INHIBITORY EFFECT OF ABRUS PRECATORIOUS EXTRACT ON BRONCHIAL HYPERREACTIVITY INDUCED BY OVALBUMIN IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objectives: This study was aimed to evaluate the bronchial hyper-reactivity of ethanolic extract of Abrus precatorious leaves in ova albumin-induced airway inflammatory responses in animal model of Asthma.

Method: Bronchial hyper reactivity was studied by using Wistar rats of either sex. The Broncho alveolar lavage fluid (BALF) was examined by sensitizing the animals with ovalbumin in (OVA).

Results: There was significant decrease in inflammatory cell count, level of nitric oxide and total protein in bronchoalveolar lavage (BAL) fluid with the treatment of ethanolic extract of Abrus precatorious (AP) leaves at the dose of 100, 200 and 400 mg/kg, p.o. Extract of AP also restored the level of lung antioxidant enzymes (LPO, GSH, SOD, Catalase). Extract of AP reduced the wet/dry weight ratio and also in histopathological examination of lung tissue protected the lungs from pathological changes which induced by OVA.

Conclusion: These results indicate that ethanolic extract of Abrus precatorious leaves decreased bronchial hyper reactivity by decreasing the infiltration of inflammatory cells in the airway. The observed effect may be due to anti-inflammatory and antioxidant activity.

Keywords: Ovalbumin, BAL, Hyperresponsiveness, Abrus precatorious.

INTRODUCTION

Asthma is a chronic respiratory disease with airway inflammation and hyper responsiveness with reversible airway obstruction, increase mucus production, infiltration of eosinophils and nonspecific airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning [1,2]. The factors that induce asthma are inhaled pollutants including allergens, viruses, bacteria, fungi, tobacco smoke and ozone. Inhalation of OVA Albumin in rats induces inflammation by activating CD4+ T helper type 2 (Th2) lymphocytes. During this process of inflammation many inflammatory mediators like eosinophils, T lymphocytes, mast cells, neutrophils, and dendritic cells (DCs), were appear in BAL fluid during the late response [3,4]. There was an also marked and characteristic pathophysiological change in the airways like thickening of the airway wall, which was indicating the restriction of airflow and the development of airway hyper responsiveness [5]. Further eosinophils release cytokines and chemokines which plays major role in airway inflammation [6]. Inflammatory mediators release reactive oxygen species (ROS) which has ability to contract smooth muscle and to release histamine from mast cells [7].

Traditionally leaves of Abrus precatorious (AP) were used to cure fever, stomatitis, asthma and bronchitis [8]. It possesses different pharmacological activities antimicrobial [9, 10], anti-implantation and anti-ovulatory [11], anti-bacterial activity [12], immunomodulatory and anti-tumor [13]. Mast cell stabilizing and antiallergy activity [14]. Anti-inflammatory activity [15]. From Abrus precatorious plant, lectins were derived which showed immunostimulant activity [16].

Thus, the present study was designed to study the effect of ethanolic extract of Abrus precatorious leaves (EAPL) on ova albumin-induced airway inflammatory responses in a management of Asthma.

MATERIALS AND METHODS

Experimental Animals

The Wistar rats of either sex weighing about 150-500 gm, were purchased from National Toxicology Center, Pune. They were housed in group of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra.) and water ad libitum. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA and experimental protocol was approved by Institutional Animal Ethics Committee (198/99/CPCSEA).

Chemicals

All chemicals were purchased from HiMedia Lab. Pvt. Ltd., India and Sigma Aldrich, USA. Ovalbumin was purchased from Central Drug House (P) LTD, New Delhi.

Plant Material

Dried leaves of Abrus precatorious were purchased from commercial supplier of Pune, India. The plant was authenticated Botanical Survey of India (BSI), Pune, India.

Preparation of Extract

About 1000gm of leaves of Abrus precatorious (AP) were dried under shed and coarsely powdered. Leaves were defatted with petroleum ether and then subjected to maceration process by using 70% ethanol for 7 days shaking occasionally. After 7 days mixture was filtered and filtrate was evaporated to dryness to give ethanolic extract of Abrus precatorious (AP). The yield obtained was 20 gm.

Preliminary phytochemical screening

After obtaining of dry extract, qualitative preliminary phytochemical screening was performed to find out the presence of various phytochemicals such as steroids, saponins, alkaloids, flavonoids, tannins, phenolic compounds, and glycosides.

Acute toxicity Study (OECD Guidelines, 423, 2001):

Albino rats of either sex weighing 200-250 gm were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. The animals were divided in 3 groups (n=3) and were...
fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The animals were given ethanolic extract of Abrus precatorious (AP) at the doses of 5, 50, 300 and 2000 mg/kg body weight orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with an ethanolic extract of Abrus precatorious (AP) and therefore was found safe up to dose of 2000 mg/kg. Doses were selected based on acute oral toxicity study. Therefore the regime for the AP dose was 100, 200 and 400 mg/kg.

Ovalbumin-induced airway inflammation [17]

Sensitization and challenge with antigen

Animals were divided into six groups (n=5) viz. NS, S, DEXA, AP-100, AP-200 and AP-400. All the animals except in the non-sensitized group (NS), were sensitized by an intraperitoneal injection of 1ml alum precipitate antigen containing 20µg of ovalbumin and 8mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum-ovalbumin mixture was given 7 days later. Non-sensitized animals were injected with alum only. Seven days after (15 day) injection, animals were exposed to aerosolized ovalbumin (1%) for 30 min. DEXA, AP-100, AP-200, AP-400 and AP-400 without OVA group of animals were received respective drug treatment 5 h before antigen challenge. The rats were sacrificed at the end of study (24 hr after sensitization) and catheter was inserted in trachea. Bronchoalveolar lavage fluid was collected by lavaging the lung with 2 aliquots of 5 ml of 0.9% sodium chloride solution total recovery volumes per rat was approximately 8 ml. Total leukocytes, eosinophils and neutrophils were counted under microscope and histopathological evaluation of lung tissue was carried out. Lung wet to dry weight ratio was taken. Estimation of biochemical parameters-Lactate dehydrogenase [40], total protein [43] and nitrite oxide [47] in BAL fluid. Superoxide dismutase, catalase [46], lipid peroxidase [44], and glutathione reductase [45] was done in lung homogenate.

**Table 1: Effects of ethanolic extract of Abrus precatorious on OVA-induced BAL inflammatory cells**

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Total leukocytes (×10^3/µl) [Mean ± SEM]</th>
<th>Differential cell counts (×10^3/µl) [Mean ± SEM]</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Alveolar Macrophages</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>8.8 ± 0.08</td>
<td>0.08 ± 0.007</td>
<td>2.1 ± 0.13</td>
<td>3.6 ± 0.12</td>
<td>7.14 ± 0.12</td>
<td>0.48 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>14.6 ± 0.15***</td>
<td>0.56 ± 0.016***</td>
<td>4.8 ± 0.12**</td>
<td>16.7 ± 0.12***</td>
<td>16.8 ± 0.14***</td>
<td>2.92 ± 0.08***</td>
<td></td>
</tr>
<tr>
<td>DEXA</td>
<td>9.6 ± 0.16***</td>
<td>0.09 ± 0.019***</td>
<td>2.9 ± 0.07**</td>
<td>5.74 ± 0.14***</td>
<td>7.64 ± 0.13***</td>
<td>0.72 ± 0.10***</td>
<td></td>
</tr>
<tr>
<td>AP-100</td>
<td>14.6 ± 0.15**</td>
<td>0.57 ± 0.007**</td>
<td>4.8 ± 0.11**</td>
<td>16.8 ± 0.10**</td>
<td>16.22 ± 0.11**</td>
<td>2.81 ± 0.11**</td>
<td></td>
</tr>
<tr>
<td>AP-200</td>
<td>11.7 ± 0.15**</td>
<td>0.35 ± 0.013**</td>
<td>3.2 ± 0.05**</td>
<td>14.14 ± 0.17**</td>
<td>13.84 ± 0.14**</td>
<td>1.5 ± 0.09**</td>
<td></td>
</tr>
<tr>
<td>AP-400</td>
<td>10.5 ± 0.23**</td>
<td>0.14 ± 0.010**</td>
<td>2.98 ± 0.12**</td>
<td>1.2 ± 0.10**</td>
<td>8.72 ± 0.08**</td>
<td>1.04 ± 0.06**</td>
<td></td>
</tr>
<tr>
<td>AP-400 (without OVA)</td>
<td>11.4 ± 0.16***</td>
<td>0.34 ± 0.012**</td>
<td>2.99 ± 0.15**</td>
<td>1.40 ± 0.15**</td>
<td>11.5 ± 0.11**</td>
<td>1.64 ± 0.08**</td>
<td></td>
</tr>
</tbody>
</table>

*** = P < 0.001 when S group compared with NS Group and *** = p<0.01, ns= not significant when AP 100, 200, 400, and AP 400 without OVA Group compared with S Group.

NS= Non-sensitized received 8mg alum in 1ml (i.p.); S= Sensitized received Ovalbumin 20µg + 8mg alum in 1ml (i.p.); DEXA= Dexamethasone (1 mg/kg, i.p.); Ovalbumin 20µg + 8mg alum in 1ml (i.p.); AP 100, 200, 400= Ethanolic extract of Abrus precatorious at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p.); AP-400 without OVA= Only Ethanolic extract of Abrus precatorious at 400 mg/kg, p.o. without OVA sensitization.

Effect of AP on LPO, GSH, SOD, and CAT level in lung tissue

Ovalbumin significantly (p<0.001) increased the level of LPO and decreased level of SOD, GSH, and CAT in sensitized group when compared with non-sensitized group (Table 2). Dexamethasone (1 mg/kg, i.p.) significantly (p<0.001) increased the level on GSH, SOD and CAT and decreased the level of LPO as compared to sensitized group. AP at a dose of 100 mg/kg significantly restored (p<0.001) the level of SOD, GSH, LPO level and did not show any change in catalase level. Animals treated with AP at the dose of 200 and 400 mg/kg significantly restored (p<0.001) the level of GSH, SOD and CAT and decreased the level of LPO as compared to sensitized group. Animals which were not sensitized with Ovalbumin but only treated with AP at the dose of 400 mg/kg showed significant reduction in nitric oxide, LDH and total protein level

Statistical analysis

The results were expressed as Mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test, with level of significance set at p<0.05 and p<0.01.

RESULTS

**Phytochemical Screening**

Preliminary phytochemical investigation of ethanolic extract of Abrus precatorious (AP) showed presence of steroids, saponins, alkaloids, flavonoids, and glycosides.

**Acute toxicity Study**

The animals were showed no mortality and safe up to the dose 2000 mg/kg body weight. Dose was selected by using acute toxicity study (OECD, 423). The present study was performed at three dose levels of ethanolic extract of Abrus precatorious (AP) at 100, 200 and 400 mg/kg body weight.

**Effect of AP on inflammatory cell counts in BAL fluid**

The number of total leukocytes, eosinophils, neutrophils, macrophages, lymphocytes and monocytes in the BAL fluid were evaluated by administrating of ovalbumin. Number of inflammatory cells were significantly (p<0.001) increased in OVA sensitized group when compared with non-sensitized group (Table No.1). Dexamethasone (1 mg/kg, i.p.) significantly (p<0.001) suppressed effect of OVA on total leukocytes, eosinophils, neutrophils, macrophages, lymphocytes and monocytes count in the BAL fluid as compared to sensitized group. AP extract at doses of 100 mg/kg, significantly (p<0.05) inhibited lymphocytes count but did not showed any decrease in total leukocytes, eosinophils, neutrophils, macrophages, and monocytes count in the BAL fluid as compared to sensitized group. AP extract at doses of 200 and 400 mg/kg, significantly (p<0.001) inhibited total leukocytes, eosinophils, neutrophils, macrophages, lymphocytes and monocytes count in the BAL fluid as compared to sensitized group. Animals which were not sensitized with Ovalbumin but only treated with AP at the dose of 400 mg/kg significantly (p<0.001) inhibited total leukocytes, eosinophils, neutrophils, macrophages, lymphocytes and monocytes count. (Table 1)
(p<0.001) when compared with sensitized group. In rats treated with AP 100 mg/kg, there was significant decrease in nitric oxide, LDH and total protein level when compared with sensitized group. Animals which were not sensitized with Ovalbumin but only treated with AP at the dose of 400 mg/kg significantly decreased nitric oxide, LDH level and total protein level (p<0.001). (Table 3)

### Table 2: Effect of Abrus precatorious on lung antioxidant status

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Antioxidant status</th>
<th>LPO/MDA nm of MDA/mg of protein</th>
<th>SOD units/mg of protein</th>
<th>GSH μg of GSH/mg of protein</th>
<th>CAT μM of H2O2 consumed/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>0.19±0.007</td>
<td>6.96±0.08</td>
<td>8.16±0.33</td>
<td>18.8±0.86</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>2.63±0.07***</td>
<td>5.60±0.202***</td>
<td>3.5±0.06***</td>
<td>5.8±0.18***</td>
<td></td>
</tr>
<tr>
<td>DEXA</td>
<td>0.8±0.04***</td>
<td>9.54±0.19***</td>
<td>8.09±0.12***</td>
<td>11.4±0.107***</td>
<td></td>
</tr>
<tr>
<td>AP-100</td>
<td>0.06±0.008***</td>
<td>7.50±0.15***</td>
<td>6.6±0.19***</td>
<td>6.6±0.12***</td>
<td></td>
</tr>
<tr>
<td>AP-200</td>
<td>0.08±0.008***</td>
<td>7.90±0.23***</td>
<td>7.2±0.14***</td>
<td>9.4±0.4***</td>
<td></td>
</tr>
<tr>
<td>AP-400</td>
<td>0.16±0.005***</td>
<td>8.5±0.14***</td>
<td>7.8±0.23***</td>
<td>10.2±0.37***</td>
<td></td>
</tr>
<tr>
<td>AP-400 without OVA</td>
<td>0.09±0.006***</td>
<td>8.18±0.13***</td>
<td>7.1±0.07***</td>
<td>6.00±0.12***</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Effect of Abrus precatorious on Nitric oxide, Lactate Dehydrogenase and Total Protein release in Bronchoalveolar lavage fluid (BALF) in rats.

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Antioxidant status</th>
<th>Nitric oxide (µMol/mg of Protein)</th>
<th>LDH (nMol NADH oxidized/min/ml)</th>
<th>Total Protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>4.00 ± 0.707</td>
<td>35.4 ± 0.81</td>
<td>0.68 ± 0.013</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>12.4 ± 0.509***</td>
<td>77.6 ± 0.92***</td>
<td>1.97 ± 0.03***</td>
<td></td>
</tr>
<tr>
<td>DEXA</td>
<td>3.8 ± 0.374***</td>
<td>41.6 ± 1.03***</td>
<td>0.67 ± 0.013***</td>
<td></td>
</tr>
<tr>
<td>AP-100</td>
<td>9.8 ± 0.244***</td>
<td>61.4 ± 0.91***</td>
<td>0.83 ± 0.0102***</td>
<td></td>
</tr>
<tr>
<td>AP-200</td>
<td>8.0±0.31 ***</td>
<td>54 ± 0.70***</td>
<td>0.788 ± 0.011***</td>
<td></td>
</tr>
<tr>
<td>AP-400</td>
<td>6.2 ± 0.37***</td>
<td>49.8 ± 0.58***</td>
<td>0.782 ± 0.011***</td>
<td></td>
</tr>
<tr>
<td>AP-400 without OVA</td>
<td>7.3 ± 0.374***</td>
<td>68.8 ± 1.15***</td>
<td>0.742 ± 0.007***</td>
<td></td>
</tr>
</tbody>
</table>

#### Effect of AP on lung wet-to-dry weight ratio

Wet/dry weight ratio was higher in sensitized group when compared with non-sensitized group. Treatment of Dexamethasone at a dose of 1 mg/kg significantly reduced the wet/dry weight ratio which was gained during the OVA induced asthma. Pretreatment with AP at the dose of 100 (p<0.05), 200 mg/kg (p<0.01) and 400 mg/kg (p<0.001) significantly reduced the wet/dry weight ratio in a dose-dependent manner (Table 4). Animals which were not sensitized with Ovalbumin but only treated with AP at the dose of 400 mg/kg significantly (p<0.001) reduced the wet/dry weight ratio. (Table 4)

### Table 4: Effect of Abrus precatorious on lung wet-to-dry weight ratio in asthma and chronic lung inflammation.

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Lung weight ratio (wet/dry) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>2.62 ± 0.102</td>
</tr>
<tr>
<td>S</td>
<td>3.94 ± 0.05***</td>
</tr>
<tr>
<td>DEXA</td>
<td>2.22 ± 0.06***</td>
</tr>
<tr>
<td>AP-100</td>
<td>3.61±0.05*</td>
</tr>
<tr>
<td>AP-200</td>
<td>3.51 ± 0.03**</td>
</tr>
<tr>
<td>AP-400</td>
<td>2.94±0.10**</td>
</tr>
<tr>
<td>AP-400 without OVA</td>
<td>3.38±0.05**</td>
</tr>
</tbody>
</table>

### Notes

- ***P < 0.001 when S group compared with NS Group and "p<0.01, ns= not significant when AP 100, 200, 400, and AP 400 without OVA Group compared with S Group.

- NS= Non-sensitized received 8mg alum in 1ml (i.p); S= Sensitized received Ovalbumin 20µg + 8mg alum in 1ml (i.p); DEXA= Dexamethasone (1mg/kg, i.p)+ Ovalbumin 20µg + 8mg alum in 1ml (i.p); AP 100, 200, 400= Ethanolic extract of Abrus precatorious at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p); AP-400 without OVA= Only Ethanolic extract of Abrus precatorious at 400 mg/kg, p.o. without OVA sensitization.

- Expression such as "p<0.001, ns= not significant when AP 100, 200, 400, and AP 400 without OVA Group compared with S Group.

- Effect of AP on lung wet-to-dry weight ratio

- Wet/dry weight ratio was higher in sensitized group when compared with non-sensitized group. Treatment of Dexamethasone at a dose of 1 mg/kg significantly reduced the wet/dry weight ratio which was gained during the OVA induced asthma.
Histopathological findings

In the present study maximum pathological changes were observed in sensitized group in which congestion, edema, cellular infiltration, emphysema, and bronchial pathology was found to be ++++, i.e., up to 75%, respectively. Dexamethasone and the ethanolic extract of *Abrus precatorious* protected the lungs from pathological changes induced by OVA. Also Animals which were not sensitized with Ovalbumin but only treated with AP at the dose of 400 mg/kg protected the lungs from pathological changes induced by OVA (Table 5) (Fig 1).

<table>
<thead>
<tr>
<th>Group(n=5)</th>
<th>Congestion</th>
<th>Edema</th>
<th>Cellular infiltration</th>
<th>Emphysema</th>
<th>Bronchial constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>DEXA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AP-100</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AP-200</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AP-400</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AP-400 without OVA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = pathological changes up to 25%; ++ = pathological changes up to 50%; +++ = pathological changes up to 75%; ++++ = pathological changes up to or more than 75%

![Images of histopathological findings](image)

**Table 5:** Effect of ethanolic extract of *Abrus precatorious* on OVA-induced histopathological changes in lung tissue.

**Fig. 1:** Effect of ethanolic extract of *Abrus precatorious* on OVA-induced histopathological changes in lung tissue.

Emphysema (white arrow), Congestion (red arrow), Edema (yellow arrow), Cellular infiltration (blue arrow), Bronchial pathology (green arrow)
DISCUSSION

In OVA-induced asthma model in rats, ovalbumin as an antigen and produces the characteristics of airway inflammation, including infiltration of inflammatory mediators like eosinophils, neutrophils, T-lymphocytes, monocytes present in tissue compartments like blood, biopsies of lung tissue, in bronchoalveolar lavage fluid and in sputum. In OVA-induced airway inflammation, the allergic response was observed in lungs 24 hr after OVA challenge. When animal re-exposed to the allergen, it binds to FcεRI receptors on mast cells and activates Th2 cytokines. This results in infiltration of inflammatory cells and mediators into the lung and increased oxidative stress. This results in decreased lung function, bronchoconstriction and airway hyperresponsiveness.

The late phase airway response in asthma is associated with the infiltration of the inflammatory cells especially eosinophils to the site of inflammation. The eosinophil is cationic protein and eosinophil derived neutrotoxin causes damage and loss of epithelium. Eosinophils adhere to bronchial epithelium and release toxic proteins which act as antagonist to M2 receptors (auto receptors) and release Acetyl choline which causes bronchial constriction and hyperresponsiveness [20]. In our study we found that treatment with Abrus precatorius in antigen challenged animal significantly inhibited antigen induced hyper reactivity by preventing increased in infiltration of total leukocyte count, eosinophils count.

After antigen challenge, airway hyper responsiveness is supported by inflammatory mediators like neutrophil which also get increased in bronchial lavage fluid in pathogenesis of asthma, but neutrophilia is generally shorter duration than eosinophilia [33]. Treatment with Abrus precatorius resulted in significant inhibition of antigen induced bronchial hyper reactivity by decreasing neutrophil count.

Large numbers of T-lymphocytes, mainly of the CD4+ subset, have also been observed in the bronchial mucosa of OVA antigen challenged rats. CD4+ T cells produce Th2-derived cytokines, such as IL-4 and IL-5, which enhance IgE synthesis [34, 35] and to act specifically on eosinophil survival, activation and secretion of proinflammatory mediators [36]. The present finding showed that treatment with Abrus precatorius in OVA sensitized animal produced significant decrease in lymphocyte count as compared to sensitized animals.

The numbers of monocyte cells were increased after antigen challenge in BALF [37, 38]. The increased level of monocytes produce level of cytokines promotes macrophages chemotaxis and stimulates macrophage phagocytosis. Abrus precatorius significantly decreased monocyte as compared to sensitized animals. Result of our study suggest that in animal airways, antigen challenge has induced eosinophil, neutrophil, monocyte and lymphocyte infiltration and activation is similar to that of reported in human asthmatics. This shows protective effect of Abrus precatorius by preventing the infiltration of inflammatory cell, thereby decreasing the release of preformed inflammatory mediators, which can prevent the direct damage to airway, which in turn prevent airway hyperresponsiveness.

Antioxidant enzymes, such as superoxide dismutases (SOD), and glutathione reductase (GSH) play an important role in offering protection to the airways against oxidative stress [24, 25]. There is a localized decrease in SOD and GSH activity within inhaled allergen sensitized airways. Superoxide dismutase converts superoxide anion to oxygen and hydrogen peroxide which is eliminated by glutathione reductase. Superoxide Dismutase (SOD) enzymes found in all cells scavenges superoxide ion and prevents its accumulation so that cells are protected from oxidative stress. Glutathione peroxidase-1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase-4 is most active with lipid hydro peroxides. Glutathione peroxidase scavenes toxic amount of peroxides and free radical which helps to reduce the inflammation and GSH play important in the protection of cells against oxidative stress. SOD and GSH get inactivated by reactive oxygen and nitrogen species which were released during oxidative stress. Thus antioxidant defense is impaired in hyperactive airways. The situation gets further aggravated during acute exacerbations of asthma. The inflammatory cells increase the production of reactive oxygen species [26]. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. This protein is localized to peroxisomes in most eukaryotic cells. It is involved in a reaction which leads to the decomposition of peroxide and then regenerated by transferring the bond oxygen to a second molecule of substrate. Thus also take part in antioxidative stress and free radical formation and oxidation. This indicates its role in antioxidative stress related to asthma. Lipid peroxidation is an important marker of oxidative stress in the lung and analyzed by MDA. Lipid peroxidation end product of malondialdehyde (MDA) is suggested to cause cell injury and death in septic shock [27]. In present study it was found that MDA i.e., LPO levels increased in sensitized group as compare to drug treated group. Abrus precatorius extract significantly restored the level of SOD, GSH, catalase and LPO. Therefore it has been suggested that Abrus precatorius extract may reduce aggravation of inflammation during asthma by providing antioxidant enzymes protection which may contribute the use of Abrus precatorius extract in asthma.

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in essentially all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death 33. If cell lysis occurs, or cell membranes are damaged, cytoplasmic enzymes, such as LDH and glutathione reductase (GR) are released into the BAL fluid [29]. The present results indicate that Abrus precatorius extract significantly reduced LDH level, thereby reduced the lysis of epithelial cell membrane in pathogenesis of asthma. Nitric oxide is a signaling molecule responsible for several diverse physiological and pathophysiological processes, and until now the prevailing hypothesis about NO has been that it contributes to toxicant-induced lung inflammation and injury [30]. Nitric oxide (NO) is a free radical playing a pivotal role as a vasodilator, neurotransmitter and immune regulator in a variety of tissues at physiological concentrations. During inflammation, many cell types express iNOS and this leads to the formation of NO radicals or 5-nitrosodih or OONO− in the host cell. This will further leads to generation of secondary reactive nitrogen and oxygen species. Studies demonstrated that, NO levels in exhaled air were increased 30 min after OVA challenge to sensitized animals, with a concomitant increase in airway constriction. This suggests that iNOS-derived NO promotes Type 2 cell expansion, bronchial hyperactivity and eosinophil infiltration in the airways by reducing T helper Type 1 cells probably IFN-γ [31]. This increased concentration of NO can be very well estimated spectrophotometrically from BALF. In this study, Abrus precatorius extract significantly reduced production of NO in BAL fluid and thus protected them from the damaging effect of excess NO production. Abrus precatorius extract significantly reduced production of NO in BAL fluid and thus protected them from the damaging effect of excess NO production. Total protein concentration in bronchoalveolar lavage fluid is marker of pulmonary edema by capillary-alveolar leakage [32, 42]. Result showed that Abrus precatorius extract reduced the level of total protein.

The water content of the lungs was determined by calculating the wet/dry weight ratio of lung tissues [28]. OVA-induced asthma, there was pulmonary edema formation i.e., fluid accumulation in the lungs, whichcollects in air sacs. Collections of fluid in air sacs of the lungs cause difficulty in breathing and thus respiratory failure. In the ethanolic extract of Abrus precatorius treated groups, there was decrease in wet/dry ratio indicating its use in pulmonary edematous conditions produced in OVA-induced asthma. Therefore, Abrus precatorius extract may prove useful & satisfactory in pulmonary edema.

In bronchial asthma, inflammatory response induces various histopathological changes in asthmatic patients. In asthma, chronic inflammation is responsible for the bronchoconstriction which leads to airway narrowing and decrease in the lumen size of the bronchile [39]. This can be clearly seen by the histopathological studies of the lung tissue by observing the cross section of bronchi. In the present study, the sections of the lung tissues of animals sensitized with egg albumin indicated marked bronchitis and severe bronchoconstriction. There was increase in haemorrhage,
hyperplasia, exudation of mucus (catarrhal and mucoid material), cell infiltration (eosinophils, neutrophils), constriction of the secondary bronchi and tertiary bronchi, infiltration of mononuclear cells around the lung blood vessels (both artery and venules) and alveolar emphysema. Thus, ethanolic extract of Abrus precatorius protected the lungs from pathological changes induced by OVA. The preliminary phytochemical investigation of ethanolic extract of Abrus precatorius showed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides. Flavonoids are known to possess anti-inflammatory effects and antioxidant activity which may be responsible for anti-inflammatory and antioxidant activity. Thus presence of these phytoconstituents in ethanolic extract of Abrus precatorius may further contribute in ova albumin-induced airway inflammatory responses in a management of Asthma.[40-42] Therefore, our data suggestive of Abrus precatorius potential in prophyllaxis and management of asthma.

REFERENCES

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