Beneficial Effect of *Achyranthes apsera* Linn. In Toluene-Di-Isocyanate Induced Occupational Asthma in Rats

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Abstract: In the present investigation, we have studied the bronchoprotective effect of ethanolic extract of *Achyranthes aspera* Linn. in toluene diisocyanate (TDI) induced occupational asthma in wistar rats. Wistar rats were divided into four different groups of eight animals each. All animals except control group were sensitized by the intranasal application of 10% TDI to induce airway hypersensitivity. At the end of the study, after provocation with 5%TDI the symptoms were observed in all animals. The total and differential leucocytes were counted in blood and bronchoalveolar (BAL) fluid. Liver homogenate was utilized for assessment of oxidative stress and lung histological examination was performed to investigate the inflammatory status in the airway. TDI sensitized rats exhibited asthmatic symptoms while *A. aspera* and dexamethasone treated rats did not show any airway abnormality. The neutrophils and eosinophils in blood were decreased significantly; the total cells and each different cell in particular eosinophils in BAL fluid were markedly decreased in treatment groups as compared to TDI sensitized rats. The antioxidant activity and histopathological observations also showed protective effect. From all above findings and observations, it can be concluded that *A. aspera* has beneficial role in occupational asthma.

Keywords: *Achyranthes aspera*, TDI, asthma.

INTRODUCTION

Occupational asthma is defined as a disease characterized by variable airflow limitation and/or bronchial hyperresponsiveness due to causes and conditions attributable to a particular working environment and not to stimuli encountered outside the workplace. Reports from several surveillance programs have suggested that occupational asthma is probably the most common type of occupational lung disease in industrialized countries. Occupational asthma accounted for 26% of all work-related respiratory disease reported to the Surveillance of Work and Occupational Respiratory Disease (SWORD) program in the United Kingdom [1] and 52% of such cases in British Columbia, where there is a particularly high prevalence due to the use of western red cedar [2]. In the United States, analysis of 1978 Social Security disability data indicated that approximately 15% of individuals disabled from asthma attributed it to workplace exposures [3].

Achyranthes aspera Linn. (*A. aspera*) is one of the medicinal plant used as an emmenagogue, antiarthritic, purgative, diuretic, antimalarial, oestrogenic, antileprotic, antispasmodic, cardiotonic, antibacterial and antiviral agent [4-7]. The ethanolic extract of the plant contained alkaloids and saponins while flavonoids and tannins were found absent [8]. Ethanolic extract of the leaves and stem of the plant are reported to inhibit *B. subtilis* and *S. aureus* bacterial strains at a concentration of 25 mg/ml [9]. The alcoholic extract of the plant is reported to have hepatoprotective [6], anti-fertility [10] and hypoglycemic activity [11]. Further salt which was prepared from the ash of *A. aspera* is reported to be efficacious in bronchial asthma [12]. A large number of medicinal plant preparations have been reported to possess anti-asthmatic effects. However, the reports of use of medicinal plants for occupational asthma are scanty. In the light of above mentioned facts, the objective of our present investigation was to evaluate anti-asthmatic action of plant extract of *A. aspera* in chemically induced occupational asthma.

MATERIAL AND METHODOLOGY

Preparation of Plant Extracts

The whole herb of *A. aspera* was uprooted from the campus of L. M. College of Pharmacy Ahmedabad, in the month of September-October at the end of flowering season. The plant was identified by comparing it morphologically and microscopically with description given in different standard texts and floras [13]. The plant was further identified and authenticated at the Dept. of pharmacognosy, Gujarat Ayurved University, Jamnagar, India and a voucher specimen was deposited at Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad. The plant material was cleaned and dried in shade. It was powdered, passed through 40 mesh sieve and stored at 25 ^{o}C. The coarse powder (1.0 kg) of the dried plant was extracted with alcohol using soxhlet extractor. The extract was concentrated under reduced pressure to yield a syrupy mass.

Drugs and Chemicals

All the required organic solvents were obtained from the S.D. Fine Chemicals Private Limited (Mumbai, India) and all were of analytical grade. 2, 4 Toluene Di-Isocyanate. Hydrogen peroxide, Trichloroacetic acid, thiobarbituric acid,

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Tris buffer, 5, 5´-dithiobis-2-nitrobenzoic acid (DTNB), epinephrine, dexamethasone were obtained from Sigma-Aldrich (St. Louis, MO).

Animals and Drugs

All animals were housed at ambient temperature (22 ± 1^0C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to standard pallet diet and water given *ad libitum*. The protocol of the experiment was approved by the institutional animal ethical committee 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.

Sensitization and Provocation of Animals

Wistar rats, weighing 200-250 g were divided into four different groups of eight animals each. Group-I: control (distilled water); Group-II: Diseased control; Group-III: dexamethasone (0.1mg/kg body weight [BW] *per os*, as reference standard); Group-IV: Ethanolic extract of *Achyranthes aspera* (500 mg/kg *per os*)

All the rats (except group-I) were sensitized by the modified method of Zheng *et al.* [14] by dropping 5µl of 10% toluene diisocyanate into each nostril for seven consecutive days. After a week of rest all the rats (except group-I) were resensitized for seven days. A week after the second course of sensitization the rats were provoked by intranasal administration of 5 µl of 5% toluene diisocyanate. All the animals of group III, and IV were given the respective drug treatment from one week prior to the initiation of sensitization till the day of final challenge with 5% toluene diisocyanate. The last dose of drug treatment was given 2 hrs prior to challenge with 5% toluene diisocyanate. The rats of group-I were treated with intranasal ethyl acetate and provoked with 5% toluene diisocyanate in the same protocol.

Blood Cell Collection and Serum Preparation

After the airway symptoms have been observed, blood was collected (under light ether anesthesia) from the retro-orbital veins of each animal and each sample was used for total and differential leukocyte count.

Bronchoalveolar Lavage and Cell Count

The tracheobronchial tree was lavaged with 10 ml of saline at 37ºC and the BAL fluid so collected was centrifuged at 2000 rpm for 5 min. at 4ºC. The pellets were resuspended in 0.5 ml saline and was used for the total and differential leukocyte count under the microscope 450 x magnifications.

Assessment of Pro-Oxidant and Antioxidant Enzyme Levels

All the animals were sacrificed by cervical dislocation and their livers isolated. Each liver was homogenized in Tris HCl buffer (0.01 M, pH. 7.4) using a REMI homogenizer (REMI Motor, Bombay, India) to generate a 10% homogen-

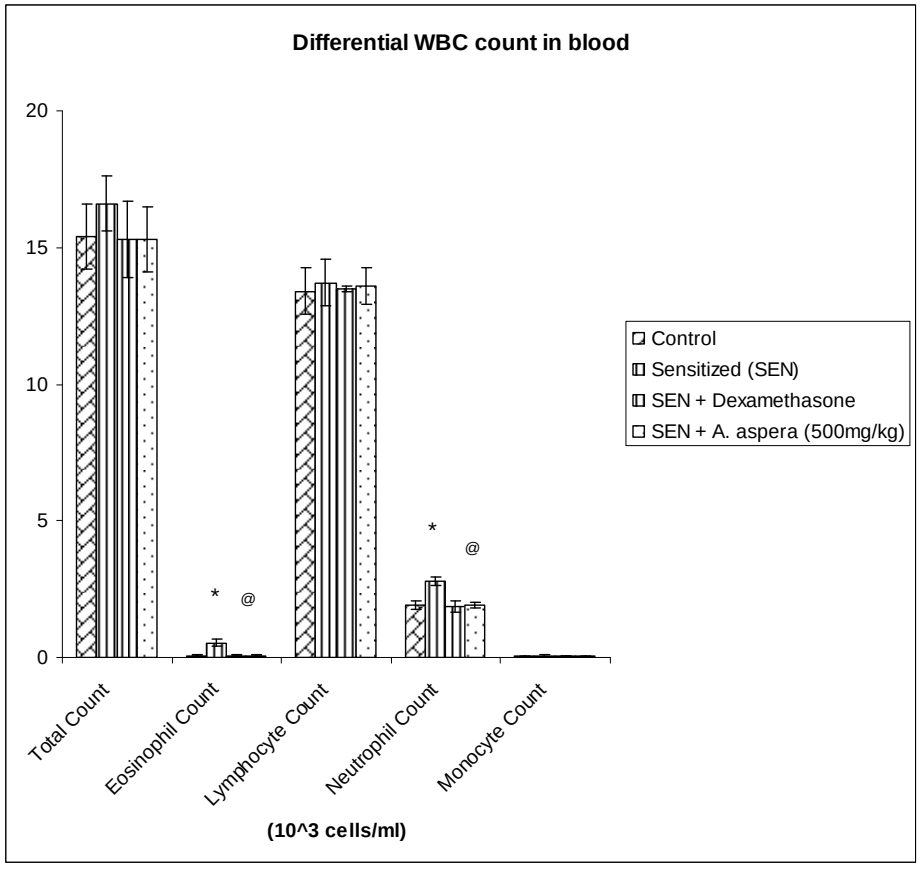

Fig. (1). Effect of *A. aspera* on change in differential leukocyte counts of the blood.

*Significantly different from control ($p < 0.01$).

@Significantly different from sensitized ($p < 0.01$).

ate [15]. The method of Devasagayam was used to measure the presence of lipid peroxides in the homogenate and is based upon the release of malondialdehyde [16]. For the estimation of antioxidant enzyme activities in the samples, superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich with the degree of inhibition of the auto-oxidation of epinephrine by SOD being used as the index of activity [17]. The method of Moron *et al.* was used for estimations of total reduced glutathione (GSH) [18]. Protein was estimated according to the method of Lowry *et al.* [19]. All the parameters were estimated spectrophotometrically (UV 1601, Shimadzu, Asia pacific Pvt. Ltd., Australia) at their respective specified wavelengths.

Histopathological Study

Histopathological study of lung tissue obtained from rats was also carried out to study the effects of ethanolic extract of *A. aspera* on bronchoconstrictive effects brought about by sensitization of the animals by TDI.

Statistical Analysis

Results were reported as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall F value was found statistically significant ($p < 0.05$), further comparisons among groups were made according to post-hoc Tukey's test. All statistical analyses were performed using Graph Pad software (San Diego, CA).

RESULTS

Effect of *A. aspera* on Airway Hyperreactivity Symptoms

After provoked with 5% TDI, the TDI- sensitized rats exhibited irritability, sneezing and hyperrhinorrhea. The exertional breathing which was similar to asthma was observed to last 8-10 minutes in TDI-sensitized rats. The control, standard and *A. aspera* treated rats did not show airway abnormality.

Effect of *A. aspera* on Circulating Leukocyte Count

The number of eosinophils and neutrophils in the blood are significantly decreased in *A. aspera* and dexamethasone treated groups as compared to TDI-sensitized group (Fig. **1**).

Effect of *A. aspera* on Cellular Content in BAL Fluid

The total cell and each leukocyte subtype in BAL fluid in TDI sensitized rats were significantly elevated, particularly the numbers of eosinophil, lymphocytes and monocytes were increased by many folds compared to those in control rats. Treatment with *A. aspera* shows significant reduction in the count (Fig. **2**).

Effect of *A. aspera* on MDA Level

MDA levels were found to be significantly increased in livers of TDI sensitized rats as compared to normal control rats. Treatment with *A. aspera* produced significant ($p < 0.01$) decrease in MDA levels. These reductions in MDA levels were comparable to that of standard dexamethasone treated rats (Table **1**).

Effect of *A. aspera* on Antioxidant Enzymes

Activities of superoxide dismutase and reduced glutathione were significantly decreased ($p< 0.01$) in TDI sensitized animals compared to control animals; however treatment with *A. aspera* increased the levels of all enzymes significantly ($p < 0.01$). In fact, this treatment brought about values nearly that of the control rats (Table **1**).

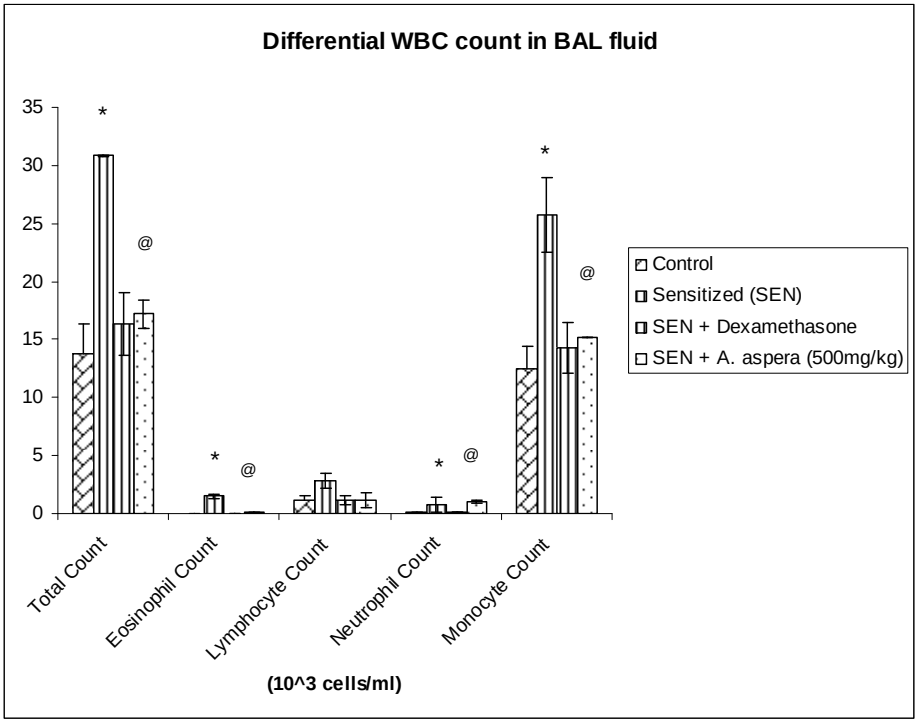

Fig. (2). Effect of *A. aspera* on change in differential leukocyte counts of the Broncho-Alveolar Lavage (BAL) fluid.

*Significantly different from control ($p < 0.01$).

@Significantly different from sensitized ($p < 0.01$).

Table 1. Effect of *A. aspera* on Change in Anti-Oxidant Enzyme Levels in Liver

	Glutathione µM/mg Protein	Malonaldehyde nM/mg Protein	Superoxide Dismutase units/min/mg Protein
Control	9.53 ± 0.25	0.16 ± 0.04	87.92 ± 2.15
Sensitized (SEN)	4.44 ± 0.37*	0.58 ± 0.14*	90.19 ± 1.52*
SEN + Dexamethasone	10.7 ± 0.23[@]	0.18 ± 0.05[@]	87.29 ± 1.23 [@]
SEN + *A. aspera*	8.19± 0.03[@]	0.2± 0.02[@]	88.29± 1.05 [@]

*Significantly different from control ($p < 0.01$).
[@]Significantly different from sensitized ($p < 0.01$).

Histological Examinations

In TDI sensitized rats, a prominent infiltration of numerous eosinophils, total leukocyte infiltration, reduced lumen size, submucosal edema, inflammatory exudation, vascular congestion, goblet cell infiltration was observed, while treatment regimen shows protective effect by less or mild pathological changes (Fig. **3**).

DISCUSSION AND CONCLUSION

The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal and other purpose. There exists a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. Large numbers of medicinal plant preparations have been reported to possess anti-asthmatic effects like *Albizzia lebbeck* [20], *Solanum xanthocarpum* [21], *Picrorhiza kurroa* [22], *Tephrosia purpurea* [23], *Tylophora indica* [24] and *Vitex negundo* [25].

Substances such as toluene diisocyanate have been reported to induce asthma in sensitized workers in the parts-per-billion range. Cessation of exposure to toluene diisocyanate in sensitized workers with occupational asthma is associated with a decrease in both the number of inflammatory cells in the airway mucosa and in the amount of subepithelial fibrosis observed with serial bronchial biopsies [26].

The airway inflammation was agreement with respiratory hyperreactivity symptoms which were similar to asthma. These results were consistent with the work of Zheng *et al*. [14]. Eosinophil infiltration of the bronchial mucosa is known as the most striking histopathological feature of asthma. Unlike mast cells that are fixed in various tissues throughout the body, eosinophils are very mobile. In association with asthma, elevated numbers of eosinophils have been

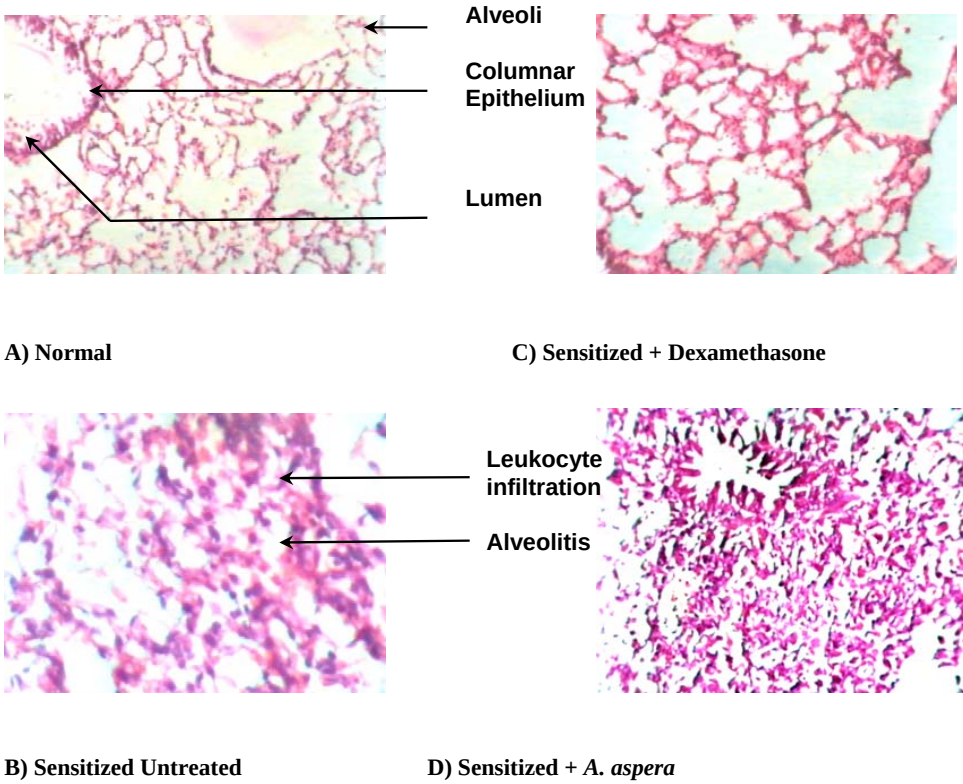

Fig. (3). Histopathological alterations and effect of *A. aspera* in sections of lung and bronchioles.

A) Shows normal view of lung pathology. **B**) Shows typical damaged lung tissue with leukocyte infiltration, inflammatory exudation vascular congestion. **C**) Shows less leukocyte infiltration by dexamethasone treatment. **D**) shows less leukocyte infiltration by *A. aspera* treatment.

identified in various tissue compartments, including circulating in the peripheral blood, in biopsies of lung tissue, in fluid specimens obtained from the lung using a bronchoscope i.e. fluid that "washes out" the bronchi and alveoli — termed *bronchoalveolar lavage* (BAL) fluid and in secretions or sputum. The mobility of eosinophils indicates that they can be stimulated to leave the bloodstream and enter the tissues. In asthma, eosinophils move from the blood into the bronchi (as documented in bronchial biopsies and in BAL fluid) and onto the surface of the airway lining (as documented in sputum). When activated, eosinophils release several pre-formed mediators from within their granules. These granules contain several proteins like eosinophilic cationic protein, eosinophil-derived neurotoxin, peroxidase, and major basic protein. Major basic protein, in particular, can directly damage airway epithelium, intensify bronchial responsiveness, and cause degranulation of basophils and mast cells. These effects increase the severity of asthma. Eosinophils release inflammatory mediators such as leukotrienes, particularly the cysteinyl leukotriene C_4, which contracts airway smooth muscle, increases vascular permeability, and may recruit more eosinophils to the airways [27]. In the present study it was found that ethanol extracts of *A. aspera* reduce the total leucocyte counts and eosinophil counts. This indicates the protective effect by preventing release several pre-formed mediators like eosinophilic cationic protein, eosinophil-derived neurotoxin, peroxidase, and cationic protein from the granules of eosinophills thereby preventing the direct damage of airway epithelium.

Neutrophils may play a pivotal role in the disease process, at least in the sudden onset fatal cases as high numbers of neutrophils have been reported to be present in the airways of patients who died from sudden onset fatal asthma. Neutrophils are source for a variety of mediators viz. PAF, PGs, TXs, LTs causing bronchial hyper responsiveness and airway inflammation [28]. T cells release a variety of cytokines that communicate with most other cells in the inflammatory process. Cytokines from T cells can, for instance, activate B cells to make antibodies (even controlling the choice between making IgM, IgG, or IgE) or activate eosinophils versus neutrophils. T cells thus initiate and orchestrate a cascade of cytokine-mediated airways inflammation. T cells and their cytokines provide a common pathway for allergic (i.e., IgE-mediated) and nonallergic asthma. They are, however, not a source of mediators of immediate hypersensitivity reactions and thus do not participate in the acute (<15 minutes) response to allergen that can cause an acute asthma attack. It is reported that nonasthmatic subjects who had been exposed acutely to ozone developed airway hyperresponsiveness in association with airway neutrophilia [29, 30]. Other clinical studies have since found that, measures of chronic asthma severity, such as FEV_1, correlate with the degree of neutrophilia in sputum or bronchial biopsy specimens [31, 32]. Neutrophilic inflammation in the airway is also increasingly recognized in acute exacerbations of asthma and in status asthmaticus [33-35]. Since the treatment with ethanol extracts of *A. aspera* reduce the neutrophil counts, it depicts that *A. aspera* may be beneficial in acute asthmatic conditions and status asthmaticus.

Oxidative stress plays an important role in the pathogenesis of airway diseases, particularly when inflammation is prominent. Increased concentration of MDA and decreased activities of antioxidant enzymes have been observed in TDI sensitized animals. Superoxide dismutase (SOD) and catalase play an important role in the detoxification of superoxide anion and H_2O_2, respectively, thereby protecting the cells against free radical induced damage [36]. Hydrogen peroxide may be reduced by enzymes glutathione peroxide, but alternatively may react again with superoxide anion to form free hydroxyl radicals, which have a greater toxicity and a longer half-life than superoxide anion [37]. Reduced Glutathione (GSH) in conjunction with glutathione peroxidase (GPx) and glutathione-S-transferase (GST) may play a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens. Treatment with *A. aspera* orally significantly reduced MDA level, and significantly improved antioxidant enzymes levels as compared to disease control rats suggesting the beneficial role in oxidative stress.

In conclusion our findings suggest that *A. aspera* at a dose of 500 mg/kg suppresses the hyperreactivity symptoms, inhibit leukocyte infiltration (particularly eosinophils and neutrophils) into the potential target sites for the disease. In addition it also shows antioxidant activity suggesting the beneficial role in TDI induced occupational asthma.

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