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Research Article

EFFECT OF ACETONE SOLUBLE AND ACETONE INSOLUBLE FRACTIONS OF *ALLIUM CEPA* ON SOME ANIMAL MODELS OF ASTHMA

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ABSTRACT

Current Asthma therapy lack satisfactory effect due to various adverse effects, hence patients are moving to Ayurveda. Researchers conducted studies in last decades on plants mentioned in Ayurveda used for asthama has shown Anti-asthamic, Anti-histaminic and Anti-allergic activity. Present study is conducted to study the anti-asthmatic activity of acetone soluble and insoluble extract of *Allium cepa.*

In this process dried peels of *Allium cepa* were immersed in 95% ethanol for 48hr and the extract obtained was further fractionated with anhydrous acetone to get acetone soluble (ASF) and acetone insoluble (AIF) fractions. Effect of the fractions was studied on isolated goat tracheal chain suspended in Kreb's solution and percentage contractile response was measured. Albino Swiss mice (22-25g) of either sex were subjected to milk- induced leucocytosis and eosinophilia to study adaptogenic and anti-allergic activity respectively. Effects of both the extracts were also studied on clonidine-induced catalepsy and clonidine induced mast cell degranulation in mice as a measure of central and peripheral mast cell stabilization respectively. Wistar rats (150-180g) were used to study effect of fractions on passive paw anaphylaxis to study immunomodulatory and anti-inflammatory activity.

KEYWORDS: Ayurveda, *Allium cepa*, Asthma, Adaptogenic, Anti-allergic.

INTRODUCTION

Asthma, a chronic relapsing inflammatory disease affecting millions of people worldwide is characterized by hyper-reactive airways, leading to reversible bronchoconstriction. There is increased hyper-responsiveness of tracheabronchial tree to various stimuli, some of these would have little or no effect on non-asthmatics. This increased responsiveness has also been described as hyper reactiveness by Luckman and Soranson[1] and is a factor in both intrinsic (non-immunogenic) and extrinsic (atopic) form of asthma. The major mechanisms proposed for the airway obstructions are i). bronchoconstriction due to histamine, leukotriene, LTC, etc. ii). mucosal oedema due to prostaglandins, bradykinin, platelet activating factor (PAF), leukotrienes, LTD_4 and LTE_4 iii). mucus secretion formed generally by submucosal gland, inflammatory exudate consisting of eosinophils and neutrophils, other mediators like histamine, prostaglandins, thromboxane A_2 - and PAF and iv). cellular infiltration and chemotaxis i.e., migration of inflammatory cells like neutrophils and eosinophils from the circulation to the site of inflammation presumably due to LTB_4.[2]

In ancient Indian system of medicine, a number of drugs of indigenous plant sources have been described for use in treatment of bronchial asthma[3,4]. *Allium cepa* has been reported to possess antiplatelet[5], anti-hyperglycemic[6,7], anti-hyper-lipidemic[8], immunosuppresant[9] and anti-asthmatic activity.[10]

Therefore, the objective was to study the anti-asthmatic effect of acetone soluble and acetone insoluble fractions and ethanolic extract of *A. cepa* and to find its possible mechanism of action.

MATERIALS

Plant material

The dried peel of *A. cepa* was obtained from commercial sources. The medicinal plant was identified and authenticated by Dr. S.C Pal of the Pharmacognosy Dept.

Preparation of extract

The extracts of medicinal plants were prepared by maceration method. The plant material was powdered into coarse powder and macerated with ethyl alcohol (95% v/v) for two days. The ethanolic extract was distilled to get concentrated extract. The concentrated extract was vacuum dried for overnight at 30°C. The ethanolic extract of *A.cepa* was further fractionated with anhydrous acetone to get acetone soluble (ASF) and acetone insoluble (AIF) fractions.

Test Animals and Tissue

Albino Swiss mice (22-25g), Wistar rats (150-180g) of either sex were housed in groups of five under standard laboratory condition of temperature (25 ± 2^0C) and 12/12 hr light/dark cycle. They had free access to standard pellets chow (Lipton, India ltd.). Goat trachea was procured from slaughter house and kept in Kreb's solution.

Drugs

Clonidine (Unichem, India), Histamine diphosphate (Sigma, USA), Dexamethasone (Merind, India) and milk.

Pharmacological Tests

Isolated Goat Tracheal Chain Preparation

Goat trachea was cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintained at 37 ±1^0C, a stream of carbogen was bubbled through the organ tube. One end was tied to an aerator tube and other attached to isotonic frontal writing lever. Tissue was allowed to equilibrate for 45min under a load of 400mg[12]. A dose response curve for histamine was taken in variant molar concentrations ranging from 16.5 x 10^{-8} to 131.8 x 10^8moles/litre. After obtaining a dose response curve of histamine on trachea, both the extracts (n=5) were added to reservoir separately and same doses of histamine were repeated.

Graph of maximum percentage of contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of ASE & AIE.

Milk-Induced Leucocytosis

Mice were divided into three groups, five animals each. Blood samples were collected from retro-orbital plexus under light ether anesthesia. Total leucocyte count was done in each group before drug administration and 24hr after milk (boiled and cooled) injection (4ml/kg, s.c.). Blood was sucked in WBC pipette and further diluted with WBC diluting fluid. Pipette was shaken for few seconds and kept aside for five minutes. Neubauer's chamber was charged with above fluid and Total Leukocyte Count was done. Group-I served as control and treated with vehicle and milk. Group-II and Group-III were treated with ASE and AIE of *A. cepa* (50mg/kg, p.o.) respectively. Milk was administered 1hr after administration of extracts. Difference in Total Leucocyte Count before and 24hr after drug administration was calculated.[13]

Milk-Induced Eosinophilia

The method as described by Brekhman[13] was followed. Blood samples were collected from each mouse from retro-orbital plexus under light ether anesthesia. Total eosinophil count was determined in each group before drug administration and 24hr after milk (boiled and cooled) administration (4ml/kg, s.c.). Group-I received vehicle and milk, served as control group. Group-II and Group-III were treated with ASE and AIE of *A. cepa* (50mg/kg, p.o.) respectively. Group-II and Group-III received milk after 1hr of drug administration. Difference in total eosinophil count before and 24hr after drug administration was calculated.

Clonidine-Induced Mast Cell Degranulation

4ml of normal saline was injected into the peritoneal cavity of mice. After a gentle massage, the peritoneal fluid was collected and transferred into the test tubes containing 3-4ml of RPMI-1640 buffer medium (pH 7.2-7.4). Mast cells were then washed by centrifugation at a low speed (400-500rpm) followed by discarding the supernatant and taking the pellets of mast cells into the medium. The mast cells were treated with clonidine (80mcg/ml) and incubated at 37^0C in a water bath for 10min. They were stained with toluidine blue and the percentage protection against degranulation was calculated.[14] ASE or AIE of *A. cepa* (50mg/kg, p.o.) were administered for 4 days, daily prior to collection of mast cells.

Clonidine-Induced Catalepsy

Bar test[15] was used to study the effect of extracts on clonidine-induced catalepsy. Clonidine (1mg/kg, s.c.) was injected to mice (n=5) pretreated with vehicle (10ml/kg, i.p.), ASE or AIE of *A .cepa* (50mg/kg, p.o.). The forepaws of mice were placed on a horizontal bar (1cm in diameter, 3cm above the table) and the time required to remove the paws from bar was noted for each animal. All the groups received clonidine after the last dose and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min.

Passive Paw Anaphylaxis

Antiserum to egg albumin was raised in rats using aluminum hydroxide gel as an adjuvant.

Animals were given three doses of 100 mcg of egg albumin (s.c.) adsorbed on 12mg of aluminum hydroxide gel prepared in 0.5ml of saline on 1st, 3rd and 5th day. On 10th day of sensitization, the animals were bled from the retro orbital plexus. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm.

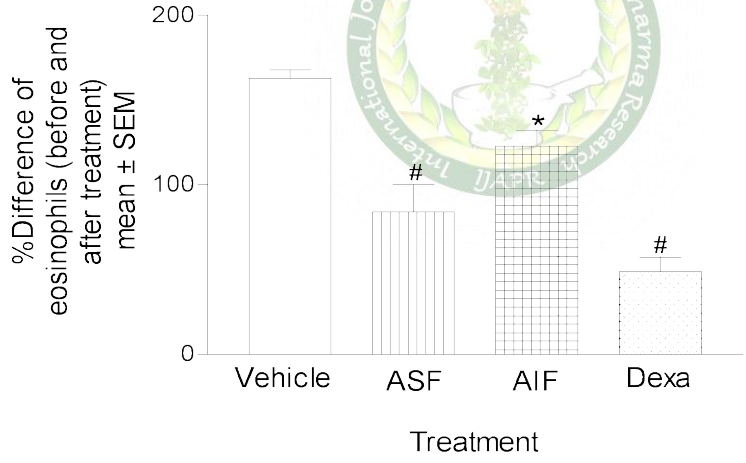
Animals were divided into 4 groups each containing 5 animals. Animals belonging too Group-I served as control and were administered only the vehicle. Group II received standard drug Dexamethasone (0.27mg/kg, s. c.). Animals belonging to Groups-III and IV received ASE & AIE of *A. cepa* (50mg/kg, p.o.). Rats were passively sensitized into the left hind paw with 0.1ml of the undiluted serum. The contra-lateral paw received an equal volume of saline. Extracts were administered 24 hour after sensitization. After 1hr of administration of extracts, the rats were challenged in the left hind paw with 10 mcg of egg albumin in 0.1ml of saline. The hind paw thickness was measured using a micrometer. The difference in the reading prior to and after antigen challenge represents the paw edema.[16]

Statistical Analysis

All observations have been presented as mean ± SEM. The data was analyzed by students t-test or One-Way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

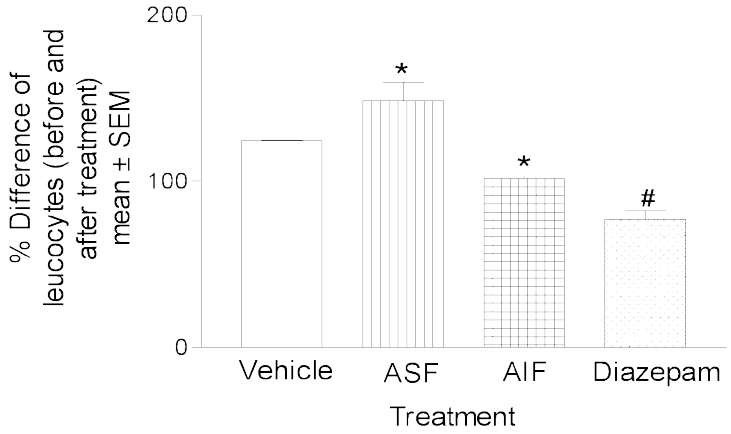
RESULTS

In the present study ASF and AIF of *Allium cepa* showed no significant inhibition of histamine induced contractions of goat tracheal chain (data not shown). ASF of *A. cepa* (148.12±10.9%) showed no significant reduction while AIF (101.56±2.27%) showed significant reduction in leucocyte count as compared to control (124.5±18.8%). In the present study, both ASF (84.16±16.06%) and AIF (122.70±9.5%) of *A. cepa* showed significant reduction of eosinophil count after milk treatment as compared to vehicle treated group (162.69±5.3%). Both the fractions of *A. cepa* significantly inhibited clonidine induced catalepsy. ASF (51±2.05%) & AIF (50±2.01%) of *A. cepa* significantly protected mast cell degranulation as compared to vehicle (25+ 1.9%). In passive paw anaphylaxis, ASF and AIF of *A. cepa* significantly reduced paw edema as compared to vehicle treated group.



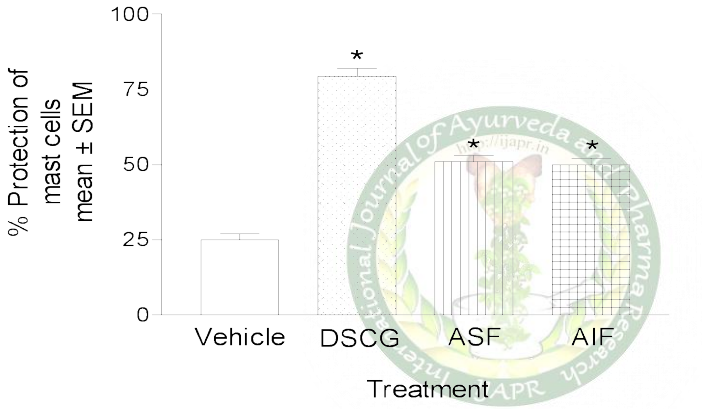
Effect of acetone soluble, acetone insoluble fractions of *A. cepa* (50 mg/kg,p.o.) and Dexamethsone on milk induced eosinophilia in mice

N = 5, #P < 0.001, *P < 0.05, one way ANOVA followed by Dunnett's test



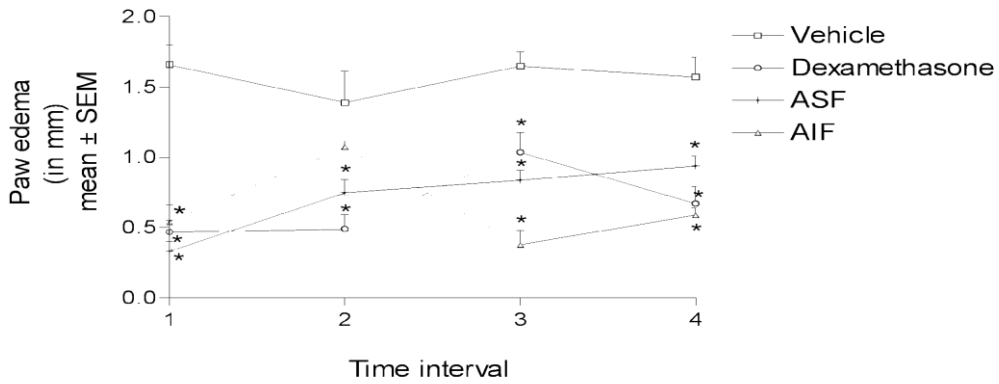
Effect of acetone soluble, acetone insoluble fractions of *A. cepa* (50 mg/kg,p.o.) and Diazepam (1mg/kg, i.p.) on milk induced leucocytosis in mice

N = 5, *P < 0.05, #P < 0.001 One way ANOVA followed by Dunnett's test



Effect of acetone soluble and acetone insoluble fractions of *A. cepa* (50 mg/kg, p.o.) on clonidine induced mast cell degranulatrion in mice

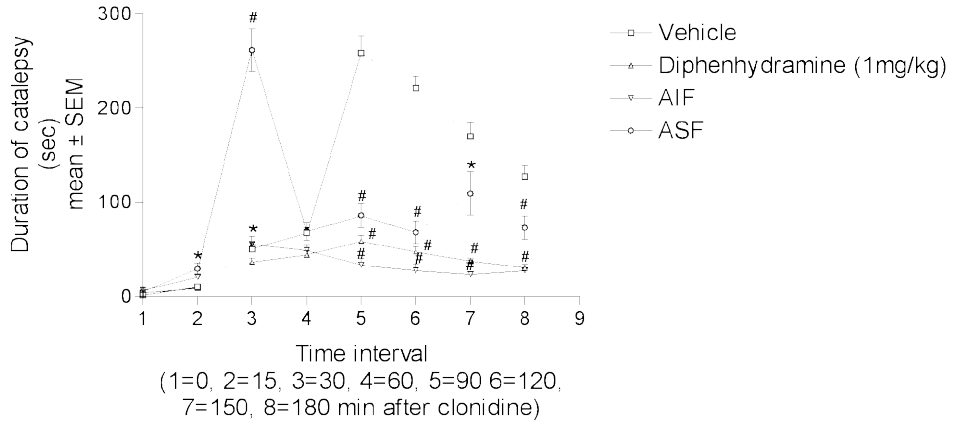
N = 5, * P < 0.001, One way ANOVA followed by Dunnett's test



(1=1/2 h, 2=1 h, 3=2 h, 4= 3 h after antigen challenge)

Effect of acetone soluble and acetone insoluble fractions of A. cepa (50 mg/kg.p.o.) on passive paw anaphylaxis in rats

N = 5,* P < 0.001, One Way ANOVA followed by Dunnett's test)



Effect of Diphenhydramine, acetone soluble and acetone insoluble fractions of *A. cepa* (50 mg/kg p.o.) on clonidine-induced catalepsy in mice

N = 5,* P < 0.05, # P < 0.001, One Way ANOVA followed by Dunnet's test

DISCUSSION

Asthma, a chronic relapsing inflammatory disease, is characterized by hyperactive airways, leading to reversible bronchoconstriction. The inflammation causes an associated increase in airway responsiveness to various stimuli[7]. Nagchaudhari and Lahiri[12] examined the suitability of goat tracheal chain for the study of action of agonist on bronchial muscle and found that both goat tracheal chain and strip preparation are suitable for screening drugs activity on respiratory smooth muscle. Kulreshtha et.al[18] demonstrated dose dependant contraction by spasmogens such as histamine (.1-.102mg) Ach (.1-12.8mcg) and $BaCl_2$ (.1-51.2mcg) using goat tracheal chain preparation. In the present study both ASF and AIF of *Allium cepa* showed no significant inhibition on histamine induced contractions of goat tracheal chain.

An adaptogen may have normalization that revealed itself irrespective of direction of previous pathological shift. Normalization effect of adaptogen is observed in milk induced leucocytosis[19]. In the present study ASF of *A. cepa (*148.61% ± 10.97) showed no significant normalization of leucocyte count after milk treatment as compared to control group (124.55%±.188), while AIF of *A. cepa* (101.56%±2.27) showed significant (P<0.05) normalization of leucocyte count indicating adaptogenic activity.

The late asthmatic phase reaction to an allergen often coincides with an increased number of eosinophils in airways[20]. Eosinophils are associated with respiratory disorders often allergic in nature together with pulmonary infiltrates that are detectable on chest film[21]. Though eosinophils can increase in number of body fluids and tissues, emphasis is placed on the number of eosinophils in the blood. Horn *et al.*[22] suggested that total eosinophil count reflect asthmatic activity and used for early detection of exacerbation. Both ASF (84.16%+16.06) (P<0.001) and AIF of *A. cepa (*122.7%+9.5) (P<0.05) showed significant reduction in eosinophil count after milk treatment as compared to vehicle treated group is suggestive of their efficacy in allergic asthma.

Unvas[23] studied the mast cell degranulation and its correlation with release of histamine after administration of compound 48/80, the mast cell degranulating agent. Both clonidine and the compound 48/80, showed dynamic expulsion of granules releasing histamine without causing any damage to cell wall[14]. It is known that DSCG, a standard mast cell stabilizer, prevents degranulation of mast cells by raising the c-AMP[24]. ASF & AIF of *A. cepa* showed significant (P<0.001) protection against clonidine- induced mast cell degranulation. This peripheral mast cell stabilizing activity signifies raised intracellular levels of c-AMP which facilitates relaxation of airway smooth muscle and inhibition of autocoids release from tissue and basophils[25]. Clonidine, an adrenoceptor agonist, induces catalepsy in mice which is inhibited by H_1 receptor antagonists, but not by H_2 receptor antagonist[26]. Muley *et.al*[27] showed that intracerebroventricular injection of histamine in conscious mice induce catalepsy, which was inhibited by H_1 receptor antagonist but not by H_2 receptor antagonist. The cataleptic effect of clonidine in mice is mediated by histamine release from mast cells that was inhibited by both ASF and AIF of *A. cepa.* This effect on catalepsy is probably due to its mast cell stabilizing or anti- histaminic activity.[28]

The major goal in asthma therapy is to treat acute exacerbation which is mainly due to immediate hypersensitivity reaction and inflammation which occurs in late phase, since inflammation is associated

with bronchial hypersensitivity and airway remodeling. Egg albumin raise antiserum when injected in to hind paw (subplanter) followed by challenge with egg albumin leads to passive paw anaphylaxis[16]. In the present study both the fractions of *A. cepa* reduced paw edema at all time intervals (P<0.001). The inhibition of primary and late phase by *A. cepa* may be due to their immune suppressant[9] and anti-inflammatory activity.

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