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Inhibitory effect of *Dolichos biflorus* extract on allergic airway inflammation and hyperresponsiveness in animal model of ovalbumin-induced asthma

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Abstract

Bronchial asthma is an allergic disorder characterized by airway hyper responsiveness, infiltration of various inflammatory mediators and remodeling of the airways. It triggered by various factors like drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine, ovalbumin. Traditional medicinal plants are the richest source of therapeutic agents for prevention and treatment of asthma and its causes. Traditionally seeds of *Dolichos biflorus* are used in the treatment of cough, edema and asthma. The present study was designed to evaluate inhibitory effect of *Dolichos biflorus* extract on allergic airway inflammation and hyperresponsiveness in animal model of ovalbumin-induced asthma.

Main findings: With the treatment of ethanolic extract of *Dolichos biflorus* (DB) seeds, there was significant decrease in inflammatory cell count, level of nitric oxide and total protein in bronchoalveolar lavage (BAL) fluid at the dose of 100, 200 and 400 mg/kg, p.o. DB also restored the level of lung antioxidant enzymes (LPO, GSH, SOD, Catalase) and reduced the wet/dry weight ratio. In histopathological examination of lung tissue, DB protected the lungs from pathological changes induced by OVA.These results indicate that ethanolic extract of *Dolichos biflorus* seeds decreased allergic airway inflammation and hyperresponsiveness by decreasing the infiltration of inflammatory cells in the airway.

Our data suggests usefulness of *Dolichos biflorus* in prophylaxis and management of asthma.

Keywords:Ovalbumin, BAL, Hyperresponsivenss, Dolichos biflorus.

Introduction

Asthma is a chronic inflammatory respiratory disease characterized by airway hyper responsiveness, infiltration of various inflammatory mediators remodeling of the airways that causes development of reversible airway narrowing [1]. In asthmatic condition, pathological findings of broncho alveolar lavage fluid (BALF) indicated presence of inflammatory mediators, including thickening of the airway wall which further cause restriction of airflow and the development of airway hyper responsiveness [2]. Although many synthetic medicines with steroidal nature are available in market, they give only symptomatic relief. They are not only affordable to buy but also have many unwanted harmful side/toxic effects on the human system disturbing the basic physiology. Traditional medicinal plants are the richest source of therapeutic agents for prevention and treatment of asthma and its causes. They are cost effective and also free from the hazardous side effects and toxicity [3].

Traditionally seeds of *Dolichos biflorus* are used in the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, asthma [4]. The seeds of *D. biflorus* have been reported to show Antioxidant [5]. Chemomodulatory [6]. antiurolithiatic [7]. hepatoprotective [8]. and hypolipidemic activities [9]. The present study was aimed to evaluate allergic airway inflammation and hyper responsiveness of *Dolichos biflorus* seeds (DB) in ovalbumin-induced airway inflammatory responses in animal model of Asthma.

Materials and Methods

Experimental Animals

The *wistar rats* of either sex weighing about 150 - 250 g were purchased from National Toxicology Center, Pune. They were housed in groups 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/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 seeds of *Dolichos biflorus* were purchased from commercial supplier of Pune, India. The plant was authenticated Agharkar Institute of India, Pune, India (Voucher no. S-137).

Preparation of Extract

About 1000gm of seeds of *Dolichos biflorus* (DB) were dried under shed and coarsely powdered. Seeds 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 *Dolichos biflorus* (DB). The yield obtained was 18 g.

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 [10].

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 *Dolichos biflorus* (DB) 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.

Ovalbumin-induced airway inflammation

Sensitization and challenge with antigen

Animals were divided into six groups (n=5) viz. non-sensitized (NS), sensitized (S), dexamethasone (DEXA), DB 100, DB 200 and DB 400. All the animals except in the NS were sensitized by an intraperitoneal injection of 1ml alum precipitate antigen containing 20µg of ovalbumin and 8 mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum-ovalbumin mixture was given 7 days later. The NS animals were injected with alum only. Seven days after (15 day) second injection, animals were exposed to aerosolized ovalbumin (1%) for 30 min. DEXA (1 mg/kg), DB-100, DB-200 and DB-400 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. Histopathological evaluation of lung tissue was carried out. Lung wet to dry weight ratio was taken[11].

Estimation of total inflammatory cell counts in BAL fluid

The total leukocyte count and differential leukocyte were counted in the bronchoalveolar lavage under microscope using a hemocytometer. For the differential white cell count, BAL fluid was centrifuged at 1500 rev/ min for 10 min using a Remi refrigerated centrifuge, supernatant liquid was discarded and cellular pellets were resuspended in 100 µl of PBS for differential count using Leishmans stain [12].

Estimation of Lung antioxidant enzymes

Whole lung samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Lung homogenates (5% w/v) were prepared in cold 50 mM Tris buffer (pH 7.4) using Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was used for the estimation of Superoxide dismutase, Catalase [13], Lipid peroxidase [14] , and Glutathione reductase [15] in lung homogenate.

Estimation of Lactate dehydrogenase Nitric oxide and Total Protein

Nitric oxide scavenging activity was performed by sodium Nitroprusside-Griess reagent (1% sulphanilamide, 2% o-phosphoric acid add 0.1% N-(1-naphthyl)-ethylenediamine hydrochloride). The absorbance was taken at 546 nm. Ascorbic acid was used as standard [16]. For the Estimation of total protein, 0.7 ml Lowry reagent was added to 0.5 ml of BAL fluid sample. It was incubated in dark at room temperature for 20 minutes. After incubation, Proteins precipitated with sodium hydroxide were estimated with Folin's phenol reagent at 750 nm [17]. Lactate dehydrogenase was estimated by additing NADH (0.02 mM), sodium pyruvate (0.01 M), sodium phosphate buffer (0.1 M, pH 7.4) in a total volume of 2 ml of BAL fluid sample. The changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol NADH oxidized/min/ml BALF [18].

Statistical analysis

The results were expressed as Mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ was considered significant.

Results

Phytochemical Screening

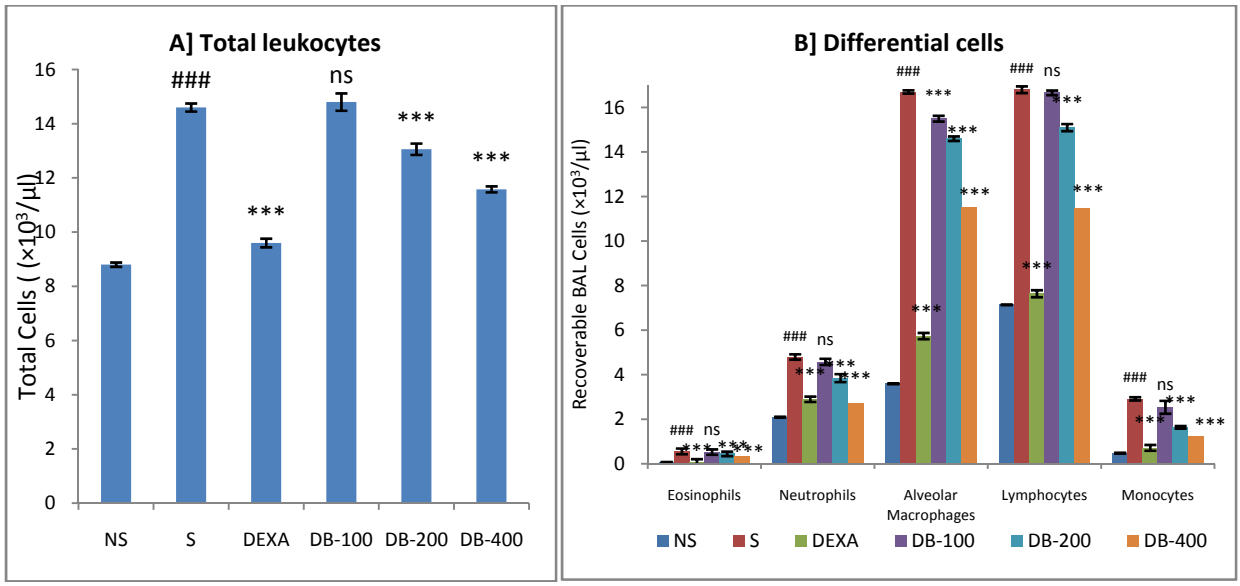
Preliminary phytochemical investigation of ethanolic extract of *Dolichos biflorus* (DB) showed presence of steroids, saponins, alkaloids, flavonoids, and glycosides.

Acute toxicity Study

The animals did not show any signs of toxicity or change in general behavior or other physiological activities. No mortality up to 7 days after treatment was observed with ethanolic extract of *Dolichos biflorus* (DB) and therefore was found safe up to dose of 2000 mg/kg. Doses were selected based on acute oral toxicity study. The present study was performed at three dose levels of ethanolic extract of Dolichos biflorus (DB) at 100, 200 and 400 mg/kg of body weight.

Effect of DB on inflammatory cell counts in BAL fluid

The number of inflammatory cells were significantly (p <0.001) increased in OVA sensitized group when compared with non-sensitized group. 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. In group of animals treated with DB at dose of 100 mg/kg, p.o., there was no significant inhibition of total leukocytes, eosinophils, neutrophils, lymphocytes and monocytes count, however, significant (p<0.001) inhibition of macrophages was observed in the BAL fluid. Animals treated with DB at doses of 200 and 400 mg/kg, showed significant (p<0.001) decrease in total leukocytes, eosinophils, neutrophils, macrophages, lymphocytes, and monocytes in the BAL fluid as compared to sensitized group. (Graph 1)



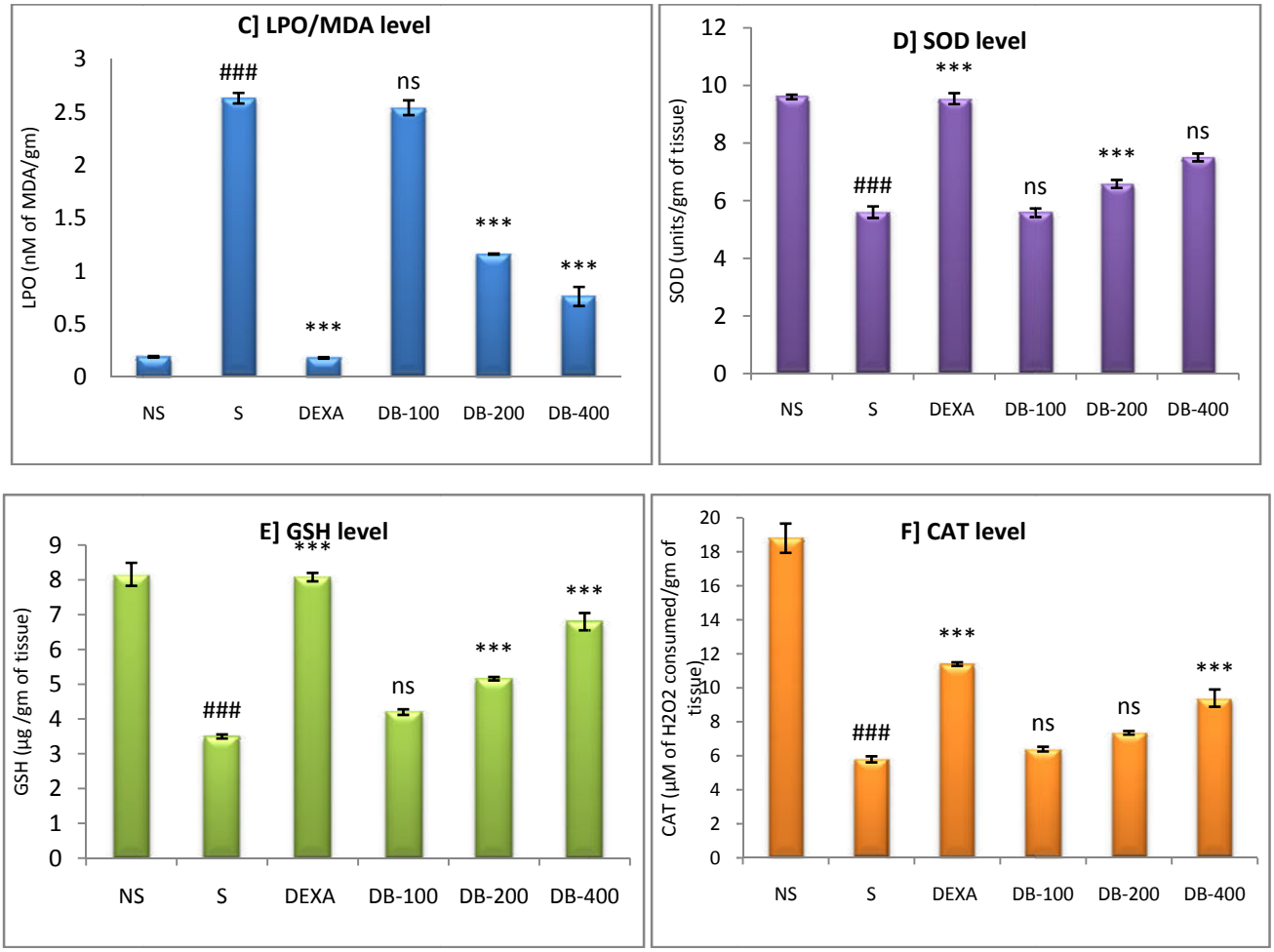
Graph 1: Effects of ethanolic extract of *Dolichos biflorus* on OVA-induced BAL inflammatory cells

###= P < 0.001 when S group compared with NS Group and * = p<0.05, ***= p<0.001, ns= not significant when DB 100, 200, 400 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.); DB 100, 200, 400= Ethanolic extract of *Dolichos biflorus* at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p.).

Effect of DB 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. Dexamethasone (1 mg/kg i.p.) significantly (p<0.001) increased the level on SOD, GSH and CAT and decreased the level of LPO as compared to sensitized group. DB at a dose of 100 mg/kg did not significantly restore the level SOD, GSH, CAT and LPO level as compared to sensitized group; DB at a dose of 200 mg/kg did not significantly restored the CAT level; but significantly restored (p<0.001) the level of SOD, GSH, LPO as compared to sensitized group. DB at a dose of 400 mg/kg significantly(p<0.001) restored the level SOD, GSH, CAT and LPO level as compared to sensitized group. (Graph 2)



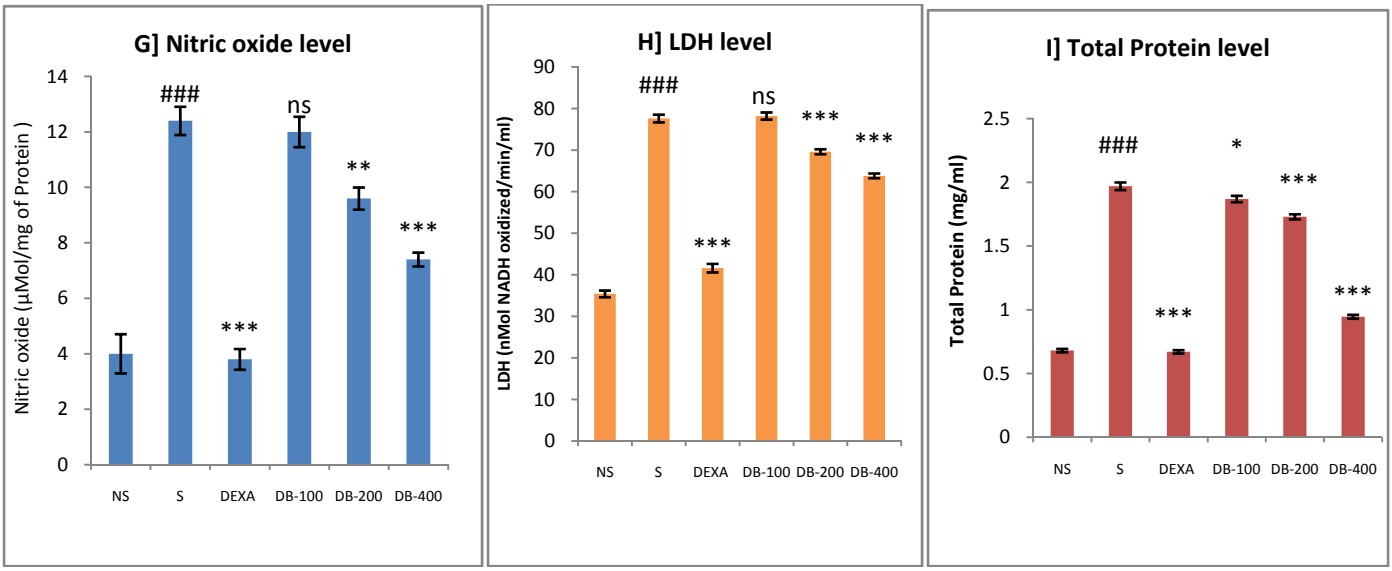
Graph 2: Effect of *Dolichos biflorus* on lung antioxidant status

###= P < 0.001 when S group compared with NS Group and *** = p<0.001, ns= not significant when DB 100, 200, 400 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.); DB 100, 200, 400= Ethanolic extract of Dolichos biflorus at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p.);

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

The nitric oxide, LDH and total protein level in BAL fluid were significantly (p<0.001) increased in sensitized group as compared to non-sensitized rats. 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 DB at a dose of 100 mg/kg, there was no significant decrease in nitric oxide and LDH level, however, significant decrease was observed in total protein (p<0.05) level when compared with sensitized group. In rats treated with DB at a dose of 200 mg/kg, there was significant decrease in nitric oxide (p<0.01), LDH and total protein level (p<0.001) when compared with sensitized group. In rats treated with DB at a dose of 400 mg/kg, there was significant (p<0.001) decrease in nitric oxide, LDH and total protein level when compared with sensitized group. (Graph 3)

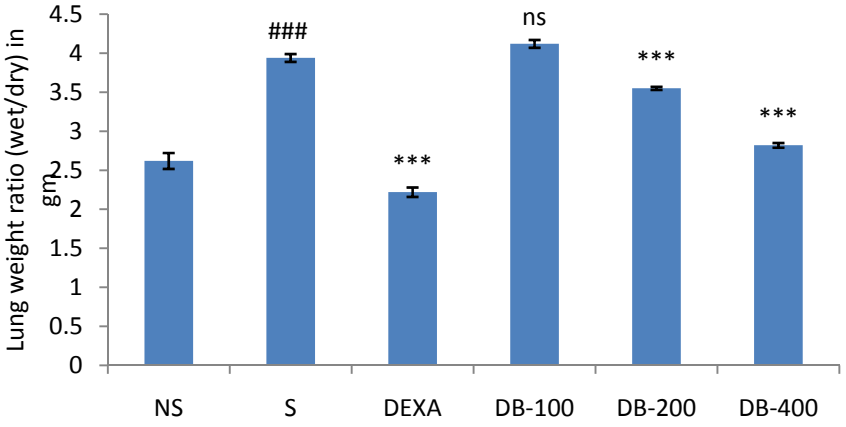


Graph 3: Effect of *Dolichos biflorus* on Nitric oxide, Lactate Dehydrogenase and Total Protein release in Bronchoalveolar lavage fluid (BALF) in rats

###= P < 0.001 when S group compared with NS Group and **= p<0.01, *** = p<0.001, ns= not significant when DB 100, 200, 400 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.); DB 100, 200, 400= Ethanolic extract of Dolichos biflorus at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p.).

Effect of DB 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 DB at the dose of 100 mg/kg, did not change the wet/dry weight ratio as compared to sensitized group. But DB at the dose of 200 and 400 mg/kg (p<0.001) significantly reduced the wet/dry weight ratio which was gained during the OVA induced asthma in a dose-dependent manner. (Graph 4)



Graph 4: Effect of *Dolichos biflorus* on lung wet-to-dry weight ratio in asthma and chronic lung inflammation

= P < 0.001 when S group compared with NS Group and ***= p<0.001, when DB 100, 200, 400 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.); DB 100, 200, 400= Ethanolic extract of Dolichos biflorus at 100, 200, 400 mg/kg, p.o. respectively with Ovalbumin 20µg + 8mg alum in 1ml (i.p.).

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 *Dolichos biflorus* protected the lungs from pathological changes induced by OVA (Figure 1).

When antigen binds to receptor-bound immunoglobulin E (IgE) antibodies, high-affinity IgE receptor i.e., FcRI receptors of mast cell localized in the airway smooth muscle, get activated. This lead to the release of inflammatory mediators and a large variety of cytokines, including TNF- and Th2-associated cytokines such as IL-4, IL-5, IL-6 and IL-10 [19]. Inflammatory mediators release reactive oxygen species (ROS) which has ability to contract smooth muscle and to release autacoid mediators derived from the mast cells like histamine, prostaglandin D_2 and the cysteinyl leukotrienec. These autacoid mediators are well known potent spasmogens of airway smooth muscle and the mast-cell–specific serine protease tryptase that induce bronchoconstriction, airway remodeling, and airway hyperresponsiveness [20, 42].

Bronchoalveolar lavage fluid (BALF) is a biofluid expressing secreted pulmonary proteins, components of the epithelial lining fluid and the products of activated cells and destructive processes. Therefore estimation of parameters in BAL fluid establishes temporal and prognostic indication of asthma [21]. In this study, OVA challenge induced airway inflammation and increase in all inflammatory mediators which worsen the asthmatic condition. Animals when firstly challenged with OVA, they start to produce IgE antibodies as the allergic response in lungs after 24 hr. When animal are re-exposed to the OVA, these IgE antibodies bind to Fc RI receptors on mast cells and activates Th2 cytokines. This results in infiltration of inflammatory cells by chemotaxis into the

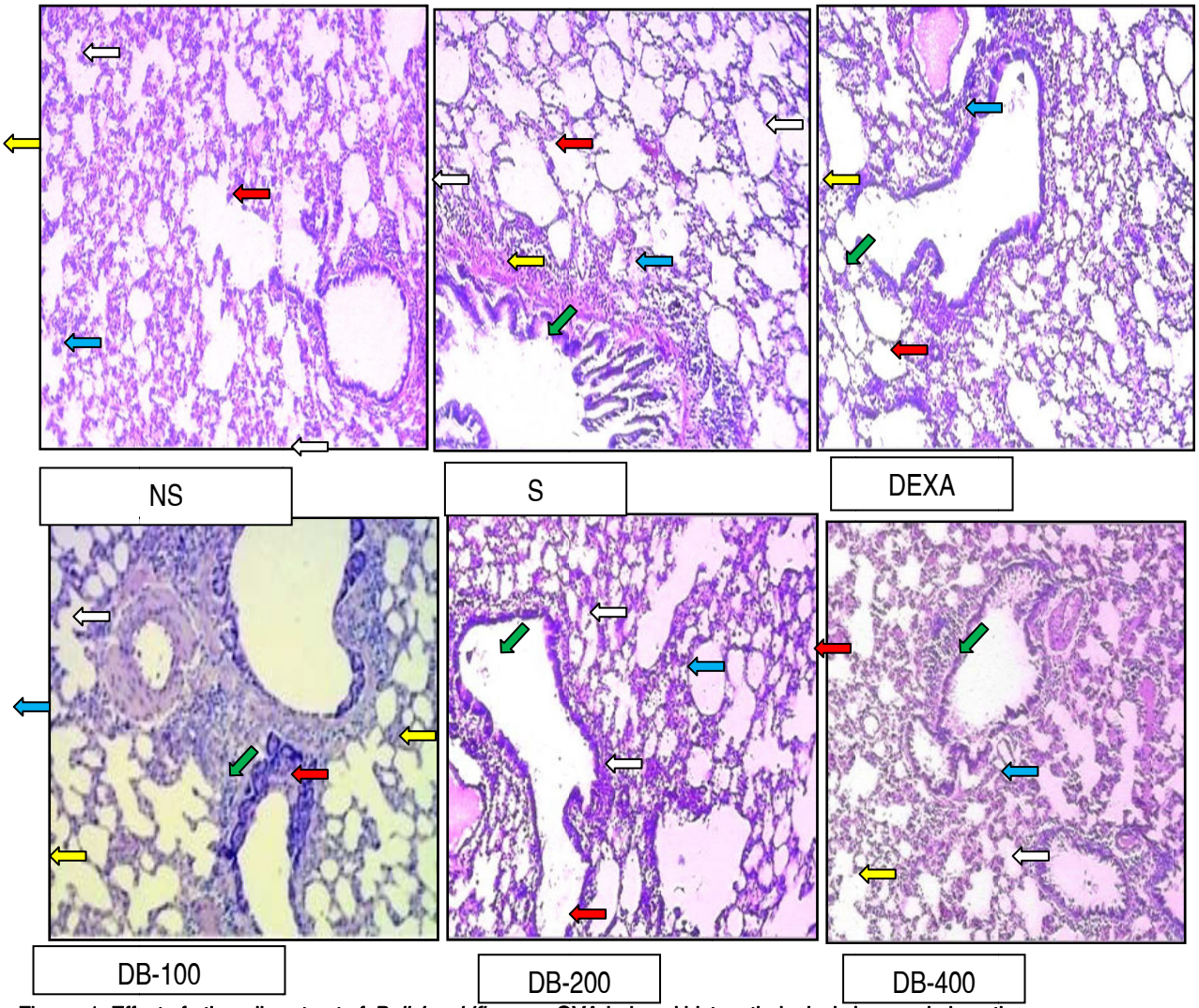


Figure. 1: Effect of ethanolic extract of *Dolichos biflorus* on OVA-induced histopathological changes in lung tissue.

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

lung which further increases oxidative stress. In the late phase airway response in asthma, in lungs there was infiltration of the inflammatory cells especially eosinophils to the site of inflammation. Adherence of eosinophils to bronchial epithelium release eosinophil derived neurotoxin which causes damage and loss of epithelium of lung tissue. Eosinophil derived neurotoxin further acting as antagonist to muscarinic M_2 receptors (auto receptors) and release acetylcholine which causes bronchial constriction and hyper-responsiveness [22].

The immune response in allergic asthma is, driven primarily by CD4+ T helper type 2 (Th2) lymphocytes. Activation of Th2 cells produce IL-4, IL-5, and IL-13, resulting in IgE production, eosinophilia, and mucus production within the lung, respectively. A more recently described subset of CD4+ T helper cells, named Th17 cells, produce IL-17A, IL-17 F, and IL-22, and seem to be involved in severe asthma involving neutrophilia. [23]. The increased level of monocytes causes increase in cytokines which promotes macrophages chemotaxis and stimulates macrophage phagocytosis. The number of monocyte cells was increased after antigen challenge in BALF [24, 25]. Monocytes produce interleukin IL-I which promotes macrophage chemotaxis and thus participate in the inflammatory process of asthmatic syndrome by macrophage phagocytosis [26].

In animal airways, OVA challenge has induced eosinophil, neutrophil, monocyte and lymphocyte infiltration and activation is similar to that of reported in human asthmatics. In present study we found that animals treated with *Dolichos biflorus* significantly inhibited OVA induced hyper reactivity by preventing infiltration of total leukocyte, eosinophils, neutrophil, lymphocyte, and monocyte counts as compared to sensitized animals. This shows protective effect of *Dolichos biflorus* 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. The similar findings were reported by Lee, et al., (2011)[43]. Ling-Yi & James (2002).

Oxidative stress in lungs is one of the reasons for airway inflammation and hyperreactivity in asthma. Oxidative stress development produce the infiltration of inflammatory cells in the airways and further produce several mediators such as superoxide radical, hydrogen peroxide, hypochlorous acid, and hydroxyl radical. Excessive production of reactive oxygen species (ROS) by blood monocytes, neutrophils, and eosinophils in turn cause characteristic increase in production of lipid peroxidation products, increased oxidized glutathione in bronchoalveolar lavage (BAL) fluid and increased production of nitric oxide (NO) [27] .

In response to oxidative stress, there was imbalance between reactive oxygen/nitrogen species production and antioxidant enzyme which include superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) [28]. Further increase of oxidative stress can induce inflammation and ultimately cell death [29]. SOD and GSH get inactivated by reactive oxygen and nitrogen species which were released during oxidative stress [30, 31]. Generation of reactive oxygen species interact with glutathione peroxidase (GSH-Px) reducing its activity. ROS interacts with NO lead to the formation of peroxynitrite ($ONOO^-$) or alternatively be rapidly converted to oxygen and hydrogen peroxide under the influence of superoxide dismutase (SOD) which is eliminated by glutathione reductase. GSH play important in the protection of cells against oxidative stress as Glutathione peroxidase scavenges toxic amount of peroxides and free radical which helps to reduce the inflammation [32]. The inflammatory cells increase the production of reactive oxygen species. Thus during the inflammatory process antioxidant defense get impaired in hyperactive airways of asthma [33, 34].

Generated hydrogen peroxide must be quickly converted into other, less dangerous substances which will be done by the Catalase enzyme which catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules. Thus also take part in oxidative stress related to asthma and free radical formation and oxidation [35]. Generation of free radical produce final products of polyunsaturated fatty acids peroxidation in the cells known as Malondialdehyde (MDA). Therefore increase in MDA i.e., LPO levels is a marker of oxidative stress [34, 36]. In present study it was found that MDA i.e., LPO levels increased in sensitized group as compared to drug treated group. *Dolichos biflorus* extract significantly restored the level of SOD, GSH, Catalase and LPO. Therefore it has been suggested that *Dolichos biflorus* extract reduces aggravation of inflammation during asthma by providing antioxidant enzymes protection which may contribute the use of *Dolichos biflorus* extract in asthma. The similar findings were observed by Jung et al., (2011)[43].

Challenge with OVA also produced excessive production of LDH, NO and total protein levels in BAL fluid which are indicative of oxidative stress in asthma. Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme which is present in all cells. When the epithelial membrane is damaged, LDH is rapidly released into the BAL fluid [37]. The present results indicate that *Dolichos biflorus* extract significantly reduced LDH level, thereby reduced the lysis of epithelial cell membrane in pathogenesis of asthma. During inflammation, Nitric oxide is produced by inducible NO synthase (iNOS) and this leads to the formation of NO radicals or S-nitrosothiols or $ONOO^-$ in the host cell. This further leads to generation of secondary reactive nitrogen and oxygen species. The iNOS-derived NO promotes Type 2 cell (Th2) expansion, bronchial hyperactivity and eosinophil infiltration in the airways by reducing T helper Type 1 cells probably IFN-γ [38]. *Dolichos biflorus* extract significantly reduced production of NO in BAL fluid and thus protected them from the damaging effect of NO production. Total protein concentration in bronchoalveolar lavage fluid is marker of pulmonary edema by capillary-alveolar leakage [39]. Results in the present study revealed that *Dolichos biflorus* extract reduced the level of total protein. The same findings were reported by Kawabata et al, (2011)[45].

The water content of the lungs was determined by calculating the wet/dry weight ratio of lung tissues [40]. In OVA-induced asthma, there was 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 *Dolichos biflorus* treated groups, there was decrease in wet/dry ratio indicating its use in pulmonary edematous conditions produced in OVA-induced asthma. Therefore, *Dolichos biflorus* extract may prove useful 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[41]. This can be clearly seen from 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, treatment with *Dolichos biflorus* protected the lungs from pathological changes induced by OVA.

The preliminary phytochemical investigation of ethanolic extract of *Dolichos biflorus* showed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides Chong et al., (2009)[50] reported mast cell stabilizing activity of saponins. Flavonoids are known to possess anti-inflammatory effects and antioxidant activity which may be responsible for anti-inflammatory and antioxidant activity. Carlo et al., (1999)[46], Geeta et al., (1981)[47] and Vijayalakshmi et al., (2011)[48] reported mast cell stabilizing and antiallergic activity of alkaloids. The seeds of *D. biflorus* have been reported to show antioxidant activity [5]. Thus presence of these phytoconstituents in ethanolic extract of *Dolichos biflorus* may further contribute in ova albumin-induced airway inflammatory responses in a management of Asthma.

Conclusion

From the above findings, we may conclude that *Dolichos biflorus* have capacity to inhibit allergic airway inflammation and hyper responsiveness in animal model of ovalbumin-induced asthma. These results may be due to presence of flavonoids and also due to antioxidant and anti-inflammatory potential of *Dolichos biflorus*. Therefore our data suggests usefulness of *Dolichos biflorus* in prophylaxis and management of asthma.

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Conflict of Interest

There is no conflict of interest

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