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Original Article

# Koenigicine, a Carbazole Alkaloid, Mitigates Type 2 Inflammation and Alleviates Asthma in Animal and Cell Models

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## ABSTRACT

**Background:** Allergic asthma is a global epidemic that significantly impacts the quality of life for both adults and children. Inhaled corticosteroids are regarded as the primary treatment for asthma, effectively alleviating symptoms over the long term; however, they do not adequately address Type 2 inflammation, which can exacerbate the condition. Therefore, we sought to evaluate the efficacy of koenigicine, a carbazole alkaloid, in mitigating Type 2 inflammation using both an ovalbumin-sensitized animal model and an LPS-induced cell model. **Materials and Methods:** BALB/c mice were sensitized with Ovalbumin+$AlOH_3$ and treated with koenigicine. Bronchoalveolar lavage fluid and pulmonary tissue were excised to examine koenigicine impact on Ova induced inflammation. Total and differential cell count, quantification of nitric oxide, myeloperoxidase activity, eotaxin and Ova specific IgE were performed to analyze efficacy of koenigicine against the inflammatory response induced due to Ovalbumin sensitization. Lipid peroxidation and the antioxidant levels were quantified to evaluate the antioxidant effect of koenigicine. To confirm the impact of koenigicine in mitigating Th2 inflammation the IL4, IL5, IL-13 and the immunomodulators TNF-α and INF-γ were quantified. Histopathological examination of pulmonary tissue was performed to further assess the koenigicine efficacy in asthmatic condition. **Results:** To evaluate the efficacy of koenigicine against the inflammatory response induced by ovalbumin sensitization, we conducted total and differential cell counts, measured nitric oxide levels, assessed myeloperoxidase activity and quantified eotaxin and ovalbumin-specific IgE. Additionally, lipid peroxidation and antioxidant levels were assessed to determine the antioxidant effects of koenigicine. We quantified interleukins IL-4, IL-5 and IL-13, along with the immunomodulators TNF-α and INF-γ, to confirm koenigicine's impact on mitigating Th2 inflammation. Furthermore, histopathological examination of pulmonary tissue was performed to further evaluate the effectiveness of koenigicine in an asthmatic context. The cytotoxicity of koenigicine was investigated in RAW264.7 macrophages with MTT assay. Koenigicine pretreated RAW264.7 macrophages were exposed to LPS stimulation and assessed for inflammatory response. **Conclusion:** Koenigicine treatment demonstrated a significant and effective response in both the animal and cell models. Our analysis confirms that koenigicine is a safe and potent antioxidant that effectively reduces Th2 inflammation and alleviates allergic asthma.

**Keywords:** Allergic Asthma, Anti-asthmatic drug, LPS stimulation, Ovalbumin sensitization, Type 2 inflammation, Koenigicine.

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## INTRODUCTION

Asthma is a global non-communicable epidemic which is marked by recurring symptoms such as sibilant rhonchi, dyspnea, tussis and thoracic constriction. These symptoms are linked to fluctuating airway blockage during expiration.[1] Globally, around 334 million individuals are suffering with asthma, creating it a widespread health issue across different age groups, races and ethnicities.[2] However, factors like socioeconomic status and ethnicity influence its prevalence, severity and death rates, particularly in the United States and other regions.[3] In accordance to the World Health Organization, the number of people with asthma may rise to 400 million by 2025, following current trends.[4] Annually, asthma leads to about 250,000 premature deaths, most of which are preventable.[5] Despite efforts to control the disease,

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the Global Initiative for Asthma (GINA) reports that asthma continues to be a major public health issue, contributing to premature death, reduced quality of life and substantial economic costs for many countries.[6]

Asthma is predicted as a multiform condition concerning the mechanisms that contribute to its morbidity. The controllable aspects has been developed as an effective strategy to identify underlying factors and tailor appropriate treatments for individual patients.[7] A key trait that can be addressed is elevated type 2 airway inflammation (type 2-high), which is marked by eosinophilic inflammation in the airways and plays a significant role in clinical issues for more than 50% of individuals with asthma.[8] Persistent type 2 (Th2) inflammation leads to ongoing damage to the epithelium, creating a cycle in which the inflammatory response intensifies, worsening the devastating medical condition associated with asthma.[9]

Recent classifications of the disease have identified two categories based on the presence of type 2 inflammation: Type2-high and Type2-low asthma. Type2-high asthma features eosinophilic airway inflammation, indicated by elevated blood eosinophil counts or increased levels of Fractional exhaled Nitric Oxide (FeNO), while Type2-low asthma contains forms such as neutrophilic asthma and paucigranulocytic asthma.[10,11] While these situations are frequently treated as separate units, their overlap is frequent and connected to collective obsessive mechanisms.[12] Individuals with allergic asthma recurrently also suffer from allergic rhinitis and those with rhinosinusitis and nasal polyps often exhibit comorbid asthma. The presence of these related conditions may result in the overuse of high-dose Inhaled Corticosteroids (ICS) for asthma treatment and inadequate disease management. As a consequence, nearly 10% of patients may suffer from severe exacerbations, decreased lung function and a lower Quality of Life (QoL).[13,14] Therefore it is need of today to formulate a drug which effectively attenuates Th2 inflammation and ameliorates asthma and its comorbidities. Consequently, there is an urgent need to develop a medication that effectively reduces Th2 inflammation and improves asthma symptoms along with its associated comorbidities.

Carbazole and its derivatives are a notable group of heterocyclic aromatic compounds known for their effective electron and charge transport properties,[15] leading to a variety of potential pharmacological applications, including antidiarrheal, antimicrobial,[16] antidiabetic,[17] anticonvulsant, antioxidative,[18] antihistaminic, anti-inflammatory,[19] neuroprotective and antitumor activities.[20] Among these, carbazole alkaloids from the Rutaceae family represent a significant class of naturally occurring compounds. Initially isolated from *Murraya koenigii*, these alkaloids exhibit potent antibacterial properties.[21] Traditionally, *Murraya koenigii* has been utilized for its stimulant, febrifuge and analgesic effects.[22] In this study, we assessed the effectiveness of koenigicine, a carbazole alkaloid obtained from *Murraya koenigii*, in mitigating allergic asthma.

## MATERIALS AND METHODS

### Chemicals

The major chemicals and reagents, including ovalbumin, aluminium hydroxide, koenigicine, etc., were procured from Sigma-Aldrich, USA. The kits for estimating the biochemical markers were acquired from Therofischer Scintific, USA and Cusabio, USA, respectively.

### Animals

Female BALB/c mice (20-25 g) were used for the present study; the mice were accommodated with the laboratory condition prescribed by international standards for laboratory animals. The mice had free access to rodent pellet diet and water. The induction of asthma and the other procedures conducted on the animals were authorized by the ethics committee, the animals were cared for appropriately. with utmost care throughout the experiment period.

### Onset of Allergic asthma in rodent model

Mice were sensitized for asthmatic attack by administering ovalbumin (10 mg/kg bwt) and aluminium hydroxide (1 mg/kg bwt). The asthmatic sensitizers were treated through intraperitoneal injections on the day 0 and 5 of the experiment.

### Grouping and Treatment regimen

Acclimatized animals were grouped into four each cluster comprised of six healthy mice. Group I are control mice were treated with saline throughout the treatment period. Group II are mice were sensitized for asthma onset with OVA+Al $(OH)_3$ and did not received any other drug treatment. Group III mice were sensitized for asthma onset with OVA+Al $(OH)_3$ and treated orally with 10 mg/kg of koenigicine for 21 days. Group IV mice were sensitized for asthma onset with OVA+Al $(OH)_3$ and treated orally with 2 mg/kg of standard anti-inflammatory drug dexamethasone for 21 days. The mice were euthanized on the subsequent day of last treatment. BALF was collected and lung tissues were excised later for subsequent analysis.

### BALF effusion

A polypropylene needle was carefully inserted into the trachea to gradually introduce 1 mL of ice-cold phosphate-buffered saline. The PBS was aspirated back and the procedure was repeated thrice to collect Bronchoalveolar Lavage Fluid (BALF). The collected BALF was then centrifuged at 5000 rpm for 5 min to isolate the supernatant, which was subsequently preserved at -80°C.

## BALF cell analysis

The cells collected from BALF was suspended again in 200 µL of PBS and subjected to total cell count using haemocytometer after staining with 0.4% trypan blue. The differential cell count was done by staining the cells with Diff-Quik staining reagent. The slides were observed for 200 cells and based on the morphology the cells were identified and the number of each cells were counted.

## Assessment of Lung inflammation

The lung index of the untreated asthma sensitized animals and the koenigicine treated asthma sensitized were analysed by dividing the lung Mass (mg) of the animals to their body mass (g). Further the inflammation induction in the experimental animals were studied via estimating the levels of nitric oxide and myeloperoxidase in the lung tissue

## Quantification of Nitric oxide

Nitric oxide was measured in the pulmonary tissue supernatants with Nitric Oxide Colorimetric Detection Kit (ThermoFisher Scientific). The stable break down production nitrate and nitrite were quantified to measure the levels of nitric oxide. The final-coloured end product was measured at 570 nm.

## Analysis of myeloperoxidase activity

Myeloperoxidase activity was analysed to estimate the level of neutrophil induced inflammation in asthma sensitized rats and the ameliorative role of koenigicine against it. The test was conducted as per the modified procedure.[23] Hexadecyltrimethylammonium bromide buffer was used to prepare tissue homogenate and analyzed for myeloperoxidase activity. The end product was measured at 460 nm.

## Assessment of Ova-sensitized asthma onset

The onset of asthma in ova sensitized untreated and the drug koenigicine and the dexamethasone treated animals were assessed through estimating the levels of eotaxin and Ova Specific IgE antibodies in the BALF. The test was performed with the Mouse Eotaxin ELISA kit and Mouse ovalbumin specific IgE, OVA sIgE ELISA Kit obtained from Invitrogen and CUSABIO respectively. The tests were done in triplicates as per the kit manual and the absorbance was measured at 450 nm immediately after the addition of stop solution.

## Assessment of redox imbalance

The occurrence of oxidative insult due to ova sensitization and the attenuating effect of koenigicine was examined via measuring antioxidants levels and the lipid peroxidation in pulmonary tissue of test subjects. The protocol of[24] was followed to assess the malondialdehyde levels in the koenigicine treated ova sensitized rats. Protocols of[25-27] were followed to quantify the activity of superoxide dismutase, catalase and glutathione levels respectively. The formation of red formazan crystals, resulting from the reaction between superoxide radicals and p-iodonitrotetrazolium violet, was quantified at 505 nm to assess SOD activity. Hydrogen peroxide decomposition was measured for 2 min at 240 nm to quantify catalase activity. The final reaction of lung homogenate with DTNB Ellman's reagent was measured at 412 nm to quantify the levels of glutathione.

## Assessment of Type 2 cytokines and immunomodulators

The type 2 cytokines IL-4, IL-5 and IL-13 which are responsible for bronchial asthma induction was quantified in the koenigicine treated and untreated ova sensitized animals using the ELISA kits procured from ThermoFisher Scientific. The immunomodulators TNF-α and INF-γ were also measured to confirm the anti-inflammatory activity of koenigicine against ova sensitization. Mouse IFN-γ ELISA Kit and Mouse Tumor Necrosis Factor α ELISA Kit obtained from Sigma Aldrich were used to quantify the levels of INF-γ and TNF-α respectively.

## Histopathological examination

Pulmonary tissue obtained from the experimental mice was carefully rinsed with Phosphate-Buffered Saline (PBS) and then fixed in 4% paraformaldehyde for 24 hr. Following fixation, the tissue underwent dehydration using a series of increasing concentrations of alcohol. Once dehydrated, the tissue was cleared with xylene and subsequently embedded in paraffin wax. The paraffin-embedded blocks were then sliced into 4-micron thick sections using a microtome. These sections were deparaffinized and stained with hematoxylin and eosin. Finally, the stained lung tissue sections were examined using a light microscope.

## Cell culture and Viability

RAW264.7 macrophages were grown in DMEM that was supplemented with 10% Fetal Bovine Serum (FBS). The cells were incubated at 37°C in an environment with 5% $CO_2$. Viability of the cells in the presence of koenigicine was assessed with MTT assay. RAW264.7 macrophages were treated with various concentration of koenigicine ranging from 0-50 µM and incubated for 24 hr. The MTT assay was performed with the MTT assay kit procured from Abcam. The final absorbance was read at 590 nm and the percentage of cell viability was calculated.

## Assessment of inflammation in LPS induced RAW264.7 macrophages

### *Quantification of nitric oxide*

RAW264.7 macrophages were cultured on 24 well plate for the quantification of nitric oxide. The cells were treated with 2.5, 5, 10 and 25 µM concentration of koenigicine an hour prior to LPS induction. Later the cells were induced with LPS for 24 hr and the supernatant of the cell culture was collected for the quantification

of nitric oxide. The test was performed as the per the kit manual using the Abcam NO colorimetric assay kit. The final absorbance was quantified at 540 nm and the NO levels of the test samples were calculated using the standard curve plot.

### Quantification of inflammatory cytokines

TNF-α and IL-6 were quantified in the LPS induced koenigicine treated RAW264.7 macrophages. The cell was cultured on 24 well plates and pretreated with different concentrations of koenigicine (2.5, 5, 10, 25 µM) for an hour and induced with LPS treatment of 24 hr. The cells were subjected to sonication and centrifugation at 4500 rpm for 5 min, supernatant was collected for the quantification of TNF-α and IL-6. The levels of TNF-α and IL-6 were measured with ELISA kits procured from ThermoFisher. The final absorbance was measured at 450 nm.

### Statistical Analysis

A minimum of 3 independent experiments were conducted for each condition and the results are expressed as mean values±Standard Deviation (±SD). Statistical analyses were carried out using one-way Analysis of Variance (ANOVA), with significance determined using SPSS version 18.0.

## RESULTS

### Impact of carbazole koenigicine on pulmonary inflammatory cell infiltration

The infiltration of immune cells in the pulmonary region was assessed to study impact of koenigicine against ovalbumin triggered inflammation. The total and differential count of leukocytes in the bronchoalveolar region of the koenigicine treated Ova sensitized animals was estimated and the results were represented in the Figure 1. The infiltration of lymphocytes were significantly triggered by the Ova sensitization which was evidenced with the increased number of leukocytes in the ova sensitized untreated mice whereas treatment with koenigicine and dexamethasone significantly decreased the infiltration of immune cells. The BALF of asthmatic induced untreated mice shown increased number of eosinophil and lymphocyte infiltration than the neutrophil and macrophages. Koenigicine significantly attenuated the infiltration of immune cells in Ova sensitized mice.

### Effect of carbazole koenigicine against inflammatory induction in Ova sensitized animals

The lung index was measured in the koenigicine treated and untreated Ova sensitized animals. The ova sensitization had significantly increased the lung index to 1.46±0.0004 whereas the koenigicine and dexamethasone treated animals exhibited 1.26±0.0002 and 1.1±0.0003 respectively. The lung indexes of control animals were found to be 0.82±0.0005.

Koenigicine exposure considerably decreased nitric oxide levels and myeloperoxidase activity to 287±0.2 µmol/g and 52±0.06 U/g correspondingly compared to the ovasensitized untreated animals which exhibited 306±0.9 µmol/g of nitric oxide and 54±0.07 U/g of myeloperoxidase activity. The dexamethasone exposure also reduced nitric oxide levels and myeloperoxidase activity to 254±0.4 µmol/g and 42±0.03 U/g respectively but it is comparatively higher than the levels of control animals which shown 122±0.7 µmol/g of nitric oxide and 32±0.03 U/g of myeloperoxidase activity (Figure 2).

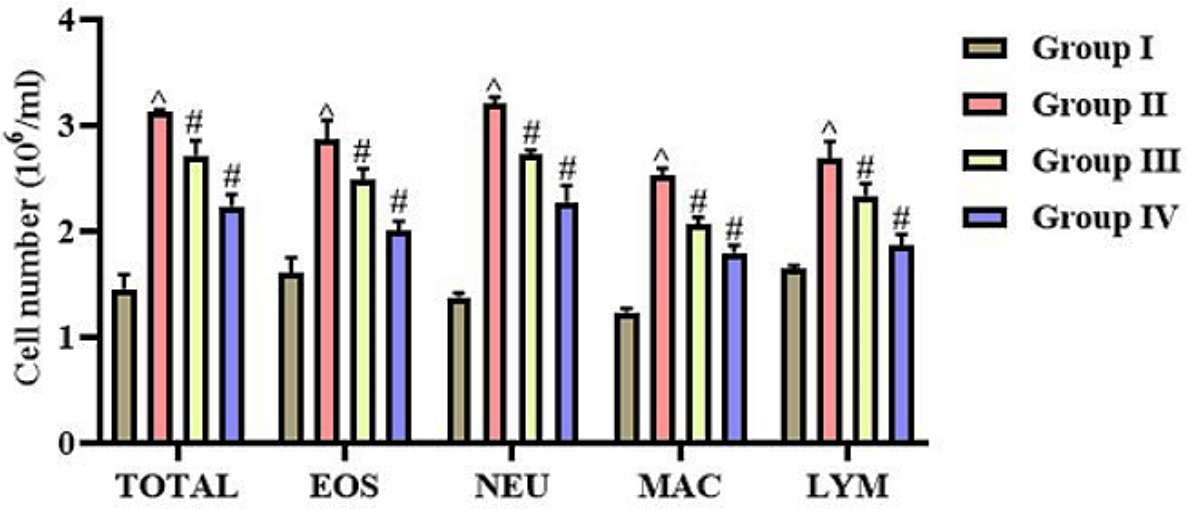


**Figure 1:** Impact of carbazole koenigicine on pulmonary inflammatory cell infiltration. BALF was assessed for total and differential leukocyte count of control, Ova sensitized asthma induced untreated animals, Ova sensitized asthma induced koenigicine and Ova sensitized asthma induced dexamethasone treated animals. Number of total, eosinophils, neutrophils, macrophages and lymphocytes were counted. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs Other groups, b-Ova sensitized untreated vs koenigicine treated and dexamethasone treated groups.

## Inhibitory effect of carbazole koenigicine on Eotaxin and Ova specific IgE in Ova sensitized animals

Figure 3A illustrates the level of Eotaxin, protein secreted during allergic condition which effectively mobilizes and activates eosinophils in the Ova sensitized untreated and koenigicine treated animals. Ova sensitization significantly enhanced eotaxin levels to 1850±5.2 pg/mL compared to control animals (1275±2.5 pg/mL) whereas both the koenigicine and dexamethasone treatment significantly attenuated the eotaxin production to 1670±4.5 pg/mL and 1430±3.2 pg/mL in Ova sensitized animals.

Ova specific IgE levels were increased to 364±0.9 ng/mL in the Ova sensitized animals compared to the other groups whereas treatment with koenigicine exhibited 292±0.5 ng/mL and dexamethasone treatment shown 283±0.4 ng/mL in the Ova sensitized animals. 215±0.6ng/mL of Ova specific IgE was quantified in the control animals (Figure 3B).

## Regulatory effect of carbazole koenigicine against redox imbalance in Ova sensitized animals

Figure 4A depicts the malondialdehyde levels in the Ova sensitized untreated and koenigicine treated animals. Koenigicine treatment significantly attenuated the lipid peroxidation in Ova sensitized animals which is depicted with decreased levels of MDA (1.42±0.0006 nm) than Ova sensitized untreated animals (1.69±0.0007 nm). The dexamethasone treated animals also exhibited decreased levels of MDA (1.2±0.0002 nm) than the Ova sensitized untreated animals. Control animals exhibited significantly decreased MDA levels (0.85±0.0009 nm) than the other experimental groups.

The antioxidants superoxide dismutase, catalase and glutathione levels were quantified in the experimental animals and the results were illustrated in the Figure 4B. Ova sensitization significantly diminished the antioxidants levels whereas treatment with both koenigicine and dexamethasone had significantly elevated the levels of antioxidants in the Ova sensitized animals.

## Immunomodulatory effect of carbazole koenigicine in Ova sensitized animals

Figure 5 A depicts the levels of type 2 cytokines IL-4, IL-5 and IL-13 in Ova sensitized asthma induced untreated and koenigicine treated animals. Ova sensitization had significantly elevated the type 2 cytokines levels compared to the control animals. Treatment with koenigicine in Ova sensitized animals had reduced the levels of type 2 cytokines. Compared to IL-5 and IL-13, the levels of IL-4 drastically increased in the Ova sensitized animals. Treatment with both koenigicine and dexamethasone had significantly reduced the IL-4, IL-5 and IL-13 intensities.

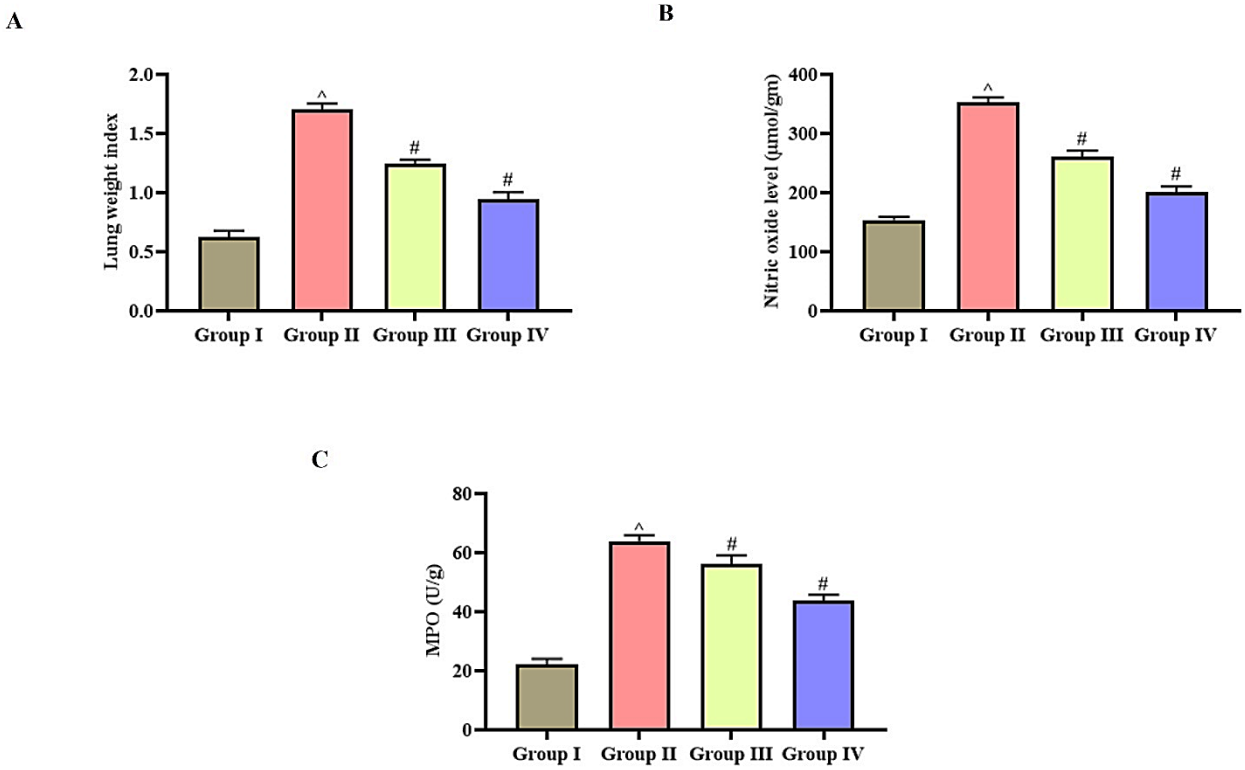


**Figure 2:** Effect of carbazole koenigicine against inflammatory induction in Ova sensitized animals. A) Lung index, B) Nitric oxide levels, C) Myeloperoxidase activity of control, Ova sensitized asthma induced untreated animals, Ova sensitized asthma induced koenigicine and Ova sensitized asthma induced dexamethasone treated animals. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs Other groups, b-Ova sensitized untreated vs koenigicine treated and dexamethasone treated groups.

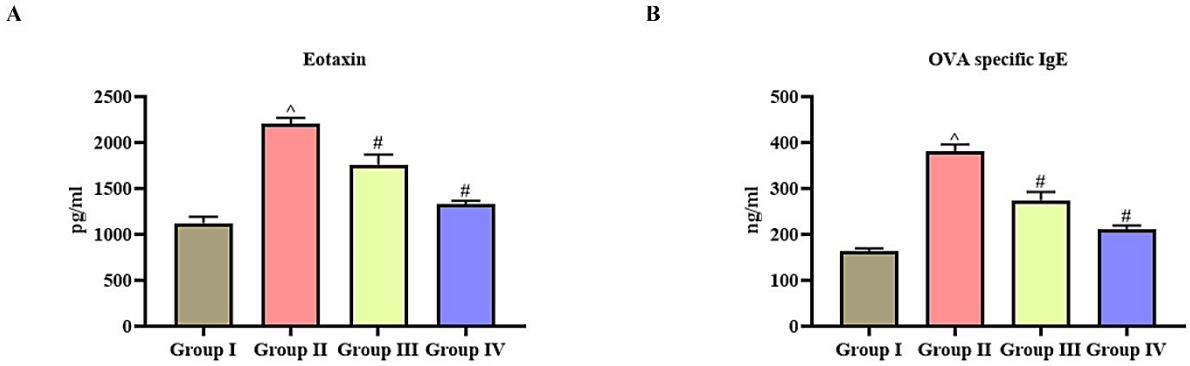


**Figure 3:** Inhibitory effect of carbazole koenigicine on Eotaxin and Ova specific IgE in Ova sensitized animals. A) Eotaxin B) Ova specific IgE levels of control, Ova sensitized asthma induced untreated animals, Ova sensitized asthma induced koenigicine and Ova sensitized asthma induced dexamethasone treated animals. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs Other groups, b-Ova sensitized untreated vs koenigicine treated and dexamethasone treated groups.

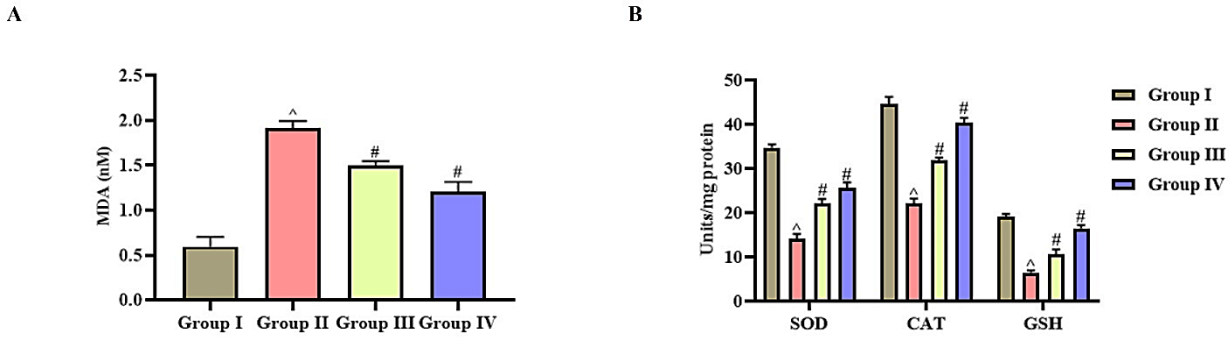


**Figure 4:** Regulatory effect of carbazole koenigicine against redox imbalance in Ova sensitized animals. Malondialdehyde levels B) Antioxidant levels-Superoxide dismutase, Catalase and glutathione levels of control, Ova sensitized asthma induced untreated animals, Ova sensitized asthma induced koenigicine and Ova sensitized asthma induced dexamethasone treated animals. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs Other groups, b-Ova sensitized untreated vs koenigicine treated and dexamethasone treated groups.

The immunomodulators TNF-α and INF-γ levels were quantified in the Ova sensitized untreated and koenigicine treated animals and the levels were represented in the Figure 5B. Ova sensitization had increased the TNF-α and decreased the INF-γ levels than the control animals. Treatment with koenigicine and dexamethasone had significantly reduced the TNF-α level and enhanced the INF-γ in Ova sensitized animals.

## Ameliorative effect of carbazole koenigicine in lung histology in Ova sensitized animals

Histopathological analysis of control animal's lung tissue exhibited normal lung tissue architecture with neatly structured alveolar spaces and slender bronchial walls (Figure 6). No sign of inflammatory cells was identified (6A). Substantial inflammatory alterations, edema, altered alveolar morphology and thickening of the bronchial wall were observed in the Ova sensitized untreated animals (6B). Ova sensitized animals treated with koenigicine shown decreased inflammatory cells and the alveolar cell disruption was reduced compared to the untreated Ova sensitized animals (6C). Dexamethasone treated Ova sensitized animals exhibited minimal number of inflammatory cells with reduced edema and altered alveolar space (6D).

## Effect of carbazole koenigicine on RAW264.7 macrophages viability

RAW264.7 macrophages were treated with different doses of koenigicine and the viability of cells after 24 hr incubation was depicted in the Figure 7. Treatment with koenigicine doesn't exhibit much cytotoxic effect on the RAW264.7 macrophages with doses upto 25 µM koenigicine treatment. Only 20% of cell death was observed with highest dose 50 µM of koenigicine treatment.

## Anti-inflammatory effect of carbazole koenigicine on LPS induced RAW264.7 macrophages

Koenigicine pretreated RAW264.7 macrophages was induced inflammation with lipopolysaccharides and assessed for inflammatory response (Figure 8). Treatment with koenigicine significantly attenuated the levels of nitric oxide, inflammatory stimulating cytokines TNF-α and IL-6 in dose dependent manner. Control cells exhibited 1.7±0.0001, 375±2.5 pg/mL and 47±0.7 pg/mL of nitric oxide, TNF-α and IL-6 respectively. Stimulation with LPS increased the nitric oxide, TNF-α and IL-6 levels to 3.4±0.0003, 635±1.2 pg/mL and 148±1.2pg/mL respectively. 25

μM koenigicine pretreated cells stimulated with LPS exhibited significantly reduced levels of 2.2±0.00008, 395±2.7 pg/mL and 64±0.6 pg/mL of nitric oxide, TNF-α and IL-6 respectively.

## DISCUSSION

Asthma is an enduring allergic ailment that affects a significant portion of the global population, making it a prominent non-transmissible disease impacting all age group. It is the prevalent enduring ailment in children and is associated with considerable morbidity, contributing to substantial asthma-related healthcare costs.[28,29] For over three decades, Inhaled Corticosteroids (ICSs) have been the mainstay of asthma treatment, effectively reducing eosinophilic airway inflammation, improving symptoms and decreasing the frequency of asthma attacks. Yet, about 2.5% of children and 10% of adults experience poor responses to ICSs, resulting in severe asthma.[30,31]

Asthma is classified into several endotypes based on factors like age of onset, allergies and genetic factors. When asthma appears after age 20, it is categorized as late-onset and is linked to two endotypes: Type2 and non-Type2.[32] While Type2 asthma isn't as allergic as early-onset asthma, it is characterized by elevated eosinophil levels, which can damage the respiratory epithelium and trigger exacerbations. Eosinophils release toxic proteins like Eosinophil Cationic Protein (ECP), contributing to this damage.[33,34] Therapies like mepolizumab, which target the cytokine IL-5, have proven effective in reducing eosinophil

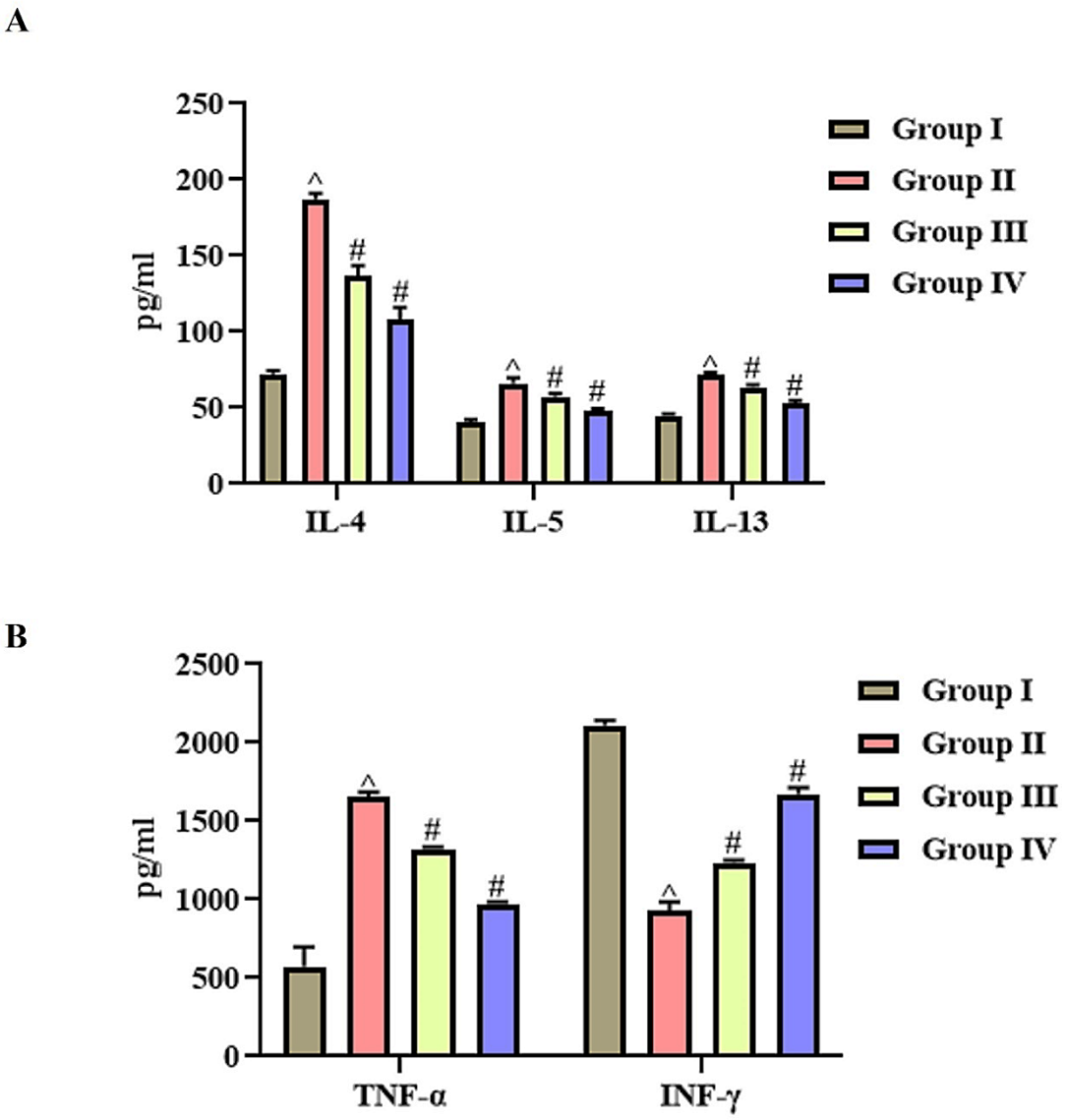


**Figure 5:** Immunomodulatory effect of carbazole koenigicine in Ova sensitized animals. A) Type 2 Cytokines - IL-4, IL-5 and IL-13, B) Immunomodulators-TNF-α and INF-γ levels of control, Ova sensitized asthma induced untreated animals, Ova sensitized asthma induced koenigicine and Ova sensitized asthma induced dexamethasone treated animals. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs Other groups, b-Ova sensitized untreated vs koenigicine treated and dexamethasone treated groups.

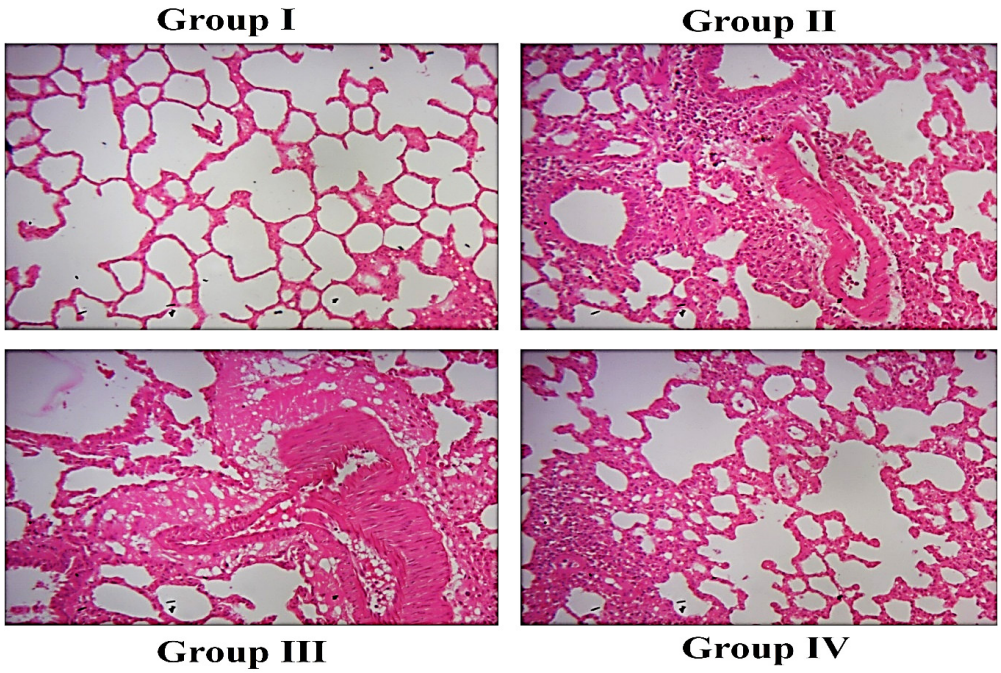


**Figure 6:** Ameliorative effect of carbazole koenigicine in lung histology in Ova sensitized animals. Representative images of HandE stained lung tissue sections of A) control, B) Ova sensitized asthma induced untreated animals, C) Ova sensitized asthma induced koenigicine, D) Ova sensitized asthma induced dexamethasone treated animals.

levels and exacerbations in T2 eosinophilic asthma patients.[35] Additionally, dupilumab, an anti-IL-4 antibody, has exhibited benefits in treating T2 asthma.[36] Therefore in this assessment our objective was to evaluate the potency of the carbazole alkaloid drug koenigicine potency to attenuate cytokines linked to type2 inflammation in Ova sensitized asthmatic rodent model.

The central cytokines in Type 2 (T2) airway inflammation are IL-4, IL-5 and IL-13. IL-4 is pivotal for the differentiation of Th2 cells and switching of B cells to produce IgE, while IL-5 aids in eosinophil differentiation, survival and recruitment to the airways.[37] IL-13 causes airway smooth muscle constriction and mucus production. Together, these cytokines drive inflammatory cell migration to the airway, leading to tissue damage and airway remodeling.[9] Eosinophil recruitment and activation are primarily influenced by IL-5 and eotaxins, with IL-4 further facilitating IgE production and Th2 cells. Besides, Innate Lymphoid Cells type 2 (ILC2) can also produce IL-5 and IL-13, further contributing to eosinophil recruitment.[38] Koenigicine treatment in Ova sensitized animals considerably enhance the IL-4, IL-5 and IL-13 levels. There was also a notable reduction in eosinophilic infiltration in BALF, along with a decrease in Ovalbumin (Ova)-specific IgE and eotaxin levels. Koenigicine exposure also elevated the INF-γ and attenuated the TNF-α levels in the Ova sensitized animals. This reduction may be attributed to the inhibitory effect of koenigicine on type 2 inflammatory cytokines, which ultimately alleviated the inflammatory response induced by Ova sensitization. Furthermore, koenigicine treatment not only diminished eosinophilic infiltration but also appeared to inhibit the infiltration of neutrophils and macrophages.

Oxidative stress is a significant factor for asthma pathogenesis, primarily due to the excessive production of reactive oxygen species.[39] This increase in ROS not only enhances the infiltration of inflammatory cells, such as eosinophils, neutrophils, monocytes and macrophages, into the lungs but also triggers the synthesis of extracellular matrix proteins and elevates pro-inflammatory cytokines in airway passages.[40,41] Asthma is often characterized by diminished antioxidant activity, contributing further to oxidative damage.[42] In children with asthma, there is often a marked increase in malondialdehyde, a biomarker for lipid peroxidation, alongside lower glutathione levels.[43] Notably, higher malondialdehyde levels correlate directly with asthma severity, indicating a relationship between oxidative stress and symptom intensity in asthma patients.[44] Given that koenigicine inhibited the inflammatory cytokines IL-4, IL-5 and IL-13, we further investigated its effects on scavenging oxidative stress, another key factor in asthma pathogenesis. Treatment with koenigicine decreased lipid peroxidation and enhanced antioxidant levels in Ovalbumin-sensitized animals.

Further we analyzed nitric oxide levels and myeloperoxidase in koenigicine treated ova sensitized animals to confirm the efficacy of koenigicine against asthmatic attack. Nitric Oxide (NO) is prime nitrogen species produced in the lungs; its autoxidation with oxygen generates nitrite, which serves as a substrate for Eosinophil Peroxidase (EPO) and Myeloperoxidase (MPO).[45]

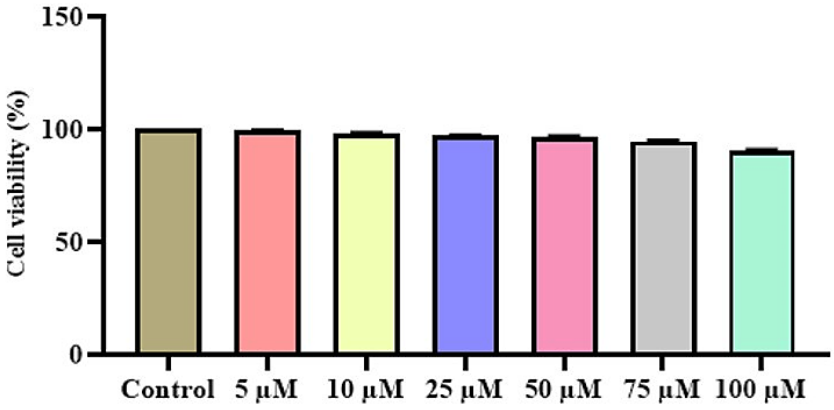


**Figure 7:** Effect of carbazole koenigicine on RAW264.7 macrophages viability. RAW264.7 macrophages were treated with 0-50 µM concentration of koenigicine for 24 hr assessed of cell viability with MTT assay. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates.

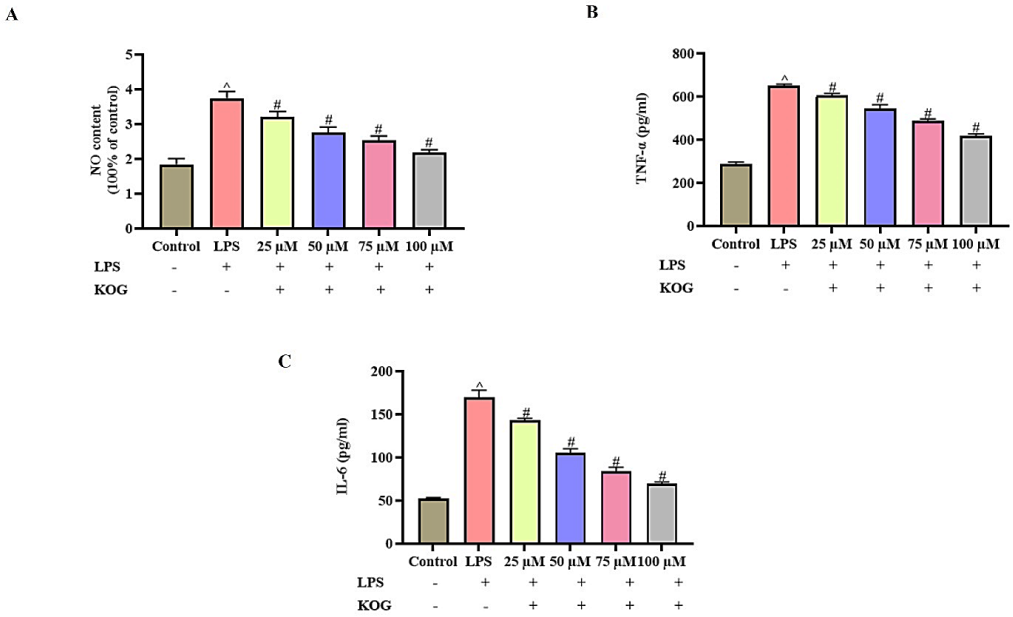


**Figure 8:** Anti-inflammatory effect of carbazole koenigicine on LPS induced RAW264.7 macrophages. A) Nitric Oxide levels B) TNF-α, C) IL-6 levels of different concentrations of Koenigicine pretreated RAW264.7 macrophages stimulated with lipopolysaccharides. Results are expressed as mean values±Standard Deviation (±SD) of the triplicates. a-Control vs other groups, b-LPS stimulated untreated vs LPS stimulated treated with different concentrations of koenigicine.

Raised levels of EPO and MPO have been associated with the activation of eosinophils and neutrophils, respectively, with MPO activity correlating with asthma severity.[46] NO can react with superoxide to form peroxynitrite ($ONOO^-$), a compound capable of nitrating tyrosine residues, leading to damage to various enzymes and proteins. Increased levels of NO have been linked to asthma risk, greater asthma severity and an augmented reaction to bronchodilators.[47] Koenigicine treatment significantly reduced nitric oxide production and myeloperoxidase activity, highlighting its potential as an effective candidate for asthma treatment. Additionally, lung histopathological analyses provided further evidence of koenigicine's efficacy in alleviating type 2 inflammation-related pulmonary damage in Ovalbumin-sensitized rats.

Our *in vitro* analysis of koenigicine on RAW264.7 macrophages demonstrated that koenigicine has no cytotoxic effects. Additionally, it reduced the levels of the inflammatory cytokines IL-6 and TNF-α induced by LPS stimulation. Cells pretreated with koenigicine showed decreased nitric oxide production in response to LPS, which aligns with our *in vivo* findings, where nitric oxide levels were similarly inhibited in ova-sensitized animals treated with koenigicine.

## CONCLUSION

In this study, we investigated the efficacy of the carbazole alkaloid koenigicine against asthmatic attacks using both animal and cell models. Treatment with koenigicine significantly reduced Type 2 inflammatory cytokines and inhibited the infiltration

of inflammatory cells into the lungs. The observed decrease in Ovalbumin-specific IgE and eotaxin levels further demonstrated koenigicine's potent anti-inflammatory effects. Additionally, koenigicine effectively enhanced antioxidant mechanisms, protecting pulmonary tissue from oxidative stress and its associated consequences. *In vitro* analyses with LPS-stimulated RAW264.7 macrophages confirmed that koenigicine is a non-cytotoxic, potent anti-inflammatory agent. In conclusion, koenigicine emerges as a promising and safe antioxidant that effectively mitigates Type 2 inflammation, representing a potential therapeutic option for alleviating allergic asthma. In addition, further in-depth works are highly warranted in the future to precisely understand the therapeutic mechanisms of koenigicine against allergic asthma.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GINA:** Global Initiative for Asthma; **Th2 inflammation:** Type 2 inflammation; **FeNO:** Fractional exhaled nitric oxide; **ICS:** Inhaled corticosteroids; **QoL:** Quality of life; **ECP:** Eosinophil cationic protein; **EPO:** Eosinophil peroxidase; **MPO:** Myeloperoxidase.

## SUMMARY

Asthma is a global non communicable epidemic which is marked by recurring symptoms such as sibilant rhonchi, dyspnea, tussis and thoracic constriction. Koenigicine treatment significantly reduced nitric oxide production and myeloperoxidase activity, highlighting its potential as an effective candidate for asthma treatment. *In vitro* analysis of koenigicine on RAW264.7 macrophages demonstrated that koenigicine has no cytotoxic effects. Additionally, it reduced the levels of the inflammatory cytokines IL-6 and TNF-α induced by LPS stimulation.

## REFERENCES

1. Reddel HK, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, *et al.* A summary of the new GINA strategy: a roadmap to asthma control. Eur Respir J. 2015 Sep;46(3):622-39. doi: 10.1183/13993003.00853-2015, PMID 26206872.
2. Enilari O, Sinha S. The global impact of asthma in adult populations. Ann Glob Health. 2019;85(1):2. doi: 10.5334/aogh.2412, PMID 30741503.
3. Ferkol T, Schraufnagel D. The global burden of respiratory disease. Ann Am Thorac Soc. 2014;11(3):404-6. doi: 10.1513/AnnalsATS.201311-405PS, PMID 24673696.
4. WHO. Asthma. 2024 https://www.who.int/news-room/fact-sheets/detail/asthma.
5. GIA, Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention; 2023. Available from: http://www.ginasthma.org.
6. GINA. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA); 2015. Available from: http://www.ginasthma.org/. [cited Dec 12, 2015].
7. Melhorn J, Howell I, Pavord ID. Should we apply a treatable traits approach to asthma care? Ann Allergy Asthma Immunol. 2022;128(4):390-7. doi: 10.1016/j.anai.2022.02.005, PMID 35172180.
8. Frøssing L, Silberbrandt A, Von Bülow A, Backer V, Porsbjerg C. The prevalence of subtypes of Type 2 inflammation in an unselected population of patients with severe asthma. J Allergy Clin Immunol Pract. 2021;9(3):1267-75. doi: 10.1016/j.jaip.2020.09.051, PMID 33039645.
9. Maspero J, Adir Y, Al-Ahmad M, Celis-Preciado CA, Colodenco FD, Giavina-Bianchi P, *et al.* Type 2 inflammation in asthma and other airway diseases. ERJ Open Res. 2022;8(3):00576-2021. doi: 10.1183/23120541.00576-2021, PMID 35923421.
10. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol. 2015;15(1):57-65. doi: 10.1038/nri3786, PMID 25534623.
11. Brusselle GG, Koppelman GH. Biologic therapies for severe asthma. N Engl J Med. 2022;386(2):157-71. doi: 10.1056/NEJMra2032506, PMID 35020986.
12. Pinart M, Benet M, Annesi-Maesano I, von Berg A, Berdel D, Carlsen KC, *et al.* Comorbidity of eczema, rhinitis and asthma in IgE-sensitised and non-IgE-sensitised children in MeDALL: a population-based cohort study. Lancet Respir Med. 2014;2(2):131-40. doi: 10.1016/S2213-2600(13)70277-7, PMID 24503268.
13. Lee LK, Obi E, Paknis B, Kavati A, Chipps B. Asthma control and disease burden in patients with asthma and allergic comorbidities. J Asthma. 2018;55(2):208-19. doi: 10.1080/02770903.2017.1316394, PMID 28586296.
14. Porsbjerg C, Menzies-Gow A. Co-morbidities in severe asthma: clinical impact and management. Respirology. 2017;22(4):651-61. doi: 10.1111/resp.13026, PMID 28328160.
15. Campbell N, Barclay BM. Recent advances in the chemistry of carbazole. Chem Rev. 1947;40(3):359-80. doi: 10.1021/cr60127a001, PMID 20251371.
16. Ding YY, Zhou H, Peng-Deng BQ, Zhang BQ, Zhang ZJ, Wang GH, *et al.* Antimicrobial activity of natural and semi-synthetic carbazole alkaloids. Eur J Med Chem. 2023;259:115627. doi: 10.1016/j.ejmech.2023.115627, PMID 37467619.
17. Eseyin OA, Edem E, Johnson E, Ahmad A, Afzal S. Synthesis and *in vitro* antidiabetic activity of some alkyl carbazole compounds. Trop J Pharm Res. 2018;17(3):537-41. doi: 10.4314/tjpr.v17i3.21.
18. Serdaroğlu G, Uludağ N, Ercag E, Sugumar P, Rajkumar PJ. Carbazole derivatives: synthesis, spectroscopic characterization, antioxidant activity, molecular docking study and the quantum chemical calculations. J Mol Liq. 2021;330:115651. doi: 10.1016/j.molliq.2021.115651.
19. Głuszyńska A. Biological potential of carbazole derivatives. Eur J Med Chem. 2015;94:405-26. doi: 10.1016/j.ejmech.2015.02.059, PMID 25794500.
20. Chen Z, Yang T, Wang W, Yao J, Han S, Tao Y. Synthesis and biological evaluation of carbazole amino alcohols as antitumor agents. Chem Select. 2018;3(44):12630-8.
21. Nandy BC, Gupta AK, Mittal A, Vyas V. Carbazole: it's biological activity. J Biomed Pharm Res. 2014;3:42-8.
22. Elseena J, Prasanth F, Mariya J, Shilpa J, Sunitha S. An insight into the chemistry and pharmacological activities of carbazole derivatives: a review. Ind J Natl Sci. 2024;13(71):40594-4060.
23. Goldblum SE, Wu KM, Jay M. Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. J Appl Physiol (1985). 1985;59(6):1978-85. doi: 10.1152/jappl.1985.59.6.1978, PMID 3001018.
24. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810.
25. Fridovich I. Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol. 1983;23:239-57. doi: 10.1146/annurev.pa.23.040183.001323, PMID 6307121.
26. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6.
27. Ali SA, Saifi MA, Pulivendala G, Godugu C, Talla V. Ferulic acid ameliorates the progression of pulmonary fibrosis via inhibition of TGF-β/smad signalling. Food Chem Toxicol. 2021;149:111980. doi: 10.1016/j.fct.2021.111980, PMID 33428986.
28. Ebmeier S, Thayabaran D, Braithwaite I, Bénamara C, Weatherall M, Beasley R. Trends in international asthma mortality: analysis of data from the WHO Mortality Database from 46 countries (1993-2012). Lancet. 2017;390(10098):935-45. doi: 10.1016/S0140-6736(17)31448-4, PMID 28797514.
29. Hossny E, Caraballo L, Casale T, El-Gamal Y, Rosenwasser L. Severe asthma and quality of life. World Allergy Organ J. 2017;10(1):28. doi: 10.1186/s40413-017-0159-y, PMID 28855973.
30. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, *et al.* Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. N Engl J Med. 2014;371(13):1189-97. doi: 10.1056/NEJMoa1403291, PMID 25199060.
31. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, *et al.* Efficacy and safety of Benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet. 2016;388(10056):2115-27. doi: 10.1016/S0140-6736(16)31324-1, PMID 27609408.
32. Kaur R, Chupp G. Phenotypes and endotypes of adult asthma: moving toward precision medicine. J Allergy Clin Immunol. 2019;144(1):1-12. doi: 10.1016/j.jaci.2019.05.031, PMID 31277742.
33. Bystrom J, Amin K, Bishop-Bailey D. Analysing the eosinophil cationic protein-a clue to the function of the eosinophil granulocyte. Respir Res. 2011;12(1):10. doi: 10.1186/1465-9921-12-10, PMID 21235798.
34. Nair P, O'Byrne PM. Measuring eosinophils to make treatment decisions in asthma. Chest. 2016;150(3):485-7. doi: 10.1016/j.chest.2016.07.009, PMID 27613975.

35. Busse W, Chupp G, Nagase H, Albers FC, Doyle S, Shen Q, *et al.* Anti-IL-5 treatments in patients with severe asthma by blood eosinophil thresholds: indirect treatment comparison. J Allergy Clin Immunol. 2019;143(1):190-200.e20. doi: 10.1016/j.jaci.2018.08.031 [ePub] 2018 Sep 8. Erratum in: J Allergy Clin Immunol. 2019;143(6):2336. doi: 10.1016/j.jaci.2019.03.014.
36. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, *et al.* Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018;378(26):2486-96. doi: 10.1056/NEJMoa1804092, PMID 29782217.
37. Gandhi NA, Bennett BL, Graham NM, Pirozzi G, Stahl N, Yancopoulos GD. Targeting key proximal drivers of type 2 inflammation in disease. Nat Rev Drug Discov. 2016;15(1):35-50. doi: 10.1038/nrd4624, PMID 26471366.
38. Bakakos A, Anagnostopoulos N, Bakakos P. Eosinophils and T2 inflammation in severe asthma. Explor Asthma Allergy. 2024;2(5):399-409. doi: 10.37349/eaa.2024.00053.
39. Ammar M, Bahloul N, Amri O, Omri R, Ghozzi H, Kammoun S, *et al.* Oxidative stress in patients with asthma and its relation to uncontrolled asthma. J Clin Lab Anal. 2022;36(5):e24345. doi: 10.1002/jcla.24345, PMID 35318723.
40. Nadeem A, Chhabra SK, Masood A, Raj HG. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol. 2003;111(1):72-8. doi: 10.1067/mai.2003.17, PMID 12532099.
41. de Groot LE, Sabogal Piñeros YS, Bal SM, van de Pol MA, Hamann J, Sterk PJ, *et al.* Do eosinophils contribute to oxidative stress in mild asthma? Clin Exp Allergy. 2019;49(6):929-31. doi: 10.1111/cea.13389, PMID 30891863.
42. Comhair SA, Erzurum SC. Redox control of asthma: molecular mechanisms and therapeutic opportunities. Antioxid Redox Signal. 2010;12(1):93-124. doi: 10.1089/ars.2008.2425, PMID 19634987.
43. Ercan H, Birben E, Dizdar EA, Keskin O, Karaaslan C, Soyer OU, *et al.* Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. J Allergy Clin Immunol. 2006;118(5):1097-104. doi: 10.1016/j.jaci.2006.08.012, PMID 17088135.
44. Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci Ö. Oxidative stress in asthma: part of the puzzle. Pediatr Allergy Immunol. 2018;29(8):789-800. doi: 10.1111/pai.12965, PMID 30069955.
45. Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. J Biol Chem. 2000;275(48):37524-32. doi: 10.1074/jbc.275.48.37524, PMID 11090610.
46. Wedes SH, Khatri SB, Zhang R, Wu W, Comhair SA, Wenzel S, *et al.* Noninvasive markers of airway inflammation in asthma. Clin Transl Sci. 2009;2(2):112-7. doi: 10.1111/j.1752-8062.2009.00095.x, PMID 20234847.
47. Britt RD Jr, Ruwanpathirana A, Ford ML, Lewis BW. Macrophages orchestrate airway inflammation, remodeling and resolution in asthma. Int J Mol Sci. 2023;24(13):10451. doi: 10.3390/ijms241310451, PMID 37445635.


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