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Evaluation of anti asthmatic activity of hydro alcoholic extract of *Citrullus colocynths* and *Cucumis trigonus* fruits

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ABSTRACT

The present study aimed to evaluate the anti-asthmatic activity of the hydro-alcoholic extract of *Citrullus colocynths* and *Cucumis trigonus* (Cucurbitaceae) fruits on Ovalbumin induced Asthma in rats. Extracts were prepared with 70% v/v ethanol using cold maceration followed by Soxhlation. Hydro alcoholic extract (200 and 400 mg/kg) of both fruits were evaluated for anti asthmatic activity on ovalbumin induced Asthma in rats with the help of parameters like, absolute eosinophil count in BALF, total leukocyte count in the BALF, absolute eosinophil count in the Blood, IgE antibodies in serum and histopathological findings of lungs. Both plants were showed significant anti asthmatic activity ($p < 0.001$) with dose of 200 & 400 mg/kg when compared with disease group. Obtained results were almost similar to that of normal group. With the same dose, *C. colocynths* was shown better anti asthmatic activity than *C. trigonus.* Hence, further detailed studies are required to evaluate the efficacy of these plants on anti-asthmatic activity.

INTRODUCTION

Asthma comes from a Greek word meaning 'panting' or 'breathless'. It is a chronic disorder of the airways that involves a complex interaction of airflow obstruction, bronchial hyper responsiveness. It is characterized by variable, recurring symptoms (wheezing, breathlessness, chest tightness, cough, sputum production) and an underlying inflammation (Busse *et al.,* 2001). 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. Asthma prevalence, an estimated 25.9 million people in U.S, including almost 7.1 million children, have asthma (NHIS, 2011). Although the prevalence of asthma in India is somewhat similar to that seen in other Asian countries, the asthma incidences have increased significantly over the years in the country. As per National family health survey of India, 2468 persons per 100,000 population are reported to be suffering from asthma, which is considerably higher in rural areas (2649 per 100,000 population) than in urban areas (1966 per 100,000 population) (Atmakuri and Dathi, 2010). The Currently available drugs for the treatment of asthma include quick relief agents (short acting inhaled β2 agonists, anticholinergics and short term systemic corticosteroids) and long term control agents (inhaled and systemic corticosteroids, long-acting β2 agonists, methylxanthenes, mast cell stabilizers and leukotrienes modifiers).

All the contemporary medicine use for the management of Asthma proves to be beneficial in acute condition and act as lifesaving remedies.

But, long term use of all these medicine can cause serious toxic side effects mainly that cause by using corticosteroid and anti-IgE antibody like omalizumab (Tripathi, 2013) (like muscle tremors, restlessness, hypotension, hyperglycemia, tachycardia, flushing, delirium, convulsions, mood changes, osteoporosis, growth retardation, adrenal crisis).

Now day's herbal medicines are the major components in Ayurvedic, homeopathic, naturopathic and other medicine systems. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on human system. The graceful advantages of herbal medicines like eco-friendly nature, less cost, great strength, less toxic and ready availability (Atmakuri and Dathi, 2010). *Citrullus colocynthis*

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and *Cucumis trigonus* L. (Cucurbitaceae) fruits are commonly known as indravaruni, used in the treatment of asthma, bronchitis, urinary discharges, jaundice, enlarged spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia, throat diseases, elephantiasis, and joint pains (Borhade *et al.,* 2013) (Arunachalam *et al.*, 2012).

MATERIALS AND METHODS

Plant material

Citrullus colocynthis and *Cucumis trigonus* (Cucurbitaceae) is collected from the sandy soil in the village Papa Raju palli, Y.S.R Kadapa district, A.P. Authentication of the plant was done by Dr. Harsha Hegde, Scientist C (RMRC, Belgaum), and a voucher specimens (RMRC-978 and 979) were deposited at RMRC (ICMR), Belgaum. The collected fruits were washed under running tap water and dried under shade, coarsely powdered and stored in neatly labeled airtight container.

Extraction

Dried powdered material was first subjected to cold maceration to extract thermo labile constituents if any with 70% v/v ethanol for 24 h, extract was filtered, and the marc was subjected for soxhlation. Filtrates of both maceration and soxhlation were combined and concentrated by using a rotary evaporator (IKA RV 10) at 40°C under reduced pressure.

Phytochemical Screening

Preliminary phytochemical tests were performed on hydro-alcoholic extract of *C. colocynthis* and *C. trigonus* for the presence of various phytoconstituents as per standard method (Trease & Evans, 2005).

Experimental Animals

Male Wistar rats (150-200 g) and housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and light (12 hr light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. Institutional Animal Ethical Committee (IAEC) as per the guidance of CPCSEA approved the experimental protocol with Resolution No. KLECOP/IAEC/Res.17-31/08/2013.

Acute oral toxicity

The acute oral toxicity of hydro-alcoholic extract of *C. colocynthis* and *C. trigonus* fruits were determined as per OECD guideline no. 425 (OECD, 2001). Based on cut off value of median lethal dose (LD_{50}), the therapeutically effective dose was derived.

Ovalbumin induced asthma in rats

Asthma is induced in rats by intra peritoneal (i.p) administration of alum precipitated Ova and Ovalbumin aerosol exposure in histamine chamber. First animals were sensitized with the alum precipitated Ovalbumin allergen through the i.p route of administration. To sensitized animals, the Ovalbumin allergen was administered through the aerosol form at 1% concentration in PBS solution for the 14^{th} day to 35^{th} day. Then the booster dose is given from the 36^{th} day to 42^{nd} day at 2% concentration of the Ovalbumin aerosol solution (Jin *et al*., 2013) (figure 1). In this method male Wistar albino, rats weighing between 150-200 gm were divided in to six groups of 6 rats in each. Group 1 serving as normal received PBS (1 ml p.o). Group 2 served as disease group received I.P administration of OVA+ OVA Aerosol. Groups 3 and 4 received (200 mg/kg, p.o) of *C. colocynthis* (CCE 1) and *C. trigonus* extract (CTE 1) + I.P administration of OVA+ OVA Aerosol. Groups 5 and 6 received (400 mg/kg, p.o) of *C. colocynthis* (CCE 2) and *C. trigonus* extract (CTE 2) + I.P administration of OVA+ OVA Aerosol.

Analysis of Broncho alveolar lavage fluid (BALF) and serum

On the 43^{rd} day, animals were anaesthetized with ether and blood collection was done by retro orbital puncture using heparin capillary tubes collected blood. Blood samples were centrifuged (15 minutes, 4°C, 2000 × g), and plasma was stored at – 70°C until use. Trachea were exposed and cannulated with polyethylene cannula for BALF that was performed by instillation of 0.5 mL of phosphate-buffered saline (PBS). The thorax was gently massaged then the BALF was withdrawn and washed with normal saline. The BALF was treated with ACK buffer, which kills RBC and WBC cells, was collected by centrifugation (10 minutes, 4°C, 5000 × g). They were stored in 20% foetal bovine serum in RPMI solution. WBC cells were counted by using WBC diluting fluid. Total leukocyte count and differential leukocyte count in BALF were counted using a hemocytometer and absolute eosinophil count was done by using indirect method (Pal & Pravati, 2011).

Measurement of IgE in serum

Enzyme immunoassays kits (ICL, immunology consultant's laboratory, Inc. USA.) according to the manufacturer's protocol determined levels of IgE in sera.

Lung tissue histopathology

Lungs were harvested after performing BALF, the right lung was fixed with 10% buffered formalin, cut into sections, stained with hematoxylin and eosin and Inflammatory parameters in lung tissue (peribroncheal, perivascular and parenchymal infiltration of inflammatory cells) were examined under microscope to evaluate the severity of inflammation. Inflammatory infiltrates were further characterized according to cell type on a morphologic basis.

Statistical Analysis

Results were expressed as Mean ± S.D., where n= 6. Differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison test (Graph Pad Prism software, version 5.01). $p<0.05$ was considered statistically significant.

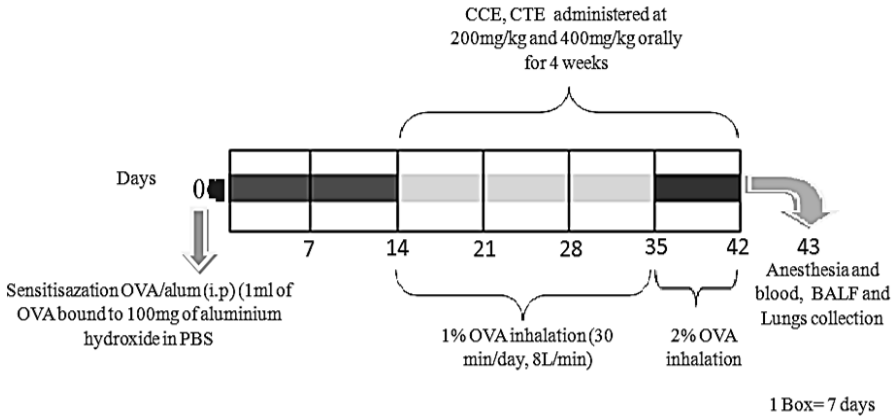

Fig. 1: Ovalbumin treatment for induction of asthma.

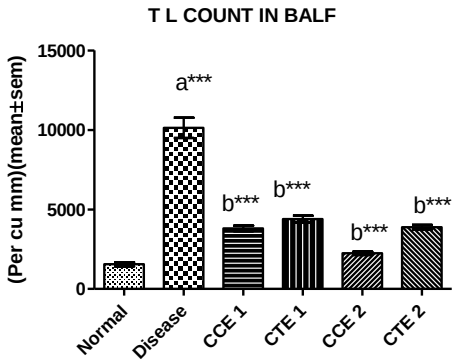

Fig. 2: Effect of hydro alcoholic extract of *Citrullus colocynthis* and *Cucumis trigonus* fruit on Total Leukocyte Count in BALF in control and experimental rats. a-when compared with Control; b-when compared with Asthma group., P<0.001; P<0.05; ns-no significance.

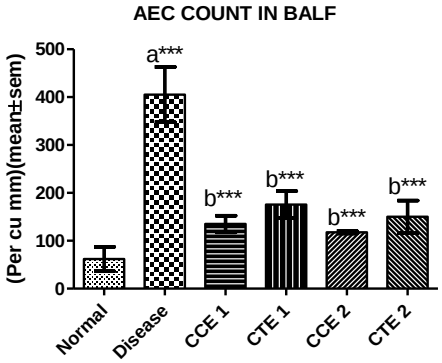

Fig. 3: Effect of hydro alcoholic extract of Citrullus colocynthis and Cucumis trigonus fruit on AEC of BALF in control and experimental rats. a-when compared with Control; b-when compared with Asthma group., P<0.001 ; P<0.05; ns-no significance.

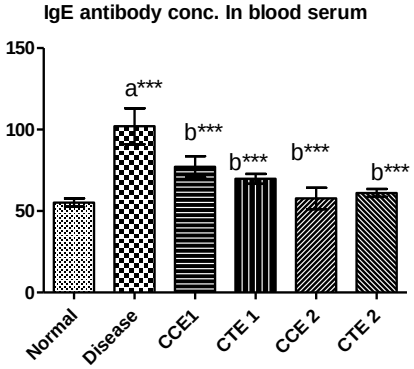

Fig. 4: Effect of hydro alcoholic extract of Citrullus colocynthis and Cucumis trigonus fruit on IgE antibody concentration in blood serum in control and experimental rats. a-when compared with Control; b-when compared with Asthma group., P<0.001 ; P<0.05; ns-no significance.

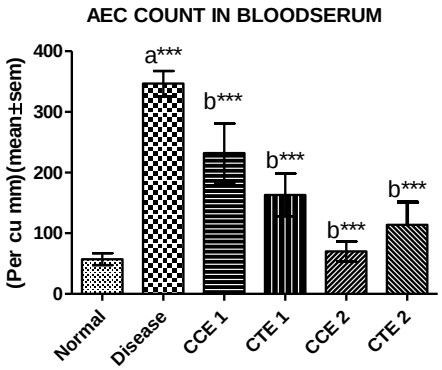

Fig. 5: Effect of hydro alcoholic extract of Citrullus colocynthis and Cucumis trigonus fruit on AEC of blood serum in control and experimental rats. a-when compared with Control; b-when compared with Asthma group., P<0.001 ; P<0.05; ns-no significance.

RESULTS

Preliminary phytochemical screening of extract of *C. colocynthis* and *C.trigonus* showed the presence of flavonoids, phenols, steroids, tannins, Diterpenes, and glycosides. There is a significant increase in eosinophil, total leucocytes count in BALF and serum IgE, eosinophil count in asthma induced group with respect to the normal group. The elevated levels are decreased to normal level in Asthma ± CCE1, Asthma ± CCE2, Asthma ± CTE1, and Asthma ± CTE2 groups, which indicate the activity of the test sample was good towards the asthma. *C. colocynthis* and *C. trigonus* (200 and 400 mg/kg) significantly reduced the total leucocytes, absolute eosinophils ($p < 0.001$) in BALF and serum IgE, absolute eosinophils ($p < 0.001$) compared with the disease group. Among all CCE2 was shown best results, which was all most comparable with normal group.

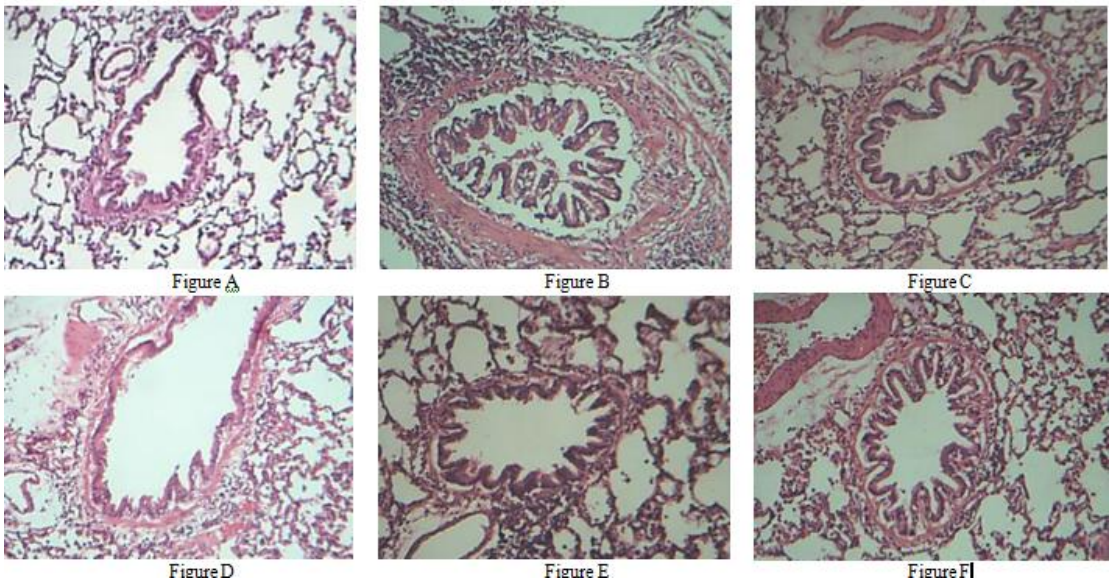

Fig. 6 : Effect of *C. colocynthis* and *C.trigonus* fruit extracts on rat lung tissue.
Figure A –normal group, Figure B- disease group, Figure C- CCE 1, Figure D – CTE 1, Figure E- CCE 2, and Figure F- CTE 2

Histopathology

Histological analysis of the lungs from normal group i.e. group I showed normal lung histology (Figure A). Histological sections of lung tissue from group II (disease group) exhibited airway inflammation, infiltration of eosinophils, lymphocytes, and sub mucosal edema of the lungs, bronchoconstriction shown as lumen plugging by mucus and cells (Figure B). Treatment with *C. colocynthis* and *C. trigonus* extracts i.e. group III, IV, V and VI prevented the tissue edema, epithelial cell hypertrophy, infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation and bronchoconstriction, which leads to normal bronchiole size (Figure C, D, E& F). Among all group V (CCE 2) Shown best results, which was all most comparable with that of normal group.

DISCUSSION

Bronchial asthma commonly characterized by increased airway reactivity to different spasmogens. An initial attack of asthma is triggered by the release of inflammatory mediators like histamine, acetylcholine, leukotrienes, prostaglandins, or specific exposure of allergens, which reflect the signals of acute bronchoconstriction (Nelson, 2003) (Bousquet *et al.*, 2000). The cross-linkage of IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that continue the airway inflammation. The elevated number of the inflammatory cells like eosinophils, lymphocytes in blood serum and biopsies of lung tissue, bronchial alveolar lavage fluid reflects the signs of asthma. These lymphocytes-induced airway inflammatory cells infiltration, eosinophils activation, IgE production, and mucus secretion, which resulted as bronchial hyperactivity (Barnes, 1998). On the contrary, serum IgE binds to allergens and triggers the release of substances from mast cells that can cause inflammation (Srinivas *et al*., 2003). In the present study, *C. colocynthis* and *C. trigonus* extracts (200 & 400 mg/kg) treated ovalbumin-sensitized rats significantly decreased the levels eosinophils and total leucocytes in the BALF and IgE levels and eosinophils concentration in serum when compared with disease group. These results shown that the protective effect of *C. colocynthis* and *C. trigonus* extracts by preventing the infiltration of inflammatory cells, by decreasing the release of preformed inflammatory mediators, which can prevent the direct damage to airway and its hyper responsiveness. Phytochemical screening of *C. colocynthis* and *C. trigonus* showed the presence flavonoids, phenols, steroids, tannins, Diterpenes, and glycosides. Several flavonoids are known to possess various biological activities, including smooth muscle relaxant, bronchodilator, antibacterial, spasmolytic, and anti-inflammatory effects (Matsuda *et al.*, 2003). Steroids, and terpenoids were responsible for broncho spasmolytic action by relaxing tracheobronchial tree of lungs (Hazekamp *et al.*, 2001).The antiasthmatic activity of *C. colocynthis* and *C. trigonus* extracts may be due to presence of the above constituents (Taur & Patil , 2011). Some of the physicians are using these two botanical sources as Ayurvedic drug ‘indravaruni’ and claimed that these two plants have similar therapeutic properties. The present experimental study is the evidence that, these two sources exhibit varying activity. Hence, based on this study results, the biological source of ‘indravaruni’ may be the fruits of *Citrullus colocynthis*.

CONCLUSION

The results obtained in the present study proved that *C. colocynthis* and *C.trigonus* (200 & 400 mg/kg) possess significant antiasthmatic activity. *C. colocynthis* was shown better anti asthmatic activity than *C.trigonus*. *C. colocynthis* may be used as ‘indravaruni’ due to its more bronchodilation, and anti-

inflammatory property, suggestive of its potential in treatment and prophylaxis of asthma. Further detailed study needs to be proceeded to evaluate the principle compounds responsible for the antiasthmatic activity.

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