p.o.) + Clonidine (1 mg/kg, s.c.)

All the groups received Clonidine, 1 hr after respective treatment and the duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 min interval [14].

#### 5.8.3. Milk induced eosinophilia

It was performed using method described by Nagore et al., (2009). Mice were divided into five groups, six animals in each group. Animals were treated as follows:

Group I served (Control): Vehicle (10 ml/kg, p.o.)

Group II (negative control): milk (4ml/kg, s.c)

Group III: HAELC (250 mg/kg p.o.) + milk (4ml/kg, s.c)

Group IV: HAELC (500 mg/kg p.o.) + milk (4ml/kg, s.c)

Group V: HAELC (1000 mg/kg p.o.) + milk (4ml/kg, s.c)

Animals belonging to group II, III, IV and V received boiled and cooled milk injection 1 hr after respective treatment. Total eosinophil count of blood samples in each group was measured [13].

#### 5.8.4. Passive paw anaphylaxis

It was performed as per procedure described previously [15] with minor modifications. Animals were divided into 5 groups each containing 6 animals. Animals were treated as follows:

Group I served (Control): 0.1ml of the undiluted serum+ Vehicle (10 ml/kg, p.o.) +10 $\mu$ g of egg albumin in 0.1ml of saline

Group II (Standard): 0.1ml of the undiluted serum+ Dexamethasone (0.27 mg/kg, i.p.) +10 $\mu$ g of egg albumin in 0.1ml of saline

Group III: 0.1ml of the undiluted serum+ HAELC (250 mg/kg p.o.) + 10 $\mu$ g of egg albumin in 0.1ml of saline

Group IV: 0.1ml of the undiluted serum+ HAELC (500 mg/kg p.o.) +10 $\mu$ g of egg albumin in 0.1ml of saline

Group V: 0.1ml of the undiluted serum+ HAELC (1000 mg/kg p.o.) +10 $\mu$ g of egg albumin in 0.1ml of saline

The vehicle, dexamethasone and HAELC were administered 24 hour after sensitization with serum. One hr after respective treatments, the animals were challenged in the left hind paw with 10mcg of egg albumin in 0.1ml of saline and the paw volume was measured by using digital plethysmometer (V. J. Instruments) and the percent inhibition of paw edema was calculated by using the formula.

$$\% \text{ Inhibition} = 1 - (V_t / V_c) \times 100 \quad [\text{Eq. 1}]$$

Where,  $V_t$  - Mean relative change in paw volume in test group and  $V_c$  - Mean relative change in paw volume in control group.

#### 5.8.5. OVA induced airway inflammation

It was carried out according to method previously described by Lee et al (2011) with minor modifications. Animals were divided into six groups with six animals in each group. On 1st day, group I to VI were sensitized with 20 $\mu$ g Ovalbumin and 2mg of alum suspended in 0.1 ml saline solution by intraperitoneal route. On 14th day, a booster injection of alum-Ovalbumin mixture was given to same group. From day 23<sup>rd</sup> to 27<sup>th</sup> respective drug treatment was given to respective group. On 28<sup>th</sup> to 30<sup>th</sup> day animals were exposed to aerosolized OVA albumin (1%) for 20 min three times a day. After 24hr of last dose of OVA, the rats were sacrificed and tracheal catheter was inserted in trachea for collecting the BAL fluid (Bronchoalveolar lavage). BAL fluid was collected by lavaging the lung with aliquots of 1ml phosphate buffer. Total recovery volume per rat was approximately 8 ml. The total cell count in of BALF was performed microscopically using Neubauer chamber. The differential leukocyte count of BALF was performed using Leishman stain. Cells

were identified as eosinophils, neutrophils, lymphocytes, monocytes, and alveolar macrophages by standard morphology and 100 cells counted.

For the histological evaluation of lung tissue, the tissues were dehydrated in various concentrations of ethanol and embedded in paraffin. A series of microsections (5 µm) were cut on a microtome and stained with H&E using standard histological techniques to assess cellular deformities due to OVA exposure [16].

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