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Anti-inflammatory effect of *Cajanus indicus* (Linn.) Mill. in a murine model of asthma

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Abstract

The present study was undertaken to explore the effects of *Cajanus indicus* (Linn.) Mill. on airway inflammation and oxidative stress in ovalbumin-induced inflammation and antilipoxygenase activity in a murine model of asthma. The protective effect of extract of whole plant of *Cajanus indicus* (Linn.) Mill. against ovalbumin induced lung inflammation and arachidonic acid induced paw inflammation was evaluated. Hydroalcoholic extract of *Cajanus indicus* (Linn.) Mill.(CI) showed significant inhibition of total as well as differential leukocytes in the bronchalveolar lavage (BAL) fluid at dose of 350 and 525 mg/kg, p.o. ($p<0.01$).Pretreatment with CI significantly restored ($p<0.01$) the level of GSH, SOD,CATALASE and LPO in lungs. In BAL fluid, treatment of CI significantly reduced nitric oxide and total protein level. CI significantly inhibited arachidonic acid induced paw edema ($p<0.01$). Hence it can be concluded that *Cajanus indicus* (Linn.) Mill. has significant anti-inflammatory, antioxidant and antilipoxygenase effect.

Keywords: *Cajanus indicus*, Asthma, Antioxidant, Ovalbumin.

1. Introduction

Asthma is an inflammatory disease of the lungs characterized by increased infiltration of leukocytes, especially eosinophils, into the airways and reduced respiratory function. The inflammation leads to bronchoconstriction, increased airway hyperresponsivenss (AHR), and mucus production [1]. The prevalence of asthma is rapidly increasing around the world, especially in young children, and it has become a significant cause of morbidity and mortality in developed countries [2]. Both eosinophils and T helper (Th2) lymphocytes play pathogenic roles in asthma [3]. Eosinophils are commonly associated with allergic inflammation, and act

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as effector cells in the pathogenesis of this disease by releasing cytotoxic granule proteins [4]. Eosinophils are known as the primary effector cells in the pathogenesis of asthma through the release of specific granule proteins and ROS [5]. Eotaxin is a potent chemoattractant for eosinophils and it is generally elevated after asthma induction. An imbalance between Th1 and Th2 leads to the clinical expression of allergic disease. Th2 cytokines, including IL-4, IL-5, and IL-13 typically increase in allergic diseases and have important effects on airway infiltration, eosinophil activation, induction of immunoglobulin E (IgE) production, mucus secretion and the release of a variety of inflammatory mediators [6].

Oxidative stress is induced by a large variety of oxygen free radicals, including reactive oxygen species (ROS). An increasing amount of clinical and experimental evidence suggests that ROS play essential role in the pathogenesis of airway inflammation [7,8,9].

The CysLTs (LTC4, LTD4 and LTE4) are potent pro-inflammatory lipid mediators that play a pivotal role in inflammation and in the contraction and remodelling of airways observed in asthmatics.CysLTs are formed by inflammatory cells such as mast cells, eosinophils, basophils and macrophages.They are potent spasmogens and they promote mucous secretion [10].

Pigeonpea [*Cajanus indicus* (Linn.) Mill., Fabaceae], is mainly distributed in semiarid and subtropical areas of the world. It is used extensively as a substitute of expensive animal protein in human diet in many developing countries. Now a days, its medical applications have provoked much interest, and its leaves have been brought to market as a product of traditional Chinese medicine (TCM) that possesses notable anti-inflammatory, anti-bacterial, abirritative properties and inhibits capillary permeability. Chemical constituent investigations showed that the major active compounds in pigeonpea leaves are flavonoids. Flavonoids are being responsible for the beneficial efficacies of *Cajanus indicus* (Linn.) Mill. leaves on human health [11]. Flavone *C*-glucosides, kind of important constituents of the flavonoid family present in foodstuffs and nutraceuticals, have received much attention recently because of their antioxidant and anticancer properties. They were found in plants and fruits. The flavonoids present in pigeonpea are Vitexin, isovitexin, luteolin, apigenin etc. [12, 13].

Various biological and pharmacological activities have been attributed to flavonoids, such as hypotensive, anti-inflammatory, antiallergic, antispasmodic, antimicrobial, antioxidant/free radical scavenging and radio protective effects.

Traditionally in India, *Cajanus indicus* (Linn.) Mill. was used in the treatment of pain, inflammation, ulcer, asthma, bronchitis, etc. [14]. Also, the whole dried plant of *Cajanus indicus* (Linn.) Mill. was given for the treatment of asthma [15]. Recent studies show that CI and the proteins derived from CI have immunomodulatory activity [16], antioxidant effects [17] and free radical scavenging activity [18].

However, no studies of CI have so far been reported on ovalbumin-induced inflammation

and its associated oxidative stress and antilipoxygenase activity of this plant in a murine model of asthma.

We investigated the effects of, *Cajanus indicus* (Linn.) Mill. extract on airway inflammation and oxidative stress in ovalbumin-induced airway inflammation and antilipoxygenase activity in a murine model of asthma.

2. Materials and methods

2.1 Chemicals

Chicken egg ovalbumin (Central Drug House Pvt. Ltd.), Arachidonic acid (Sigma Aldrich, USA), Biochemical estimation kits (Nirmal Laboratories Pvt.Ltd.).All other chemicals and solvents were of the highest grade commercially available.

2.2 Collection and extraction

The medicinal plant (procured from Narayangaon, India) specimen was identified and authenticated by Dr. Rajesh Dabur at "Regional Research Institute (Ay.)" Pune with specimen voucher no. 185 and catalogued. The technique used for the extraction purpose was cold maceration. The whole plant was washed with distilled water and shed dried and latter powdered. This powder was then defatted with petroleum ether and then macerated with aqueous-ethanol (20:80 % v/v) solution for 7 days with occasional shaking. It was then filtered and the solvent was evaporated under vacuum. The yield of hydroalcoholic extract of plant of *Cajanus indicus* (Linn.) Mill. was found to be 7.5 % w/w.

2.3 Animals

Albino rats of Wistar strain weighing 200-250 g were obtained from National Toxicology Center (NTC), Pune. Animals of either sex were housed under standard laboratory conditions of 22 ± 3 ^{0}C temperature and relative humidity 30% and 12 h light and dark cycle maintained, free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra) and water *ad libitum*. The protocol of this study was approved by the Institutional Animal Ethics Committee **(**IAEC registration no. 198/99/CPCSEA).

2.3 Acute toxicity study (OECD, 425)

The acute toxicity study for hydroalcoholic extract of plant of *Cajanus indicus* (Linn.) Mill. was performed using albino mice (OECD 425, 2001). The animals were fasted overnight prior to the experiment and maintained under standard conditions. Initially limit test at 2,000 mg/kg was carried out, and then monitored for any mortality for the 14 days. CI was administrated orally in increasing doses and was found safe up to dose of 5,000 mg/kg.

3. Experimental protocol

3.1 Bronchoalveolar lavage in rats

3.1.1 Sensitization and challenge with antigen

Wistar rats were divided into six groups (n=6) (200 to 250 g). The rats were sensitized (S) by an intra-peritoneal injection of 1ml alum precipitate antigen containing 20 µg of ovalbumin and 8 mg of alum suspended in 0.9% sodium chloride solution [19]. A booster injection of this alum-ovalbumin mixture was given 7 days later. Non sensitized (NS) animals were injected with alum only. Seven days after (i.e. on 15th day) second injection animals were exposed to aerosolized ovalbumin (1%) for 30 min into a closed plexiglass chamber. One group received Dexamethasone (1mg/kg, i.p) as standard and

remaining three groups were received *Cajanus indicus* (Linn.) Mill. extract at dose 175, 350, 525 mg/kg, p.o. before 5 hr of antigen challenge. Bronchoalveolar lavage (BAL) fluid was collected by lavaging the lungs with 2 aliquots of 5 ml of 0.9% sodium chloride solution. Total recovery volume per rat was approximately 8 ml. The total leucocyte count in the bronchoalveolar lavage fluid was performed using a haemocytometer. For the differential leucocyte count, BAL fluids were centrifuged at 1350 rpm for 10 mins using a Remi refrigerated centrifuge, supernatant liquid was discarded and cellular pellets were resuspended in 100 µl of PBS for total and differential cell counts by using Leishmans stain [20].

3.1.2 Lung tissue histopathology

For the histological evaluation of lung tissue, the lungs were fixed in formalin and embedded in paraffin wax. Sections of lung tissue were cut at 5 µm thickness, mounted on glass slides and stained with hematoxylin and eosin (H × E) to assess lung histopathology. Asthmatic lung injury was graded from 0 (normal) to 4 (severe) in each four categories: Integrity of alveoli, Infiltration of leucocytes, type inflammatory exudates, Status of bronchi, Perivascular status of lung blood vessels, activation of alveolar macrophages. The total asthmatic lung injury score (TALIS) was calculated by adding the individual scores for each category.

3.1.3 Lung antioxidant enzymes assay (Estimation of MDA, GSH, SOD and CAT)

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 GSH [21], malondialdehyde (MDA) [22], superoxide dismutase (SOD) [23] and catalase [24] levels.

3.1.4 Nitric oxide, Total Protein and LDH analysis in BALF

The pulmonary production of nitric oxide in the BALF was spectrophotometrically determined by assaying BALF for nitrite using the Griess reagent (1% sulfanilic acid, 0.1% *N*-1 naphthyl ethylenediamine dihydrochloride, 5% phosphoric acid). Absorbance was measured at 525 nm and nitrite concentration was determined using sodium nitrite as a standard [25]. Total protein was measured by Bradford assay (Biolab diagnostic, Pvt. Ltd., India) according to the manufacturer's instructions with bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as standard. Lactate dehydrogenase was determined by buffered pyruvate substrate, NADH reagent and the absorbance change per minute at 340 nm was read.

3.1.5 Wet to Dry Lung Ratio

Four to five animals by each group were used to determine the wet-to-dry lung ratio as an indicator of pulmonary edema. The left lung was excised and immediately weighed using a precision balance, then re-weighed after being dried for 24 hours in an oven at 90°C. The wet/dry ratio was calculated by dividing the wet weight by the dry weight [26].

3.2 Arachidonic acid-induced rat paw edema

Wistar rats were divided into six groups (n=6)

weight from 200 to 250 g. Paw edema was induced by subplantar injection of 0.1 ml 0.5% arachidonic acid dissolved in 0.2M carbonate buffer (pH 8.5) into the right hind paw. Indomethacin (10 mg/kg, i.p.) and Montelukast (10 mg/kg, i.p.) used as reference standard were administered 30 min. while hydroalcoholic extract of *Cajanus indicus* (Linn.) Mill. (175, 350, 525 mg/kg, p.o.) were administered 1 hr. before arachidonic acid injection. Edema volume was measured by a plethysmometer (UGO Basile 7140, Italy) immediately after arachidonic acid injection at 30, 60, 90 and 120 min. [27].

4. 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 $p<0.05$.

5. Results

5.1 Effects of CI on OVA-induced total and differential leucocytes in BAL fluid

We summarized the OVA-induced airway inflammation in a murine model of asthma and examined the change of total and differential leucocyte count in the BAL fluid following OVA sensitization and challenge. Number of total and differential leucocytes in BAL fluid were significantly ($p<0.01$) increased in OVA sensitized group when compared with non-sensitized group. Pretreatment with CI (350 mg/kg, p.o.) and (525 mg/kg, p.o.) significantly reduced ($p<0.01$) the total leucocytes, eosinophils, neutrophils, macrophages, lymphocytes and monocytes in the BAL fluid when compared to sensitized group while there was no significant inhibition at dose of (175 mg/kg, p.o.). Dexamethasone (1 mg/kg, i.p.) significantly reduced ($p<0.01$) the total as well as differential leucocyte count in BALF when compared to sensitized group. These results suggest that CI inhibited the OVA-induced inflammatory response in a murine model of asthma (Graph 1, Graph 2).

5.2 Effects of CI on OVA- induced histopathological changes in lung tissue

To examine effects of CI on OVA-induced asthmatic lung injury in rat, we observed the histopathological changes in lung tissue by using microscopy. After 24 hr of ovalbumin (1%) challenge we found that there was increased in infiltration of leucocytes, type of inflammatory exudates i.e. catarrhal and mucoid material, constriction of the secondary bronchi and tertiary bronchi, infiltration of mononuclear cells around the lung blood vessels, integrity of alveoli affected , activation of alveolar macrophages in sensitized group. Dexamethasone 1 mg/kg and CI at dose of 175, 350 and 525 mg/kg significantly reduced ($p<0.01$) ovalbumin induced histolopathogical changes as comparable to sensitized group. Total asthmatic lung injury score was less in Dexamethasone and CI as compared to sensitized group. These results suggest that CI inhibited the OVA-induced lung inflammatory changes in a murine model of asthma (Figure 1-6, Graph 11).

5.3 Effects of CI on LPO, GSH, SOD and CAT level

We analyzed the levels of LPO, GSH, SOD, and CAT level in lung homogenate samples by using biochemical estimation kits. Ovalbumin significantly ($p<0.01$) increased the level of LPO and decrease level of GSH, SOD and CAT in OVA sensitized group when compared with

non-sensitized group. Dexamethasone (1 mg/kg i.p.) has not shown any effect on GSH, SOD and CAT but significantly ($p<0.01$) decreased level of LPO as compare to sensitized group. Pretreatment with CI at a dose of 175, 350 and 525 mg/kg significantly reduced ($p<0.05$, $p<0.01$) the LPO level, also significantly restored ($p<0.05$, $p<0.01$) the level of GSH, SOD and CAT when compared with sensitized group. These results suggest that CI restored the level of antioxidant enzymes in OVA -induced lung inflammation and its associated oxidative stress (Graph 6, Graph 7, Graph 8 and Graph 9).

5.4 Effect of CI on nitric oxide, total protein and LDH level in BALF

We analyzed the levels of nitric oxide, total protein and LDH level in BALF by using biochemical estimation kits. Result demonstrated that the nitric oxide and total protein level in BAL fluid were significantly ($p<0.01$) increased in sensitized group compared to non-sensitized rats . The nitric oxide, total protein and LDH level was significantly decreased ($p<0.05$, $p<0.01$) in rats treated with CI 175, 350 and 525 mg/kg when compared with sensitized group. Treatment of Dexamethasone at a dose of 1 mg/kg showed significant reduction in nitric oxide, total protein and LDH level ($p<0.01$) when compared with sensitized group. These results suggest that CI decreased the level of NO, an early marker of lung inflammation, injury or asthma [28], LDH and total protein level which are biochemical indices of pulmonary damage [29] in OVA -induced lung inflammation and its associated oxidative stress in a murine model of asthma (Graph 3, Graph 4 and Graph 5).

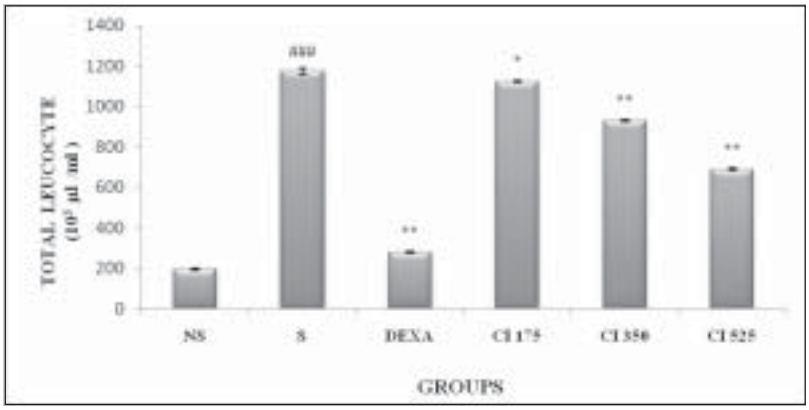
5.5 Effects of OVA on the lung wet-to-dry weight ratio

We analyzed the lung wet-to-dry weight ratio which was significantly higher in sensitized group when compared with non-sensitized group ($p<0.01$). Pretreatment with CI 350 and 525 mg/kg significantly reduced ($p<0.05$, $p<0.01$) the lung wet/dry weight ratio. Treatment of Dexamethasone at a dose of 1 mg/kg showed significant reduction in lung wet-to-dry weight ratio ($p<0.01$) when compared with sensitized group. These results suggest that CI decreased gain in weight of lung due to increase in inflammatory cells, tissue necrosis in the in OVA -induced lung inflammation in a murine model of asthma (Graph 10).

5.6 Effect of CI on arachidonic acid induced paw edema in rats

We observed the effect of of CI on arachidonic acid induced paw edema in rats to predict antilipoxygenase activity. In the control group, arachidonic acid increased the paw volume which was measurable up to the time period of 1.5 hrs. Pretreatment with CI (525 mg/kg, p.o.) significantly reduced ($p<0.01$) the paw volume when compared to control group and the percentage inhibition was 38.90 % where as montelukast (10 mg/kg i.p) showed 61.11 % reduction in the paw edema while Indomethacin (10 mg/kg i.p) doesn't showed any significant reduction in the paw volume when compared to control group. . These results predict that CI may inhibit arachidonic acid induced paw edema by inhibiting lipoxygenase pathway [30] in a murine model of asthma (Graph 11, Graph 12).

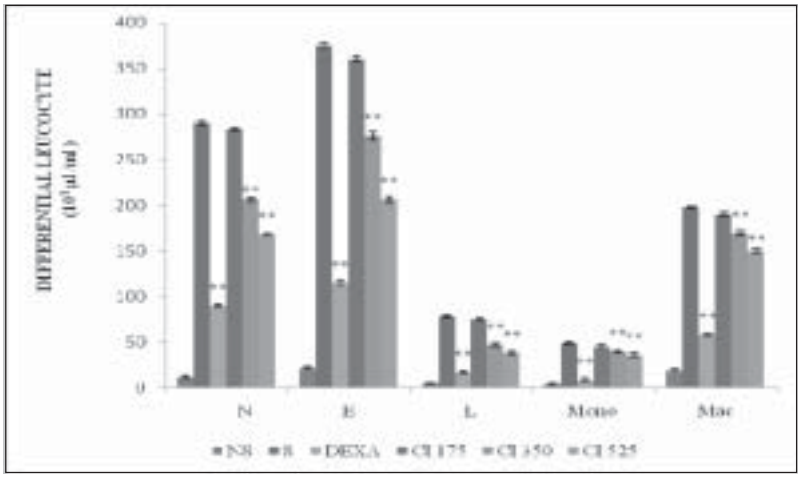
Graph 1: Effect of *Cajanus indicus* (Linn.) Mill. on Total leucocyte



Where, n=6, Values are expressed as Mean ± SEM , Group-I (NS) = Nonsensitized = 8 mg alum in 1ml saline (i.p.), Group-II (S) = Sensitized = Ovalbumin 20 µg + 8 mg alum in 1ml saline (i.p.), Group III (DEXA) = Dexamethasone (0.5 mg/kg, i.p.), Group-IV (CI 175) = *Cajanus indicus* extract (175 mg/kg, p.o.), Group-V (CI 350) = *Cajanus indicus* extract (350 mg/kg, p.o.), Group-VI (CI 525) = *Cajanus indicus* extract (525 mg/kg, p.o.) , Statistical analysis done by using ANOVA followed by Dunnett's test, Group III, IV, V,VI compared with Group II, Group I compared with Group II.

*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

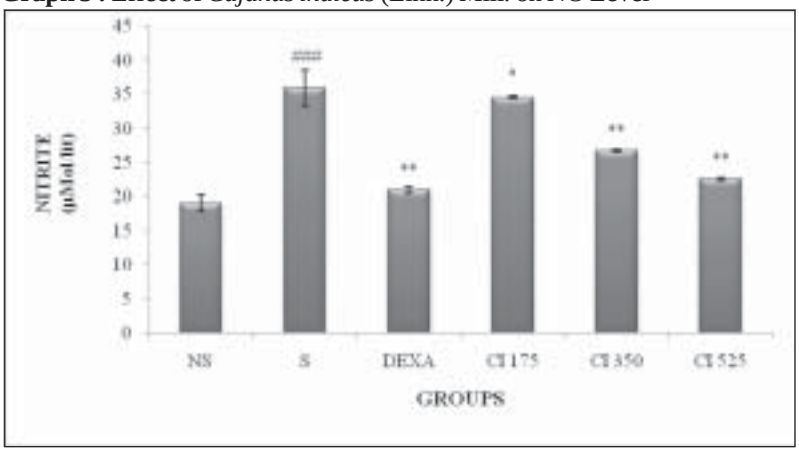
Graph 2 : Effect of *Cajanus indicus* (Linn.) Mill. on Differential leucocyte



Where, n=6, Values are expressed as Mean ± SEM, Group-I (NS) = Nonsensitized = 8 mg alum in 1ml saline (i.p.), Group-II (S) = Sensitized = Ovalbumin 20 µg + 8 mg alum in 1ml saline (i.p.), Group III (DEXA) = Dexamethasone (0.5 mg/kg, i.p.), Group-IV (CI 175) = *Cajanus indicus* extract (175 mg/kg, p.o.), Group-V (CI 350) = *Cajanus indicus* extract (350 mg/kg, p.o.), Group-VI (CI 525) = *Cajanus indicus* extract (525 mg/kg, p.o.), Statistical analysis done by using ANOVA followed by Dunnett's test., Group III, IV, V,VI compared with Group II, Group I compared with Group II.

*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 3 : Effect of *Cajanus indicus* (Linn.) Mill. on NO Level



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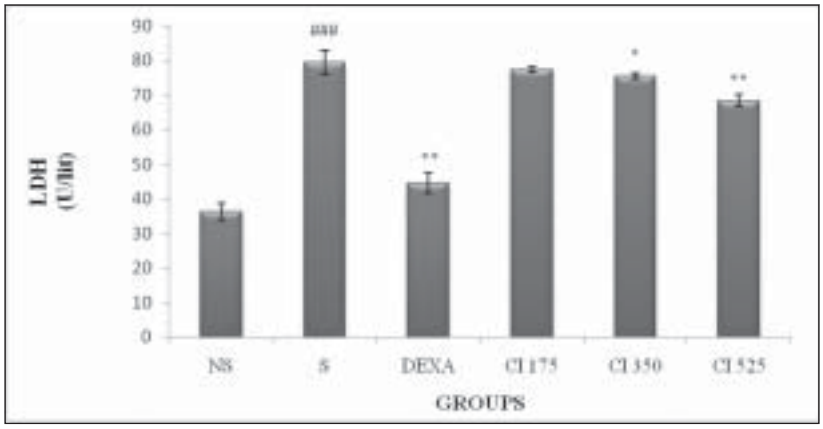
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 4 : Effect of *Cajanus indicus* (Linn.) Mill. on LDH Level



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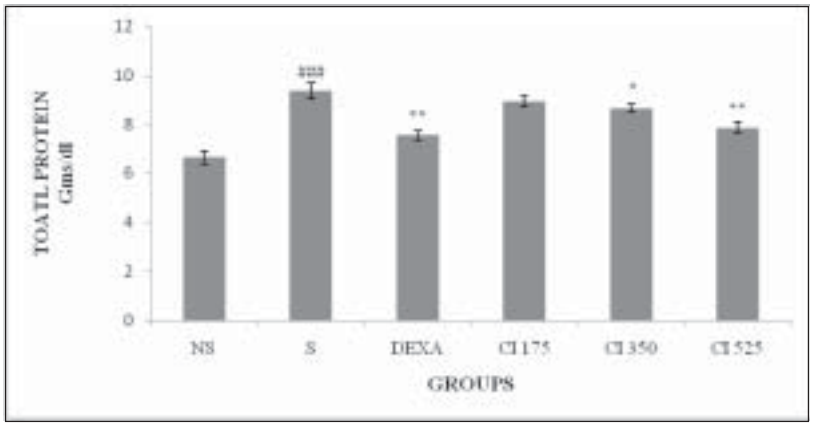
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 5 : Effect of *Cajanus indicus* (Linn.) Mill. on TOTAL PROTEIN Level



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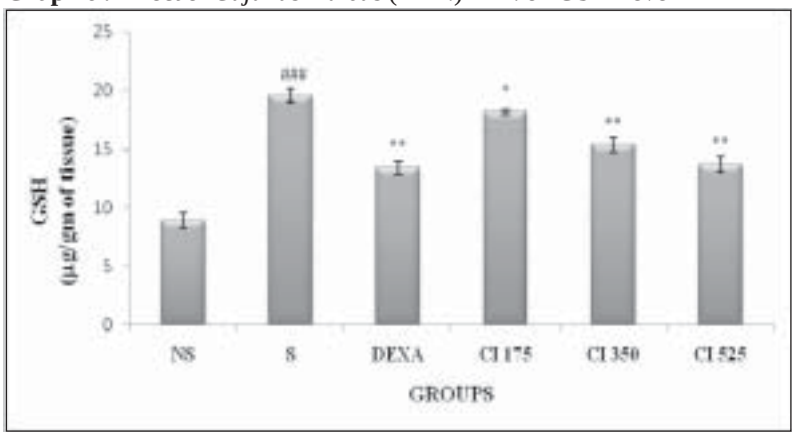
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 6 : Effect of *Cajanus indicus* (Linn.) Mill. on GSH Level



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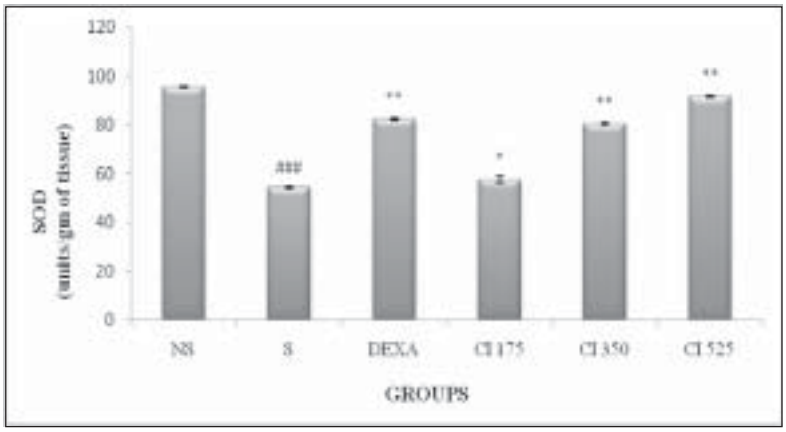
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 7 : Effect of *Cajanus indicus* (Linn.) Mill. on SOD Level



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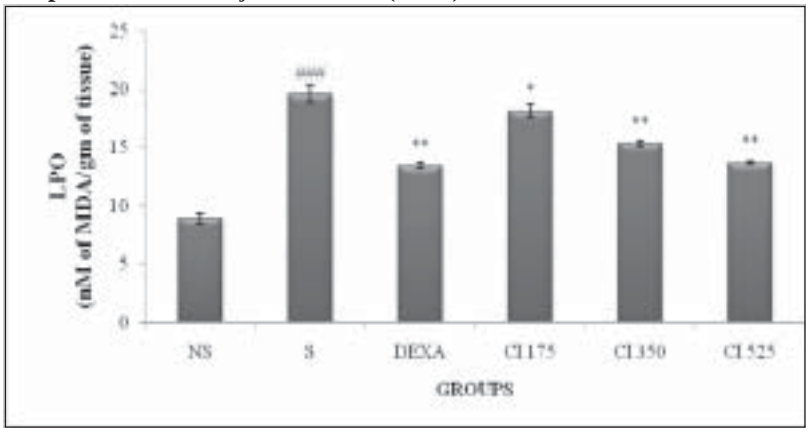
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 8 : Effect of *Cajanus indicus* (Linn.) Mill. on LPO Level



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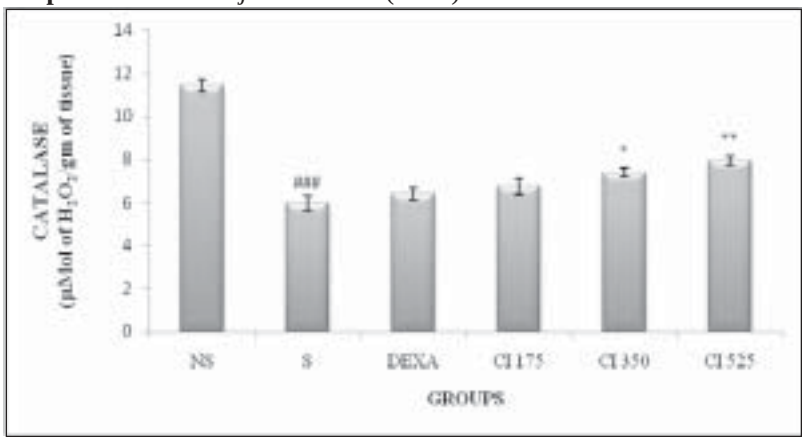
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 9 : Effect of *Cajanus indicus* (Linn.) Mill. on CATALASE Level



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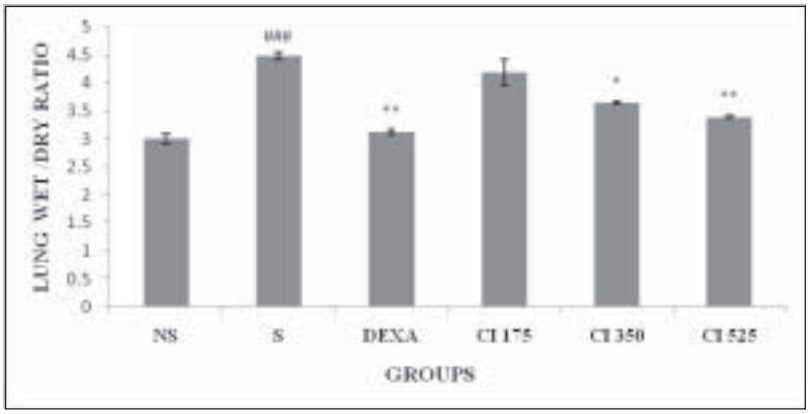
*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

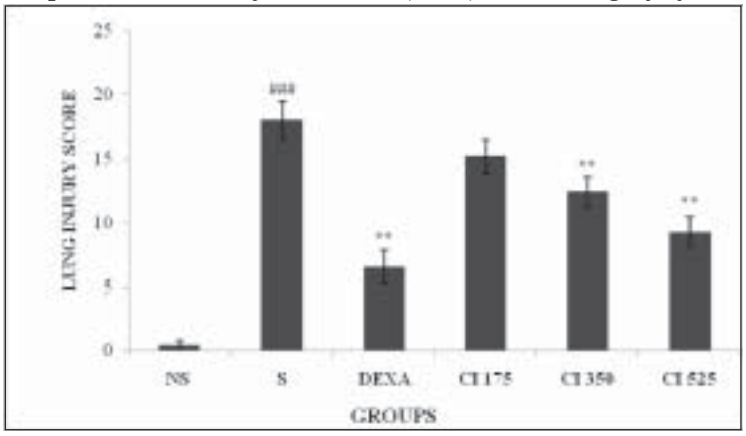
Graph 10 : Effect of *Cajanus indicus* (Linn.) Mill. on lung wet/dry ratio



Where, n=6, Values are expressed as Mean ± SEM, Group-I (NS) = Nonsensitized = 8 mg alum in 1ml saline (i.p.) Group-II (S) = Sensitized = Ovalbumin 20 µg + 8 mg alum in 1ml saline (i.p.), Group III (DEXA) = Dexamethasone (0.5 mg/kg, i.p.), Group-IV (CI 175) = *Cajanus indicus* extract (175 mg/kg, p.o.), Group-V (CI 350) = *Cajanus indicus* extract (350 mg/kg, p.o.), Group-VI (CI 525) = *Cajanus indicus* extract (525 mg/kg, p.o.), Statistical analysis done by using ANOVA followed by Dunnett's test, Group III, IV, V, VI compared with Group II, Group I compared with Group II.

*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

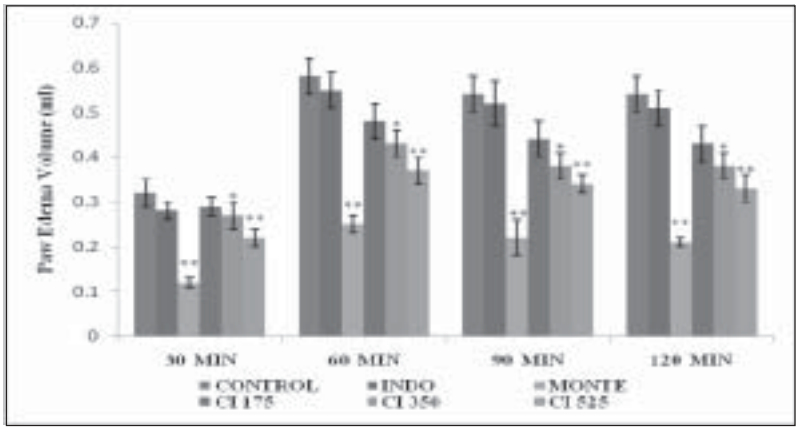
Graph 11 : Effect of *Cajanus indicus* (Linn.) Mill. on lung injury score



Where, n=6, Values are expressed as Mean ± SEM, Group-I (NS) = Nonsensitized = 8 mg alum in 1ml saline (i.p.) Group-II (S) = Sensitized = Ovalbumin 20 µg + 8 mg alum in 1ml saline (i.p.), Group III (DEXA) = Dexamethasone (0.5 mg/kg, i.p.), Group-IV (CI 175) = *Cajanus indicus* extract (175 mg/kg, p.o.), Group-V (CI 350) = *Cajanus indicus* extract (350 mg/kg, p.o.), Group-VI (CI 525) = *Cajanus indicus* extract (525 mg/kg, p.o.), Statistical analysis done by using ANOVA followed by Dunnett's test, Group III, IV, V, VI compared with Group II, Group I compared with Group II.

*p<0.05, **p<0.01 when compared with Sensitized group; ###p<0.01 when compared with Nonsensitized group.

Graph 12 : Effect of *Cajanus indicus* (Linn.) Mill. on arachidonic acid induced paw edema



Where, n=6, Values are expressed as Mean ± SEM, Group-I (Control) = Vehicle (0.5% CMC, 1 ml/ kg, p.o.), Group-II (Standard I) = Indomethacin (10 mg/kg, i.p), Group-III (Standard II) = Montelukast (10 mg/kg, i.p), Group-IV (CI 175) = *Cajanus indicus* extract (175 mg/ kg, p.o.), Group-V (CI 350) = *Cajanus indicus* extract (350 mg/ kg, p.o.) , Group-VI (CI 525) = *Cajanus indicus* extract (525 mg/ kg, p.o.), Statistical analysis done by using ANOVA followed by Dunnett' test, Group II, III, IV, V, VI compared with Group I

*p<0.05, **p<0.01 when compared with Group I

Figure 1-6: Effect of *Cajanus indicus* (Linn.) Mill. on Histopathological studies of Lung tissue in Bronchoalveolar Lavage in Rats.

1.

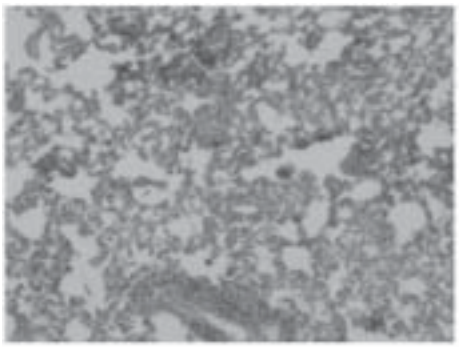


Fig. 1: Photomicrograph of Nonsensitized group showing well-inflated alveoli, normal texture of Lung tissue (H & E 100X)

2.

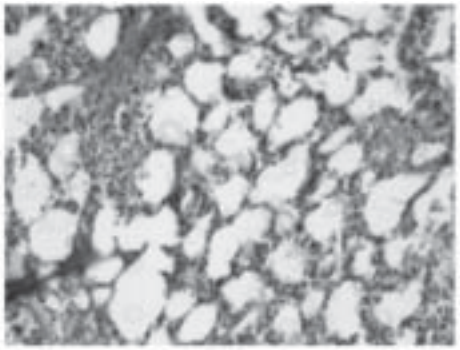


Fig. 2: Photomicrograph of Sensitized group showing massive perivascular edema, recruitment of inflammatory cell in Lung tissue (H & E 100X)

3.

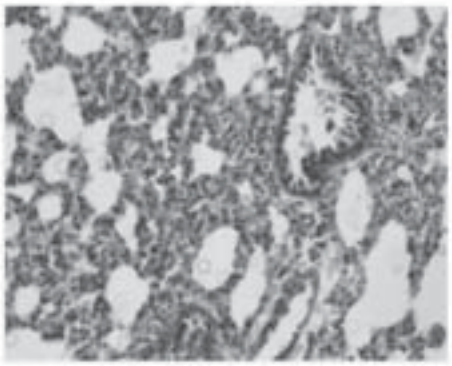


Fig. 3: Photomicrograph of DEXA group showing absence of perivascular edema ,normal texture of Lung tissue (H & E 100X)

4.

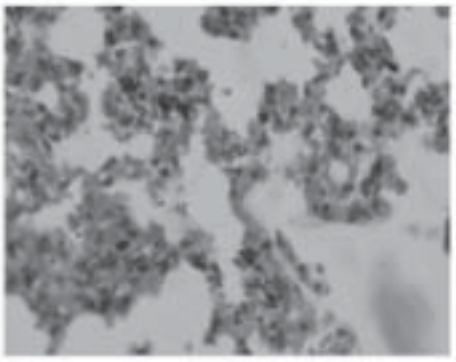


Fig. 4: Photomicrograph of CI175 group showing edema, recruitment of inflammatory cells in Lung tissue (H & E 100X)

5.

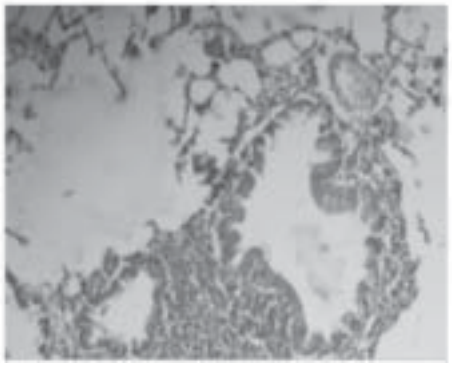


Fig. 5: Photomicrograph of CI350 group showing less necrosis ,less recruitment of inflammatory cells in Lung tissue (H & E 100 X)

6.

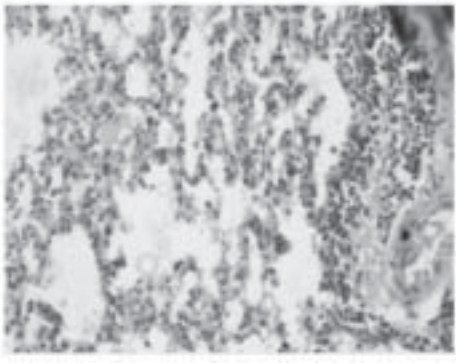


Fig. 6: Photomicrograph of CI525 group showing normal texture of Lung tissue with less necrosis (H & E 100 X)

6. Discussion

Cajanus cajan (Linn.) Mill., which was used traditionally in inflammations, asthma, bronchitis, etc., nevertheless the effect of CI on airway inflammation and its associated oxidative stress , arachidonic acid induced paw edema in a murine model of asthma remains unknown.

OVA-induced asthma is a disease that results from chronic airway inflammation characteristically associated with the infiltration of macrophages, lymphocytes, mast cell, neutrophils and eosinophils into the bronchial lumen [31,32]. Inflammatory cells recruited to asthmatic airways have an exceptional capability to produce ROS. At site of inflammation, multiple inflammatory cells, including eosinophils, neutrophils, and macrophages, are capable of generating ROS and NO, an early marker of lung inflammation, injury or asthma which can participate in the development of a variety of diseases, including allergic asthma [33,34,28].

In this study, we observed that CI significantly inhibited the characteristics of airway inflammation, including infiltration of inflammatory cells by decreasing total as well as differential leucocyte in BALF. For further evaluating the anti-inflammatory efficiency of CI on OVA-induced lung inflammation model, we compared the effect of CI with Dexamethasone. The data demonstrated that CI showed significant activity.

In addition, CI decreased the activity of reactive oxygen and nitrogen species (ROS/RNS) in OVA-induced airway inflammation reaction.Consistent with these findings, our present results showed that ROS and RNS generation in BAL fluid, which mainly consists of recruited inflammatory cells, was significantly increased in the OVA-induced group. The increased ROS and RNS generation was substantially reduced by CI.

Leukotrienes are potent proinflammatory mediators in the pathogenesis of asthma. LTs antagonists are beneficial in patients with aspirin-sensitive asthma. Arachidonic acid-induced paw oedema in rats is an in vivo model to distinguish between cyclooxygenase and lipoxygenase inhibitors [30]. Subplantar injection of arachidonic acid produced significant edema which was measurable up to the time period of 1.5 hrs. It is well known that rat paw oedema induced by arachidonic acid is more sensitive to the LOX inhibitor that to the COX inhibitors [30, 35]. The rat paw edema induced by arachidonic acid is perceptibly reduced by inhibitors of arachidonic acid metabolism and by corticosteroids and is insensitive to selective cyclooxygenase inhibitors [27].

The present study showed that intraperitoneal administration of indomethacin, a COX inhibitor did not inhibit edema formation, but edema was inhibited by montelukast, a LOX inhibitor and CI extract. This suggested that the CI may be useful in asthma by inhibition of lipoxygenase pathway of arachidonate metabolism.

The inhibition of OVA-induced lung inflammation and its associated oxidative stress, antilipoxygenase effect can be attributed to a great extent to the presence of flavonoids, as many of these compounds have been reported to possess this activity.

Hence, further studies are required to prove the described effects are due to the presence of flavonoids.

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