Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

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

ANUPAMA A. SURALKAR[1*], SANJAY B. KASTURE[2]

[1]Padmashree Dr. D.Y Patil Institute of Pharmaceuticals Science and Research, Pimpri, Pune. [2]Department of Pharmacology, Sanjivani College of Pharmaceutical Education & Research, Kopargaon. Email: anupamaas@rediffmail.com

Received: 28 Nov 2012, Revised and Accepted: 17 Dec 2012

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 (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, Hyperresponsivenss, *Abrus preacatorious.*

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 hyperresponsiveness [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 bronchititis [8]. It possesses different pharmacological activities antimicrobial [9, 10], anti-implantation and antiovulatory [11], antibacterial activity [12], immunomodulatory and anti-tumor [13], Mast cell stabilizing and antiallergic activity14, Anti-inflammatory activity15. 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) in 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 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 400mg/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) second 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 hr 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 aquilots of 5 ml of 0.9% sodium chloride solution total recovery volume 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 [48], 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.

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 of 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)

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

Groups (n=5)	Total leukocytes ($\times10^3$/µl) (Mean ± SEM)	Differential cells ($\times10^3$/µl) (Mean ± SEM)				
		Eosinophils	Neutrophils	Alveolar Macrophages	Lymphocytes	Monocytes
NS	8.8 ± 0.08	0.08 ± 0.007	2.1 ± 0.13	3.6 ±0.12	7.14 ±0.12	0.48 ± 0.10
S	14.6 ±0.15$^{###}$	0.56±0.016$^{###}$	4.8 ± 0.12$^{###}$	16.7±0.12$^{###}$	16.8±0.14$^{###}$	2.92±0.08$^{###}$
DEXA	9.6±0.16***	0.09±0.019***	2.9±0.07***	5.74±0.14***	7.64±0.13***	0.72±0.10***
AP-100	14.6±0.15 ^{ns}	0.57±0.007 ^{ns}	4.8±0.11 ^{ns}	16.86±0.18 ^{ns}	16.22±0.11*	2.81 ±0.11 ^{ns}
AP-200	11.7±0.15***	0.35±0.013***	3.2±0.05***	14.14±0.17***	13.84±0.14***	1.5 ±0.09***
AP-400	10.7±0.23***	0.14±0.010***	2.98±0.12***	12.8 ±0.10***	8.7±0.08***	1.04 ±0.06***
AP-400 without OVA	11.4 ±0.16***	0.34±0.012***	2.99±0.15***	14.0±0.15***	11.5 ±0.11***	1.64±0.08***

$^{###}$= $P < 0.001$ when S group compared with NS Group and * = $p<0.05$, ***= $p<0.001$, 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-400without 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 No.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 significantly restored LPO, SOD and GSH level ($p<0.001$) but did not restored the Catalase level significantly. (Table 2)

Effect of AP on nitric oxide and total protein level in BAL fluid:

Result demonstrated that the nitric oxide, LDH and total protein level in BAL fluid were significantly ($p<0.001$) increased in sensitized group compared to non-sensitized rats (Table No.3). Treatment of Dexamethasone at a dose of 1 mg/kg showed significant reduction in nitric oxide, LDH and total protein level

(p<0.001) when compared with sensitized group. In rats treated with AP 100 mg/kg, there was significant decrease in nitric oxide (p<0.01), LDH and total protein (p<0.001) level when compared with sensitized group. In rats treated with AP 200 and 400 mg/kg, there was significant (p<0.001) 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

Groups (n=5)	Antioxidant status			
	LPO/MDA nM of MDA/mg of protein	SOD units/mg of protein	GSH µg of GSH/mg of protein	CAT µM of H2O2 consumed/mg of protein
NS	0.19± 0.007	9.6± 0.08	8.16 ± 0.33	18.8± 0.86
S	2.63±0.05###	5.60±0.202 ###	3.5 ± 0.06###	5.8±0.18###
DEXA	0.18± 0.007***	9.54±0.19***	8.08 ± 0.12***	11.4± 0.107***
AP-100	0.06± 0.008***	7.58± 0.15***	6.6 ±0.19 ***	6.6± 0.12 ns
AP-200	0.08± 0.008***	7.90± 0.23***	7.2 ± 0.14 ***	9.4± 0.4***
AP-400	0.16± 0.005***	8.5± 0.141***	7.8 ± 0.23***	10.2± 0.37***
AP-400 without OVA	0.09± 0.006***	8.18±0.13***	7.1 ± 0.07 ***	6.00± 0.12 ns

###= P < 0.001 when S group compared with NS Group and *** = p<0.001, 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-400without OVA= Only Ethanolic extract of *Abrus precatorious* at 400 mg/kg, p.o. without OVA sensitization.

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

Groups (n=5)	Antioxidant status (Mean ± SEM)		
	Nitric oxide (µMol/mg of Protein)	LDH (nMol NADH oxidized/min/ml)	Total Protein (mg/ml)
NS	4.00 ± 0.707	35.4 ± 0.81	0.68 ± 0.013
S	12.4 ± 0.509###	77.6 ± 0.92###	1.97 ± 0.03###
DEXA	3.8 ± 0.374***	41.6 ± 1.03***	0.67 ± 0.013***
AP-100	9.8 ±0.244**	61.4 ± 0.81***	0.83 ± 0.0102***
AP-200	8.00±0.31 ***	54 ±0.70***	0.788 ± 0.011***
AP-400	6.2 ± 0.37***	49.8 ±0.58***	0.782 ± 0.011***
AP-400 without OVA	7.3 ± 0.374***	68.8 ± 1.15***	0.742 ± 0.007***

###= P < 0.001 when S group compared with NS Group and **= p<0.01, *** = p<0.001, 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-400without OVA= Only Ethanolic extract of *Abrus precatorious* at 400 mg/kg, p.o. without OVA sensitization.

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), 200mg/kg (p<0.01) and 400 mg/kg (p<0.001) significantly reduced the wet/dry weight 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.

Group (n=5)	Lung weight ratio (wet/dry) (Mean ± SEM)
NS	2.62 ± 0.102
S	3.94 ± 0.05###
DEXA	2.22 ± 0.06***
AP-100	3.61± 0.05*
AP-200	3.51 ± 0.03**
AP-400	2.94± 0.10***
AP-400 without OVA	3.38± 0.05***

= P < 0.001 when S group compared with NS Group and * = p<0.05, **= p<0.01, ***= p<0.001, 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-400without OVA= Only Ethanolic extract of *Abrus precatorious* at 400 mg/kg, p.o. without OVA sensitization.

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

Group(n=5)	Congestion	Edema	Cellular infiltration	Emphysema	Bronchial constriction
NS	-	-	+	+	-
S	+ + +	+ + +	+ + +	+ +	+ + +
DEXA	+	+	+	+	++
AP-100	+ + +	+ +	+ + +	+ + +	+ + +
AP-200	+ +	+ +	+ +	+ +	+ +
AP-400	+	+	+	++	+
AP-400 without OVA	+	+	+	+	-

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

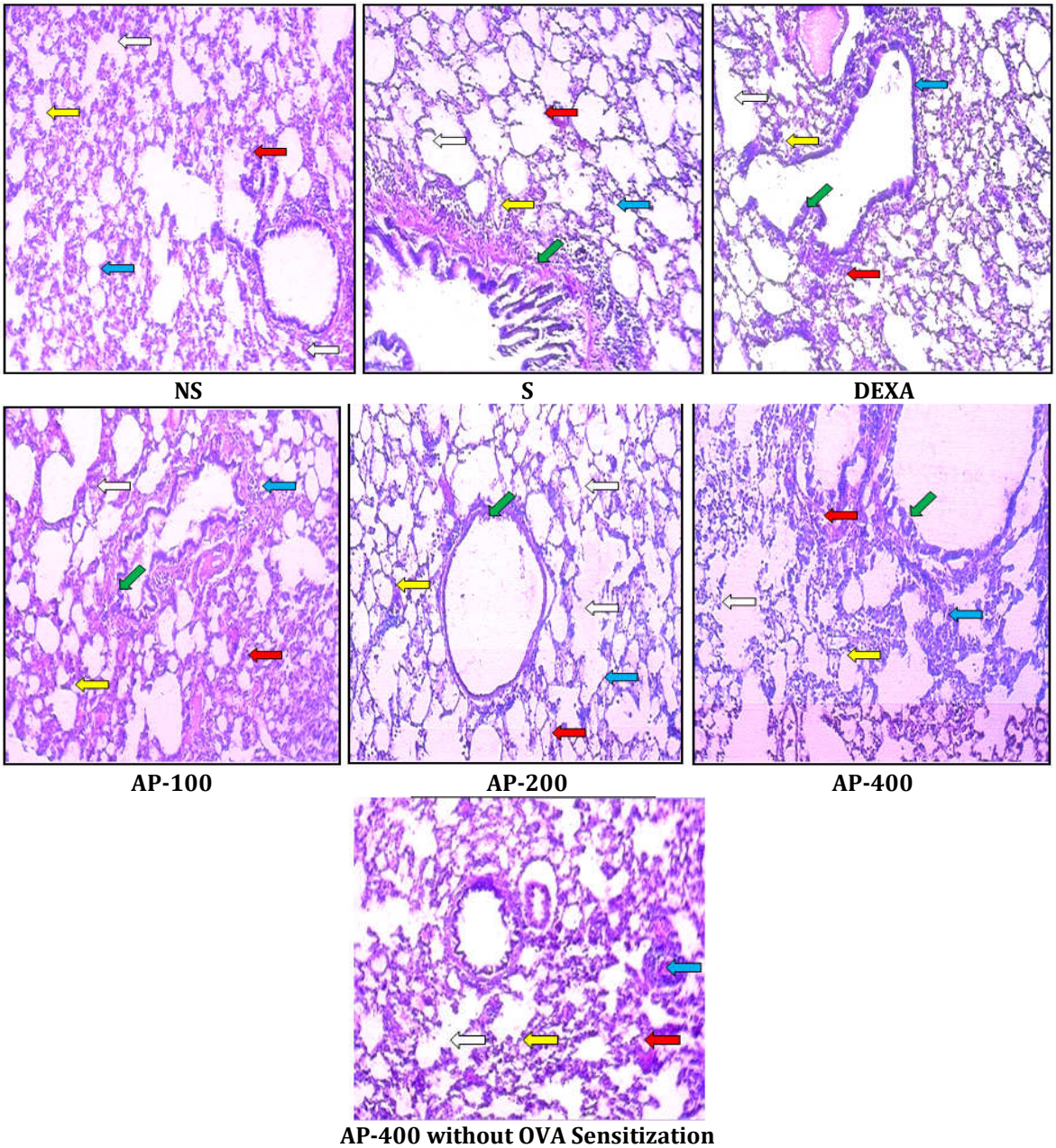

Fig. 1: Effect of ethanolic extract of *Abrus precatorious* on OVA-induced histopathological changes in lung tissue.

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, ova albumin act 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 hyper responsiveness.

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 neurotoxin causes damage and loss of epithelium. Eosinophils adhere to bronchial epithelium and release toxic proteins which act as antagonist to M_2 receptors (auto receptors) and release Acetyl choline which causes bronchial constriction and hyper responsiveness [20]. In our study we found that treatment with *Abrus precatorious* in antigen challenged animal significantly inhibited antigen induced hyper reactivity by preventing increase 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 precatorious* 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 precatorious* 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 precatorious* significantly decreased monocyte as compared to sensitize in OVA 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 precatorious* 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 inflamed asthmatic 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 scavenges 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 oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Thus also take part in oxidative stress and free radical formation and oxidation. This indicates its role in oxidative 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 maladionaldehyde (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 precatorious* extract significantly restored the level of SOD, GSH, catalase and LPO. Therefore it has been suggested that *Abrus precatorious* extract may reduced aggravation of inflammation during asthma by providing antioxidant enzymes protection which may contribute the use of *Abrus precatorious* 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 precatorious* 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 S-nitrosothiols or ONOO- 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 precatorious* extract significantly reduced production of NO in BAL fluid and thus protected them from the damaging effect of excess NO production. *Abrus precatorious* 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 precatorious* 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, which collects 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 precatorious* treated groups, there was decrease in wet/dry ratio indicating its use in pulmonary edematous conditions produced in OVA-induced asthma. Therefore, *Abrus precatorious* 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 bronchiole [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 bronchus and tertiary bronchi, infiltration of mononuclear cells around the lung blood vessels (both artery and venuoles) and alveolar emphysema. Thus, ethanolic extract of *Abrus precatorious* protected the lungs from pathological changes induced by OVA.

The preliminary phytochemical investigation of ethanolic extract of *Abrus precatorious* 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 precatorious* may further contribute in ova albumin-induced airway inflammatory responses in a management of Asthma.[40-42] Therefore, our data suggestive of *Abrus precatorious* potential in prophylaxis and management of asthma.

REFERENCES

1. Djukanoviae R., W.R. Roche, J.W. Wilson, C.R. Beasley, O.P. Twentyman and R.H. Howarth,. Mucosal inflammation in asthma. Am Rev Respir Dis 1990; 142(2): 434-457.
2. Beasley R., C. Burgess, J. Crane, N. Pearce and W. Roche. Pathology of asthma and its clinical implications. J Allergy Clin. Immunol 1993; 92(12): 148-154.
3. Corrigan, C.J., Kay, A.B. The roles of inflammatory cells in the pathogenesis of asthma and of chronic obstructive pulmonary disease. American Journal of Respiratory Disease 1991; 143: 1165-1178.
4. Roh SS, Kim SH, Lee YC, Seo YB. Effects of radix adenophorae and cyclosporine A on an OVA-induced murine model of asthma by suppressing to T cells activity, eosinophilia, and bronchial hyper-responsiveness. Mediators Inflamm. 2008; 78(1425): 1-11.
5. Salib, R.J., A. Drake Lee and P.H. Howarth. Allergic rhinitis: past, present and the future. Clin. Otolaryngol. Allied Sci. 2003; 28(4): 291-303.
6. Miloš Filipović, Snežana Cekić. The role of eosinophils in asthma. Medicine and Biology 2001; .8 (1): 6 – 10.
7. Peter J. Barne,. Reactive oxygen species and airway inflammation. Free Radical Biology & Medicine 1990; 9: 235-243,
8. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Dehradun: International Book Distributor; 1987, 763-767.
9. Adelowotan O, Aibinu I, Adenipekun E, Odugbemi T. The in vitro antimicrobial activity of *Abrus precatorious* (L) fabaceae extract on some clinical pathogens. Niger Postgrad Med J 2008; 15(1): 32-37.
10. Bobbarala V, Vadlapudi V. Abrus precatorious L. seed extracts antimicrobial properties against clinically important bacteria. Int J PharmTech Res 2009; 1(4): 1115-1118.
11. Okoko II, Osinubi AA, Olabiyi OO, Kusemijiu TO, Noronha CC, Okanlawon AO. Anti-ovulatory and anti-implantation potential of the methanolic extract of seeds of *Abrus precatorious* in the rat. Endocr Pract 2010; 16: 554-560.
12. Zore GB, Award V, Thakre AD, Halad UK, Meshram NS, Surwase BS, et al. Activity-directed-fractionation and isolation of four antibacterial compounds from *Abrus precatorious* L roots. Nat Prod Res 2007; 21(9): 838-5.
13. Ghosh D, Maiti TK. Immunomodulatory and anti-tumor activities of native and heat denatured Abrus agglutinin. Immunobiology 2007; 212(7): 589-599.
14. Taur DJ, Patil RY. Mast cell stabilizing and antiallergic activity of *Abrus precatorious* in the management of asthma. Asian Pac J Trop Med 2011; 4(1): 46-49.
15. Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of *Abrus precatorious*. East J Med 2009; 14: 23-25.
16. Ramnath V, Kuttan G, Kuttan R. Immunopotentiating activity of abrin, a lectin from *Abrus precatorious* Linn. Indian J Exp Biol 2002; 40(8): 910-913.
17. Chapman RW, Howard AH, Richard J, Celly C. Effect of inhaled roflumilast on the prevention and resolution of allergen-induced late phase airflow obstruction in Brown Norway rats. European Journal of Pharmacology; 2007; 571: 215-221.
18. Smith, J. A. Neutrophils, host defense, and inflammation: a double-edged sword. J. Leukoc. Biol., 1994; 56(6): 672-686.
19. S. Franovaa, M. Joskovaa, M. Sutovskaa, E. Novakovab, K. Adamicovac, O. Pechanovad, G. Nosalovaa. The efficiency of polyphenolic compounds on allergen induced hyperreactivity of the airways. Biomedicine & Preventive Nutrition, 2011, 232–235.
20. Williams, T.J., The eosinophil enigma, J Clin Invest, 2004; 113: 507–509.
21. Nishida, S., Teramoto, K., Kimoto-Kinoshita, S., Tohda, Y., Nakajima, S., Tomura, T.T., Irimajiri, K., Change of Cu, Zn-superoxide dismutase activity of guinea pig lung in experimental asthma. Free Radical Research, 2002; 36: 601–606.
22. Zhang, M., Nomura, A., Uchida, Y., Iijima, H., Sakamoto, T., Iishii, Y., Morishima, Y., Mochizuki, M., Masuyama, K., Hirano, K., Sekizawa, K. Ebselen suppresses late airway responses and airway inflammation in guinea pigs. Free Radical Biology & Medicine, 2002; 32: 454–464
23. MacPherson JC, Comhair SA, Erzurum SC, Klein DF, Lipscomb MF, Kavuru MS, Samoszuk MK, Hazen SL. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. J Immunol. 2001; 166: 5763-5772.
24. Comhair SA, Ricci KS, Arroliga M, Lara AR, Dweik RA, Song W. Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. Am J Respir Crit Care Med. 2005; 172: 306-313.
25. Kinnula VL, Cropo JD. Superoxide dismutase in the lung and human lung diseases. Am J Respir Crit Care Med. 2003; 167: 1600- 1619.
26. Jarjour NN, Calboun WJ. Enhanced production of oxygen radicals in asthma. J Lab Clin Med. 1994; 123: 131-136.
27. Gawel S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek. 2004; 57: 453-5.
28. Moss DW, Henderson AR. Enzymes. In: Tietz Textbook of Clinical Chemistry, 2nd ed. Burtis CA, Ashwood ER, eds. Philadelphia: W.B. Saunders Company. 1994: 735-896.
29. Henderson, RF, Muggenburg, B.A. Use of Bronchoalveolar Lavage to Detect Lung Injury. Current Protocols in Toxicology. 2004; 265–287.
30. Van der Vliet, A., Eiserich, J.P., Cross, C.E. Nitric oxide: a pro-inflammatory mediator in lung disease. Respir Res, 2000; 1: 67–72.
31. Smith, N., Broadley, K.J. Optimisation of the sensitisation conditions for an ovalbumin challenge model of asthma. International Immunopharmacology, 2007; 7: 183–190.
32. Visser, Y.P., Walther, F.J., Laghmani, E.H. Sildenafil attenuates pulmonary inflammation and fibrin deposition, mortality and right ventricular hypertrophy in neonatal hyperoxic lung injury. Respir Res. 2009 Apr 29; 10: 30.
33. Diaz, P., M.C. Gonzalez and F.r. Gallenguillos,. Leukocytes and mediators in bronchoalveolar lavage during allergen induced late-phase asthmatic reactions. Am. Rev. Respir Dis.1989; 139: 1383-1388.
34. Metzger, W.J., D. Zavala and H.B. Richerson. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs: description of the model and local airway inflammation. Am. Rev. Respir Dis. 1987; 135: 433-40.
35. Walker, C., J.C. Virchow, P.L.B. Jr. Bruijnzeel, K. Blaser. T-cell subsets and their soluble products regulate eosinophilia in allergic and non-allergic asthma. J. Immunol. 1991; 146(6): 1829-1835.
36. Pene, J., F. Rousset, F. Brire, I. Chrotien, J.Y. Bonnefoy, H. Spits, et al. IgE production by human B cells is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci U S A. 1988 Sep;85(18):6880-4
37. Sanderson, C.J.,. Interleukin-5, eosinophils and disease. Blood 1992; 79(12): 3101-3109.

38. Lapa e Silva, J.R., C.M. Bachelet, M. Pretolani, D. Baker, R.J. Scheper and B.B. Vargaftig. Immunopathologic alterations in the bronchi of immunized guinea-pigs. Am. J. Respir Cell Mol. Biol., 1993; 9(1): 44-53.
39. Kelly, H.W. and C.A. Sorknes,. Asthma. In: Pharmacotherapy- A Pathophysiological Aproch, Dipiro JT, R.L. Talbert, G.C. Yee, T.R. Matzke, B.G. Wells, L.M. Posey, Eds. Sixth Edition, The McGraw-Hill, 2005: 508.
40. Kim, H.K., B.S. Cheon, Y.H. Kim, S.Y. Kim, H.P. Kim. Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. Biochem. Pharmacol 1999; 58: 759-765.
41. Srinivas, K.V.N.S., Y. Koteswara Rao, I. Mahender, B. Das, K.V.S.R. Krishna and K.H. Kishore. Flavonoids from *Caesalpinia pulcherrima*. Phytochem 2003; 63: 789-93.
42. Matsuda, H., T. Morikawa, S. Ando, I. ToguchidaI and M. Yoshikawa. Structural requirements of flavonoids for nitric oxide production inhibitory activity and mechanism of action. Bioorg. Med. Chem 2003; 11: 1995-2000.
43. Lowry O.H., N.J. Rosenbrough, A.C. Farr, R.J. Randell. Protein measurement with folin-phenol reagent. Journal of Biological Chemistry 1951; 193: pp. 265–275
44. Baskar R, Lavanya R, Mayilvizhi S, Rajasekaran P. Free radical scavenging activity of antitumour polysaccharide fractions isolated from *Ganoderma lucidum* (Fr.) P. Karst. Natural Product Radiance 2008; 7(4): pp.320-325.
45. Premanand R., Santhosh Kumar P.H., Alladi Mohan, Study of Thiobarbituric Reactive Substances and Total Reduced Glutathione as Indices of Oxidative Stress in Chronic Smokers with and Without Chronic Obstructive Pulmonary Disease. Indian J Chest Dis Allied Sci 2007; 49: 9-12
46. Babre N, Debnath S., Manjunath Y., Parameshwar P., Wankhede S., Hariprasath K. Antioxidant potential of hydroalcoholic extract of *Barringtonia acutangula* linn roots on streptozotocin induced diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2: 4, 201-203.
47. D. Taleb-Senouci, H. Ghomari, D. Krouf, S. Bouderbala, J. Prost, M.A. Lacaille-Dubois, M. Bouchena. Antioxidant effect of *Ajuga iva* aqueous extract in streptozotocin-induced diabetic rats. Phytomedicine 2009; 16: 623–631.
48. Wajhul Qamar, Rehan Khan, Abdul Quaiyoom Khan, Muneeb U Rehman, Abdul Lateef,Mir Tahir, Farrah Ali, Sarwat Sultana. Alleviation of lung injury by glycyrrhizic acid in benzo(a)pyrene exposed rats:Probable role of soluble epoxide hydrolase and thioredoxin reductase. Toxicology, 2012; 291: 25-31.