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Review Article

EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF *CENTRATHERUM ANTHELMINTICUM* LINN. IN EXPERIMENTAL ANIMALS

Samir Shah*, Kintu Patel and Kerai Suraj

Department of Pharmacology, Sardar Patel College of Pharmacy, Bakrol - 388 315, Dist-Anand, Gujarat, India.

ABSTRACT

Purpose: The aim of study was to evaluate the scientific basis for the traditional use of *Centratherum anthelminticum* Linn. seeds in asthma.

Design/Methodology: In the present study, methanolic extract of *Centratherum anthelminticum* seeds was evaluated for preliminary phytochemical study and antiasthmatic activity by Histamine induced preconvulsion dyspnoea (PCD), Mast cell degranulation (MCD) by compound 48/80 and Egg albumin induced asthma at different dose levels. In PCD animals were divided into three groups and % increase in time of PCD was recorded. In MCD, animals were divided into seven groups and % protection of mast cells was measured. In egg albumin induced asthma, animals were divided into five groups. Broncho alveolar fluid was collected and evaluated. Dunnett's t-test and post hoc test were used for statistical analysis.

Findings: Phytochemical study revealed the presence of flavonoids, proteins, Saponins, tannins, steroids, glycosides and phenols. The results of pharmacological studies showed that the methanolic extract of *C.anthelminticum* significantly decreased bronchospasm induced by histamine and protected the mass cell degranulation as compared to the control groups. It also significantly decreased the total leukocyte and different WBC count in egg albumin induced asthma.

Conclusions: Methanolic extract of *C.anthelminticum* seeds possess antiasthmatic activity. Antiasthmatic action of *C.anthelminticum* could be due to its antihistaminic, mast cell stabilizing property and preventive effect on infiltration of inflammatory cells.

Key words: *Centratherum anthelminticum Linn. , Histamine induced preconvulsion dyspnoea (PCD), Mast cell degranulation (MCD), Egg albumin induced asthma, compound 48/80.*

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INTRODUCTION

Bronchial asthma is heterogeneous pulmonary disorder characterized by recurrent episodes of cough, breathlessness and wheezing, which may resolve spontaneously or after the use of bronchodilator medication.[1] The global prevalence of asthma is anticipated to be approximately 4.5 percent.[2,3] There are about 334 million patients with asthma affecting all age groups, across the world. The prevalence of asthma has increased over time and an additional 100 million people worldwide are expected to develop asthma by the year 2025. In the Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH), a survey conducted in two phases across 16 centers in India, the prevalence of asthma in adults was 2.05 per cent, with an estimated burden of 17.23 million.[4] A recent analysis using three

Correspondence Address:

Dr. Samir K. Shah

Department of Pharmacology,
Sardar Patel College of Pharmacy,
Bakrol - 388 315,
Dist-Anand, Gujarat, India.
Contact No: 9924990795
Email ID: samirkshah77@gmail.com

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different estimate models (INSEARCH, GINA and WHO survey) suggests that the prevalence of asthma in India varies between 2.05 to 3.5 per cent (17-30 million patients).[5] The estimated cost of asthma treatment per year for the year 2015 has been calculated to be approximately 139.45 billion. An estimated 15 million disability adjusted life in years (DALYs) are lost due to asthma.

Currently synthetic drugs such as bronchodilators, methylxanthines, Mast cell stabilizers, Leukotriene inhibitors, steroids are used for treatment. Although synthetic drugs give instant relief from symptoms of asthma, but they cause lot of undesirable effects (like those of steroids). Moreover, their efficacy goes on decreasing with their continuous use. So, Patients are seeking for complementary and alternative medicine for asthma because of side effect like tachycardia muscle tremor and convulsions. Herbal drugs like on the other hand, provide prolonged effects and have less side effect.[6]

Various herbal plants like Bacopamonnieri L., Eclipta alba Linn, Lepidiumsativum Linn, Menthaspicata L, Piper betel Linnand Euphorbia hirta possesses antiasthmatic activity.[7]

C.anthelminticum has been traditionally applied as anthelmintic, stomachic, digestive, diuretic, tonic, alterative, anti-phlegmatic, anti-asthmatic, anti-phlegmatic treatment, as well as a therapeutic agent for cough, diarrhoea, skin diseases, ulcers, leucoderma and fevers.[8, 9,10.]

Experimental investigations on the extracts or pure compounds isolated from the plant indicated a vast variety of pharmacological effects, including anti-inflammation/anti-pyretic[10], anti-helminthic[11], anti-filarial[12], anti-cancer[13], anti-diabetic[14], melanogenesis[15]. But the anti-asthmatic activity of *Centratherum anthelminticum* was not scientifically proved. The present study was carried out to evaluate the antiasthmatic activity of *Centratherum anthelminticumin* experimental

MATERIALS AND METHODS

Procurement and Authentification of Plant:

The Centratherum anthelminticum seeds were collected from the commercial market. The Plant was authenticated by Medicinal Plants Survey and Collection unit, Government Agriculture University, Anand, India. A sample was preserved at institute as herbarium No. SPCP/Herbarium-002/2013.

Preparation of extract :

Seeds were washed with water and dried. Coarse powder of desired particle size was subjected to successive extraction in a soxhlet apparatus using methanol. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste. The extract was coded as MCA (Methanolic extract of *Centratherum anthelminticum*). Percentage yield was obtained 1.3 gm. It was used to further experimental study.

Phytochemical screening :

The phytochemical tests for Flavonoids, Proteins, Saponins, Tannins, Steroids, Glycosides and Phenols were done for preliminary screening of the extract.

Experimental animals :

Experiment was conducted according to the CPCSEA guideline and the study was approved by the institutional animal Ethics Committee (SPCP/IAEC/RP-006/2012-13). Wistar rats (175-200 g) and guinea pigs (400-600 g) of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and light (12 hrs. light/ dark cycles) were used. Rats were fed with standard pellet diet and water ad libitum and Guinea pigs were fed with fresh vegetables and water ad libitum. Throughout the experiments, animals were processed according to the suggested ethical guideline for the care of laboratory animals.

Models used for antiasthmatic activity :

Histamine induced preconvulsion dyspnoea (PCD) Guinea pigs were selected and divided into three groups each containing six animals in each group.

Table 1: Experimental design for Histamine induced preconvulsion dyspnoea (PCD)

Groups	Treatments
STD	0.25 % Histamine HCl aerosol +Ketotifen (1 mg/kg, p.o) for 7 days
Test I	0.25 % Histamine HCl aerosol + Methanolic extract of C. anthelminticum (200 mg/kg, p.o) for 7 days
Test II	0.25 % Histamine HCl aerosol + Methanolic extract of C. anthelminticum (400 mg/kg, p.o) for 7 days

On day 0, the experimental animals were kept in a closed chamber and exposed to an aerosol of 0.25% histamine dihydrochloride and preconvulsion-

dysponea time was measured. As soon as dysponea occurs, it leads to the appearance of convulsion and PCD time was measured. Animals were removed from the chamber and placed in fresh air to recover. Thereafter animals of different groups were given respective treatment for 7 days. On the 7th day, after 2 hrs of the last dose, the time for the induction of PCD was recorded. The % increase in time of PCD was calculated using following formula.[16]

Calculation:

The% increase in time of PCD was calculated using following formula

% increase in time of PCD = (1-T1/T2) ×100

Where: T1 =PCD at day 0, before treatment

T2 = PCD at on day 7 after treatment

Mast cell degranulation by compound 48/80

Collection of mast cell:

Albino rats of either sex were selected and were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution into the peritoneal cavity and massage gently in this region for 90 s, to facilitate cell recovery. The peritoneum was exposed by making midline incision. The pale fluid was collected with the help of tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant liquid was discarded and pale cell palette was collected. The cell pellets were resuspended in fresh buffer and recentrifuged.

The peritoneal cell suspension was divided in seven parts.

Table 2: Experimental design forMast cell degranulation by compound 48/80

Groups	Treatments
Normal control	-
Model control	Compound 48/80
STD	Compound 48/80 + Disodium chromoglycate (10 µg/mL)
T1	Compound 48/80+Methanolic extract of C. anthelminticum(100 mg/kg)
T2	Compound 48/80+Methanolic extract of C. anthelminticum(200 mg/kg)
T3	Compound 48/80+Methanolic extract of C. anthelminticum(500 mg/kg)
T4	Compound 48/80+Methanolic extract of C. anthelminticum(1000 mg/kg)

Each containing 0.1 ml of cell suspension and incubated at 37°C in water bath for 15 min. Then, 0.1 mL of compound 48/80 was added in all samples except in Normal control group and suspensions was again incubated for 10 min at 37°C. The cells were then stained with 10% of toluidine blue solution and observed under the higher magnification by microscope. The percent granulated and degranulated mast cells were counted in each group. [17]

Method of measurement of granulated/ degranulated mast cell:

For the observation of the cells under light microscope, small drop of the cell suspension was put on the slide. Then, 10% of toluidine blue stain solution was poured drop wise (care should be taken such that the stain is not getting dried up). A drop of cedar wood oil was placed. The slide was examined under oil immersion lens. An observation chart having 100 squares was prepared and each cell was identified. All the cells in the view were identified without ignoring any cell. The Granulated and degranulated mast cell count was obtained directly from the observation chart by summing up total number of granulated and degranulated mast cell.

Egg albumin induced asthma

Guinea pigs were selected and divided in five groups and each group containing six animals.

Table 3: Experimental design for Egg albumin induced asthma

Groups	Treatment
Normal control	-
Model control	Sensitized with egg albumin (1 ml, 10% w/v, i.p.)
STD	Egg albumin + Dexamethasone (5 mg/kg; p.o.)
Test I	Egg albumin + Methanolic extract of C. anthelminticum(200 mg/kg; p.o.)
Test II	Egg albumin + Methanolic extract of C. anthelminticum(400 mg/kg; p.o.)

All groups were sensitized with egg albumin (1 ml, 10% w/v, i.p.) except group I on the 1st day. The animals of group III was dosed dexamethasone and group IV and group V were dosed methanolic extract of seeds of C. anthelminticum for 15 days. Two hour after the last dose of drug administration (on 15th day), all the groups were again challenged with egg albumin (0.5 ml, 2% w/v , i.v.) except group I through saphenous vein. After 3 h of the challenged of the egg albumin or just prior to death of animals which ever was earlier. The trachea was immediately cannulated after anaesthetization and the airways lavage with saline at 25°C (two aliquots of 1mL/100g body weight). Broncho alveolar cells were collected in two successive lavages using saline and recovered through a tracheal cannula. The BALF

was stored on ice and total WBC cell count was performed using light microscope. Dilutions of lavage fluid (1 in 10) were made in saline and differential WBC was counted by light microscopy stained with Leishman's stain. At least 200 cells were counted on each slide. Cells were differentiated using standard morphological criteria. All differential cell counts were performed blind and in randomized order at the end of the study. The result obtained where compared with controlled with sensitized group and sensitized with treated groups.[18]

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test in mast cell degranulation and Egg albumin induced asthma and t-test in PCD model using graph pad prism software.

RESULTS

Phytochemical screening:

The methanolic extract of *Centratherum anthelmin-ticum* was subjected to phytochemical evaluation. The results revealed the presence of Flavonoids, Proteins, Saponins, Tannins, Steroids, Glycosides and Phenols.

Histamine induced preconvulsion dyspnoea (PCD)

Methanolic extract of *C. anthelminticum* significantly increased the time of PCD following exposure to histamine aerosol induced bronchospasm in guinea pigs. Extract of *C. anthelminticum* dose dependently increased in PCD. Percentage PCD was significantly (#P<0.05) increased in animal groups treated with standard ketotifen 1 mg/kg and test drug 200 and 400 mg/kg than before treatment.

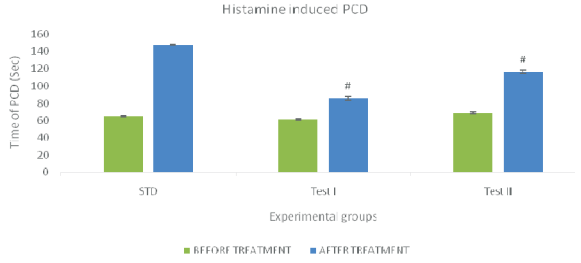

Fig. 1: Effect of Methanolic extract of *Centratherum anthelminticum* on PCD.

All values are expressed as Mean± SEM for each group (n=6).Statistical analysis was carried out by t-test.
#indicates significant difference from before treatment at P<0.05

Mast cell degranulation by compound 48/80

Methanolic extract of *C. anthelminticum* and disodium chromoglycate significantly ($p < 0.05$) inhibit in vitro rat peritoneal mast cell degranulation induced by compound 48/80 as compared to base line value i.e. model control group. (Fig. 2; Table 4)

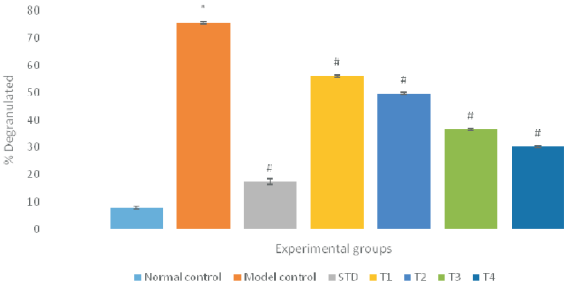

Fig. 2 : Effect of Methanolic extract of *Centratherum anthelminticum* on compound 48/80 induced mast cell degranulation.

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.
*indicates significant difference of Granulated mast cells and Degranulated mast cells from Normal control at P<0.05
#*indicates significant difference of Granulated mast cells and Degranulated mast cells from Model control at P<0.05

Table 4: Effect of *C. anthelminticum* on Compound 48/80 induced mast cell degranulation

Groups	% Granulated
Normal control	92.13 ± 0.468#
Model control	24.37 ± 0.444*
STD	82.59 ± 1.032#
T1	43.81 ± 0.511#
T2	51.23 ± 0.417#
T3	63.41 ± 0.349#
T4	69.77 ± 0.533#

Egg albumin induced asthma :

Animals from model control group showed significant increase in the total leukocytes, Neutrophil, Lymphocyte, Eosinophil and Monocyte count as compared to normal control group. While, the same was significantly decrease in treatment groups (STD, Test I and Test II) as compared to model control group. (Fig. 3; Table 5)

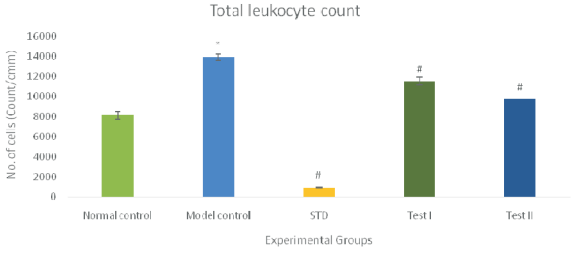

Fig.3 : Effect of Methanolic extract of *Centratherum anthelminticum* on Egg albumin induced Asthma.

All values are expressed as Mean± SEM for each group. Statistical analysis was carried out by One way ANOVA followed by Dunnet's-Post hoc test.
*indicates significant difference of Granulated mast cells and Degranulated mast cells from Normal control at P<0.05
# indicates significant difference of Granulated mast cells and Degranulated mast cells from Model control at P<0.05

Table 5: Effect of C. anthelminticum(p.o., for 15 days) on BALF in egg albumin sensitized guinea pigs

Group	Neutrophil count/cmm	Lymphocyte count/cmm	Eosinophil count/cmm	Monocytes count/cmm
Normal control	2,417±670.9	3,911±311.2	177.3±241.32	107.7±14.3
Model control	3,891±161.7*	9,239±281.3*	471.7±261.03*	271.3±25.11*
STD	2,833±40.79#	4,709±20.9#	287.1±16.81#	140.8±18.05#
Test I	3,697±217.9#	6,709±331.6#	417.1±359.15#	213.2±21.09#
Test II	3,117±23.11#	5,937±18.1#	363.5±11.26#	157.4±11.3#

DISCUSSION

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators like histamine triggered by exposure to allergens that directly cause acute bronchoconstriction.[19]

In the present study, histamine was used as spasmogens in the form of aerosol to cause immediate bronchoconstriction in the form of PCD in guinea pigs. Bronchodilating effect of methanolic extract of *C. anthelminticum* was evaluated by observing its effects on time of PCD. In our study, we found that the time of occurrence of PCD was significantly increased that suggestive of bronchodilating activity following treatment with *C. anthelminticum* against 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.[20] The cross-linkage of IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that continue the airway inflammation. In our study, treatment with methanolic extract of *C. anthelminticum* dose dependently reduce the degranulation of mast cells, which may be indicative of mast cell stabilizing property of *C. anthelminticum*.

The elevated number of the inflammatory cells like eosinophils, lymphocytes in blood serum, 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.[21] On the contrary, serum IgE binds to allergens and triggers the release of substances from mast cells that can cause inflammation.[22] In the present study, *C. anthelminticum* extract (200 & 400 mg/kg) treated animals significantly decreased the levels eosinophils and total leucocytes in the BALF and eosinophils concentration in serum when compared with disease group. These results shown that the protective effect of *C. anthelminticum* extract 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. anthelminticum* 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.[22] Steroids, and terpenoids were responsible for broncho spasmolytic action by relaxing tracheobronchial tree of lungs.[22] The antiasthmatic activity of *C. anthelminticum* extract may be due to presence of the above constituents.[22]

CONCLUSION

In the light of above finding and discussion, it can be concluded that methanolic extract of *C. anthelminticum* Linn. Increased in the time of PCD, stabilized the mast cell and also gave desired effect on the differential WBC which is indicative of its bronchodilator, mast cell stabilizing and preventive effect. These properties of the test drug are therefore may be advantageous in preventing asthma.

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