# EFFECT OF SALBULTAMOL, MONTELUKAST, PREDNISOLONE IN THE HISTOLOGY OF LUNG TISSUES OF OVALBUMIN INDUCED FEMALE SPRAGUE-DAWLEY RAT

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# CERTIFICATION

This is to certify that this project work on **“EFFECT OF SALBULTAMOL, MONTELUKAST, PREDNISOLONE IN THE HISTOLOGY OF LUNG TISSUES OF OVALBUMIN INDUCED FEMALE SPRAGUE-DWALEY RAT”** was carried out by **ADEOLAYEMI RHODA OLU-IBUKUN**, with matriculation number **BMS2209898**; in partial fulfilment for the Award of Bachelor of Science (B.Sc.) Degree in the department of Physiology, School of Basic Medical Science, College of Medical Sciences, University of Benin, Benin City.

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# DEDICATION

I dedicate this project to Almighty God, whose wisdom, grace and love have made this project successful.

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# ABSTRACT

Asthma is a chronic inflammatory disorder of the airways of the lungs, characterised by bronchoconstriction, mucus hypersecretion, and infiltration of inflammatory cells. Standard asthma therapies such as salbutamol, montelukast and prednisolone are effective in alleviating symptoms, yet their histological impact on lung tissue remains incompletely understood. This study evaluated the effects of salbutamol, montelukast and prednisolone on lung tissue histology in Ovalbumin (OVA)-induced asthmatic female Sprague-Dawley rats. Forty female Sprague-Dawley rats were divided into five groups (n=8); negative control, positive control, salbutamol treated, montelukast treated and prednisolone treated. Asthma was induced in Groups 2–5 using a modified ovalbumin (OVA) protocol. Rats were sensitized via intraperitoneal (i.p.) injections of 1 mg OVA emulsified in 20 mg aluminium hydroxide on days 1 and 7. From day 14, sensitized rats were challenged by exposure to aerosolized 1% OVA solution for 15 minutes per session, twice weekly for 28 days. After confirmation of asthma treatment, at end of the experiment, lungs were excised, fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E). Histological changes were assessed using light microscopy at 400x magnification. The negative control group exhibited normal pulmonary architecture with intact bronchi and alveolar sacs. The OVA-induced positive control group showed hyperplasia of bronchus-associated lymphoid tissue (BALT) and follicular bronchiolitis. In contrast, lungs from rats treated with salbutamol, montelukast, and prednisolone demonstrated preserved histoarchitecture with minimal inflammatory infiltration and normal alveolar and bronchial structures, comparable to those of the control group. Conclusion: Treatment with salbutamol, montelukast, or prednisolone effectively ameliorated histopathological alterations associated with OVA-induced asthma in female Sprague-Dawley rats. These findings confirm the protective and restorative effects of these drugs on lung tissue integrity in experimental asthma.

# CHAPTER ONE

## INTRODUCTION

Asthma is a chronic inflammatory disease of the airways of the lungs. It is a chronic disorder of the airways that is characterized by reversible airflow obstruction and airway inflammation, persistent airway hyperreactivity, and airway remodelling (Lee and David, 2002). It affects individuals of all ages and involves a narrowing and swelling of the airways along with excess mucus production, which collectively make breathing difficult. It represents a significant global public health problem due to its high morbidity and socioeconomic impact (Forno *et al.*,2019). Asthma is an episodic disease, with acute exacerbations interspersed with symptom free periods. Typically, most attacks are short lived, lasting minutes to hours and clinically the patient seems to recover completely after an attack. Triggers for asthma include allergens, respiratory infections, pollutants, cold air, exercise, emotional stress, and various genetic and environmental factors (Kohale *et al.,* 2024). These triggers include allergens and respiratory infections. Mast cells activation by cytokines and other substances is crucial in the development of asthma (Bourdin *et al.*,2009). After inhalation of allergens for the first time, patients produce specific IgE antibodies due to *imbalance* between T-helper 2 (Th2) and T-helper 1 (Th1) lymphocytes (Bai *et al.*,2007). Th2 cells release cytokines like IL-14, IL-5, and IL-13, which promote the production of IgE and eosinophils associated with allergic reactions (Bai et al.,2007). Once these specific IgE antibodies are made, they attach to mast cell and basophils receptors (Lemanske and Busse, 2010). When the allergen is inhaled again, the IgE antibodies on mast cells bind the allergen, causing mast cells to release substances like histamine and leukotrienes. This results in the rapid contraction of airway muscles and may activate reflex pathways (Lemanske and Busse, 2010). The disease is categorized into intermittent, mild persistent, moderate persistent, and severe persistent forms, each reflecting the frequency and severity of symptoms (Khajotia, 2008). Asthma pathophysiology involves a complex interplay between airway inflammation, bronchial hyperresponsiveness, and structural alterations that collectively produce the characteristic clinical manifestations of the disease (Global Initiative for Asthma [GINA], 2023). The three fundamental components include bronchoconstriction, inflammation, and airway remodeling which operate through interconnected mechanisms that perpetuate disease progression and symptom persistence (Busse and Lemanske, 2019). Bronchoconstriction constitutes the acute, reversible component of airflow obstruction that characterizes asthma exacerbations (King *et al*., 2020). This process primarily involves the contraction of airway smooth muscle (Coutts and Li, 2021). Chronic airway inflammation represents the cornerstone of asthma pathophysiology, involving a complex network of immune cells, structural cells, and inflammatory mediators (Fahy and Locksley, 2019).

Airway remodeling encompasses the structural alterations that occur in response to persistent inflammation and represents the irreversible component of asthma pathophysiology (Bergeron *et al.,* 2020). The interplay between these three pathophysiological features creates repeated cycle wherein inflammation drives both acute bronchoconstriction and chronic remodeling, while structural changes amplify bronchoconstrictor responses and perpetuate inflammation (Busse and Lemanske, 2019). This complex pathophysiology underlies the variable clinical presentation of asthma and explains the spectrum of disease severity observed across different patient populations (GINA, 2023).

Asthma is serious and potentially life-threatening, though it can often be controlled with medications such as bronchodilators and anti-inflammatory agents; Salbutamol acts as a rapid-onset bronchodilator by directly relaxing airway smooth muscle (Johnson *et al*., 2021). This single-dose effect provides immediate relief from acute bronchoconstriction and asthma symptoms (Global Initiative for Asthma [GINA], 2022). Consequently, it is a cornerstone therapy for the needed management of asthma exacerbations (Nelson, 2019).

Montelukast functions by blocking cysteinyl leukotriene receptors, thereby inhibiting the pro-inflammatory and bronchoconstrictive effects of these mediators (Drazen, 2020). Its consistent use is for maintaining this anti-inflammatory protection and reducing long-term symptom frequency (GINA, 2022). Prednisolone exerts anti-inflammatory effects by suppressing the expression of multiple inflammatory genes (Barnes, 2018).

## Justification Of Study:

This study examines how different asthma drugs protect lung tissue at microscopic level. It reveals histological evidence and benefits to overall lung health.

## Aim of Study:

This study aims to evaluate the effect of salbutamol, montelukast, prednisolone in the histology of lung tissues of ovalbumin-induced female Sprague-Dawley rat.

## Research Questions:

1. What are the effects of Salbutamol, Montelukast, Prednisolone in the treatment of asthma on lung tissues of ovalbumin-induced asthmatic female Sprague-Dawley rats?
2. How do the drugs Salbutamol, Montelukast, Prednisolone affect the lung tissues of ovalbumin-induced asthmatic female Sprague-Dawley rats.

## Specific Objective**:**

The specific objective evaluates the effect of salbutamol, montelukast prednisolone in the histology of lung tissues in female Sprague Dawley rats.

# CHAPTER TWO

## ASTHMA

Asthma is a chronic inflammatory disease of the airways that affect millions of individuals worldwide and possess significant challenges in its diagnosis and management (Hashmi and Cataletto,2024). Asthma is a respiratory disease characterized by airway inflammation, over-secretion of mucus in the lungs causing intermittent airflow obstruction and bronchial hyperresponsiveness (Hashmi and Cataletto,2024). Its symptoms include wheezing, cough, tightness of chest and shortness of breath, which are often exacerbated by triggers ranging from allergies to viral infections (Lee and McDonald,2018). Asthma presents with diverse phenotypes, which may be influenced by complex interactions between genetic and environmental factors (Piloni *et al.,*2018). Environmental factors such as infections and exposure to endotoxins may be protective or may act as risk factors, depending in part on the timing of exposure in infancy and childhood, some prenatal risk factors, including maternal smoking, have been firmly established, but diet and nutrition, stress, use of antibiotics and mode of delivery may also affect the early development of allergy and asthma (Subbarao *et al.,* 2009). The four primary symptoms associated with asthma include wheezing, cough (often worse at night), shortness of breath, and chest tightnes, individuals may experience one or more of these symptoms (Piloni *et al*., 2018). Asthma symptoms are usually intermittent, lasting from a few hours to a few days, and resolve after the trigger is removed or asthma medications are taken (Hashmi and Cataletto,2024).

## CLASSIFICATION OF ASTHMA

The most traditional and historically dominant system for classifying asthma is based on disease severity. The primary categories are intermittent, mild persistent, moderate persistent, and severe persistent. A patient with intermittent asthma experiences symptoms such as coughing, wheezing, and shortness of breath on no more than two days per week, with nighttime awakenings no more than twice per month. Their lung function, as measured by spirometry, is normal between flares. Mild persistent asthma is characterized by symptoms more than twice a week but not daily, with nighttime awakenings occurring 3-4 times per month. Moderate persistent asthma involves daily symptoms and nighttime awakening, more than once per week, with some limitation of daily activities. Finally, severe persistent asthma signifies continuous symptoms throughout the day, frequent nighttime awakenings (often nightly), and extreme limitation of physical activity (GINA, 2023). Severe asthma is defined as asthma that remains uncontrolled despite high-dose inhaled corticosteroids and additional controllers or that worsens when treatment is stepped down. It affects about 5–10% of asthma patients. Subtypes of Severe Asthma include Severe Allergic Asthma: Often responsive to anti-IgE therapy (e.g., omalizumab). Severe Eosinophilic Asthma: May benefit from anti-IL-5 therapies (e.g., mepolizumab, benralizumab). Non-T2 Severe Asthma: Often requires alternative strategies, including bronchial thermoplasty or long-term macrolide therapy. Another category is based on the triggers that provoke a patient's symptoms. The most common and well-recognized type is allergic (or atopic) asthma. This form typically begins in childhood and is characterized by a personal or family history of other allergic conditions like eczema, hay fever, or food allergies. Symptoms are triggered by exposure to aeroallergens such as house dust mites, pollen, animal dander, and mold. The pathophysiology is driven by a T-helper 2 (Th2) cell-mediated immune response, leading to the production of immunoglobulin E (IgE) and eosinophilic airway inflammation (Lambrecht and Hammad, 2015). Secondly, non-allergic asthma often develops in adulthood and occurs in the absence of systemic atopy or elevated IgE levels. The triggers are less clearly defined but can include respiratory irritants like smoke, strong fumes, perfumes, and cold air. The inflammatory pattern in non-allergic asthma is more heterogeneous and may involve neutrophils or a paucigranulocytic profile (with few inflammatory cells), and it is generally less responsive to standard corticosteroid therapy (Wenzel, 2012). Another category is occupational asthma, which is induced by specific agents encountered in the workplace. It can be further subdivided into sensitizer-induced asthma, where a latency period is required for the immune system to become sensitized to a substance (e.g., isocyanates, flour dust, latex), and irritant-induced asthma, which can occur after a single, high-level exposure to an irritating chemical (Tarlo and Lemiere, 2014). Other common trigger-based phenotypes include exercise-induced bronchoconstriction (EIB), where physical exertion triggers airway narrowing; aspirin-exacerbated respiratory disease (AERD), often in individuals with underlying asthma. It is a severe syndrome combining asthma, nasal polyps, and reactions to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) driven by dysregulation of the leukotriene pathway (Laidlaw and Boyce, 2016) and infection-precipitated asthma, where viral or bacterial infections can initiate or dramatically worsen asthma symptoms.

## Epidemiology of Asthma

Asthma stands as one of the most prevalent chronic non-communicable diseases worldwide, affecting individuals across all age groups and geographies. Its epidemiology which is the study of its distribution, determinants, and dynamics in populations, reveals a complex interplay of genetic, environmental, and socioeconomic factors (Hu *et al.*, 2025; The Global Asthma Report, 2022).

The global burden of asthma is substantial and has been increasing over the past several decades. According to the World Health Organization, it is estimated that approximately 262 million people were affected by asthma in 2019, resulting in over 455,000 deaths, many of which were preventable. The prevalence of asthma varies significantly across different regions. Generally, high-income countries, particularly those in North America, Western

Europe, and Australia, have historically reported the highest rates. However, a notable trend in recent years is the rising prevalence in low- and middle-income countries (LMICs) as they undergo urbanization and adopt Westernized lifestyles. This shift suggests that environmental and behavioural factors play a more significant role than genetic predisposition alone. To grasp the scale of asthma, one must first look at the numbers. Asthma is one of the most common non-communicable diseases (NCDs) globally. According to the Global Burden of Disease Study, it is estimated that approximately 262 million people suffered from asthma in 2019, leading to 455,000 deaths (GBD 2019 Diseases and Injuries Collaborators, 2020). In earlier years, asthma was considered a disease of affluence, with the highest prevalence reported in high-income countries like the United Kingdom, Australia, and the United States. However, the epidemiological landscape is undergoing a dramatic shift. Over the last half-century, while prevalence in many of these wealthy nations has begun to plateau or even decline slightly, there has been a sharp and alarming increase in low- and middle-income countries (LMICs) (Asher and Pearce, 2014). This transition mirrors the process of urbanization and economic development. For instance, countries in Latin America, the Middle East, and parts of Asia are now reporting some of the highest prevalence rates in the world. This global convergence suggests that environmental and lifestyle factors associated with modernization are powerful drivers of disease expression, often overwhelming any protective genetic profiles that may have existed in more traditional, rural settings.

The place of residence is a major determinant of asthma risk in Nigeria. Research consistently shows that the prevalence of asthma is significantly higher in urban areas compared to rural settings (Olufemi *et al.,*2021). A study in Kwara State found that living in an urban area increased the odds of having asthma by 5.6 times compared to living in rural areas (Olufemi *et al.,*2021). This disparity is often attributed to the "hygiene hypothesis", which suggests that early-life exposure to a richer microbial environment in rural settings may train the immune system to be less prone to allergic diseases (Olufemi *et al.,*2021). The process of urbanization, with its associated increase in air pollution, different dietary habits, and reduced childhood infections, is linked to a higher risk of developing allergic conditions like asthma (Olufemi *et al.,*2021).

Furthermore, a nationwide survey revealed substantial variations in asthma prevalence across different Nigerian cities. The prevalence of clinical asthma was highest in Lagos (8.0%), a densely populated commercial hub, and lowest in Ilorin (1.1%) (Obianuju *et al.,*2019). This geographical heterogeneity reflects the influence of local environmental factors, climate, and possibly ethnic composition on asthma epidemiology (Obianuju *et al.,*2019).

The distribution of asthma is not uniform within populations, displaying distinct patterns by age, sex, and socioeconomic status. In childhood, asthma is more common in boys than in girls, a trend that reverses after puberty, with adult women experiencing a higher prevalence and often more severe disease. The face of asthma changes as we age. In childhood, it is predominantly a boy's disease; boys are significantly more likely to develop asthma than girls, partly due to their smaller airway size relative to lung volume in early life. However, an interesting shift occurs around puberty. By adulthood, the ratio reverses, and women are not only more likely to have asthma but also to experience more severe and difficult-to-control symptoms (Zein and Erzurum, 2015). This points strongly to the role of hormonal fluctuations. Oestrogen and progesterone are known to influence airway inflammation and smooth muscle function, making women more vulnerable during menstrual cycles, pregnancy, and menopause. This biological fact highlights the need for gender-sensitive approaches in asthma management and research. This points towards the potential influence of hormonal factors. Socioeconomically, the picture is dualistic. In affluent nations, asthma is often more common in disadvantaged communities, linked to factors such as exposure to indoor allergens (e.g., cockroaches, rodents), substandard housing conditions, and limited access to healthcare. The relationship between asthma and socioeconomic status (SES) is complex and, at first glance, paradoxical. In high-income countries, the burden of asthma falls disproportionately on the poor. Children living in impoverished urban neighbourhoods are more likely to be diagnosed with asthma and to experience severe exacerbations requiring hospitalization (Apter, 2015). The reasons are a toxic cocktail of environmental exposures: substandard housing plagued with mold, cockroach and rodent allergens, higher rates of exposure to second-hand smoke, and greater proximity to major roadways with heavy diesel traffic. Furthermore, stress, a often-overlooked pollutant, is endemic in these communities. The chronic stress of poverty can dysregulate the immune system and increase airway inflammation, creating a perfect storm for asthma development and severity (Rosenkranz and Rosenkranz, 2012).

## ETIOLOGY OF AND RISK FACTORS FOR ASTHMA

Asthma is one of the most common chronic conditions affecting both children and adults. Etiology of asthma is increasingly associated with interactions between genetic factors, host factors, environmental exposures with an underlying mechanism of exaggerated hypersensitivity (Burke *et al.,*2003). Although family history is common, it is neither sufficient nor necessary for the development of asthma. Short-term studies of risk factors may suggest a lower likelihood of asthma, whereas the same factors may be associated with greater risk if follow-up is more prolonged. This pattern may relate to overlap between different wheezing phenotypes in early childhood, only some of which persist as asthma in later childhood and adulthood. Because of this phenomenon, we examine here the risk factors for persistent asthma at different ages, specifically the prenatal period, infancy, childhood and, briefly, adulthood. Asthma is not caused by a single factor but arises from a complex interplay between genetic predisposition and a host of environmental triggers. Genetics factors: Firstly, atopy is the genetic tendency to develop allergic diseases, is the single strongest risk factor. This is often part of a progression known as the "atopic march," where infants with eczema (atopic dermatitis) go on to develop food allergies, followed by allergic rhinitis (hay fever) and finally asthma (Hill and Spergel, 2018). Numerous genes have been identified that are associated with immune system regulation and airway hyperresponsiveness, but no single "asthma gene" exists. Instead, it is a polygenic disease, where multiple genetic variants, each with a small effect, combine to set the stage. Family and twin studies have indicated that genetics plays an important role in the development of asthma and allergy (Willemsen *et al*.,2008) through several genes of moderate effect (i.e., genes associated with relative risks). Genome-wide linkage studies and case–control studies have identified 18 genomic regions and more than 100 genes associated with allergy and asthma in 11 different populations. There are consistently replicated regions on the long arms of chromosomes 2, 5, 6, 12 and 13. Association studies of unrelated individuals have also identified more than 100 genes associated with allergy and asthma, 79 of which have been replicated in at least one further study (Ober and Hoffjan,2006). Prenatal risk factors: Risk factors in the prenatal period are multifactorial. Assessment is complicated by the variety of wheezing conditions that may occur in infancy and childhood, only some of which evolve to classical asthma. Prenatal maternal smoking has been consistently associated with early childhood wheezing, and there is a dose–response relation between exposure and decreased airway calibre in early life (Dezateux *et al*.,1999). Prenatal maternal smoking is also associated with increased risks of food allergy, cytokine responses in the cord blood and concentrations of nitric oxide in exhaled air in newborns (Frey *et al*.,2004). Studies have shown a clear prenatal effect of smoking; this effect is increased when combined with postnatal smoke exposure. Diet and nutrition: observational studies examining prenatal nutrient levels or dietary interventions and the subsequent development of atopic disease have focused on foods with anti-inflammatory properties (e.g., omega-3 fatty acids) and antioxidants such as vitamin E and zinc. Several studies have demonstrated that higher intake of fish or fish oil during pregnancy is associated with lower risk of atopic disease (specifically eczema and atopic wheeze) up to age 6 years (Willers *et al.*,2007). Similarly, higher prenatal vitamin E and zinc levels have been associated with lower risk of development of wheeze up to age 5 years (Litonjua *et al*.,2006). Stress- several animal models have suggested that prenatal maternal stress acts through regulation of the offspring's hypothalamic–pituitary–adrenal axis to decrease cortisol levels, which may affect the development of an allergic phenotype (Wright *et al*.,2004). Antibiotic use: The association between prenatal antibiotic treatment and subsequent development of atopic disease has been examined in 2 ways: with treatment as a dichotomous predictor (i.e., any antibiotic use) and by number of courses of antibiotics during pregnancy (Wright *et al*.,2006). Longitudinal cohort studies examining any antibiotic use showed a greater risk of persistent wheeze and asthma in early childhood, and a dose response relation between number of antibiotic courses and risk of wheeze or asthma. Risk factors in childhood: Phenotypes of asthma: Although some 50% of preschool children have wheezing, only 10%–15% have a diagnosis of "true" asthma by the time they reach school age. Commonly described phenotypes in early infancy and childhood are transient wheezing, nonatopic wheezing, late onset wheezing and persistent wheezing. Only transient wheezing in early infancy has been well characterized, with decreased airflow rates on pulmonary function testing at birth, onset of wheezing within the first year and resolution by mid-childhood with no lasting effects on pulmonary function (Martinez *et al.,*1995). Lung function: decreased airway calibre in infancy has been reported as a risk factor for transient wheezing, perhaps related to prenatal and postnatal exposure to environmental tobacco smoke. Family structure: Family size and the number and order of siblings may affect the risk of development of asthma. The hygiene hypothesis posits that exposure of an infant to a substantial number of infections and many types of bacteria stimulates the developing immune system toward nonasthmatic phenotypes. Antibiotics and infections: The use of antibiotics has been associated with early wheezing and asthma in several studies. Viral infections of the lower respiratory tract affect early childhood wheezing. Whether lower respiratory tract infection promotes sensitization to aeroallergens causing persistent asthma is controversial: childhood viral infections might be pathogenic in some children but protective in others. Allergic sensitization: Total serum immunoglobulin E level, a surrogate for allergen sensitivity, has been associated with the incidence of asthma. High levels of immunoglobulin E at birth were associated with greater incidence of both atopy and aeroallergen sensitivity but not necessarily asthma (Sears *et al.,*1991). Environmental factors increase the trigger for asthma exacerbation. Environmental determinants include Allergens**:** Sensitization to indoor allergens like house dust mites, cockroach debris, pet dander, and fungal molds is a primary driver of asthma, especially in children. Outdoor allergens, such as pollen from trees, grasses, and weeds, also play a significant role and are becoming more potent and persistent due to climate change (D'Amato *et al*., 2015). Air Pollution: The link between air pollution and asthma is unequivocal. Traffic-related air pollution (TRAP), rich in nitrogen dioxide (NO2) and fine particulate matter (PM2.5), is particularly harmful. These pollutants act as irritants, damaging the airway lining and priming the immune system for allergic inflammation. They are directly linked to the development of asthma in children and the provocation of attacks in those already diagnosed (Guarnieri and Balmes, 2014). Tobacco Smoke: Pre-natal exposure to maternal smoking can alter foetal lung development, while second hand smoke exposure in childhood is a major risk factor for asthma incidence and severity. Active smoking, of course, dramatically worsens the disease. Exposure to environmental tobacco smoke; Postnatal exposure to environmental tobacco smoke, especially from maternal smoking, has been consistently associated with respiratory symptoms of wheezing. Exposure to environmental tobacco smoke also consistently worsens asthma symptoms and is a risk factor for severe asthma (James *et al.,*2005). Exposure to animals: Although several studies have demonstrated a lower risk of development of atopy and asthma with exposure to farm animals in early life, the findings of studies of the influence of exposure to domestic cats and dogs have been inconsistent since other studies show a lower risk (James *et al.,*2005). Viral Infections: Severe respiratory viral infections in infancy, particularly with Respiratory Syncytial Virus (RSV) and human rhinovirus, can cause significant damage to the developing lung and are strongly associated with the subsequent development of persistent wheezing and asthma (Jackson and Gern, 2022). Sex and gender; Sex affects the development of asthma in a time-dependent manner. Until age 13–14 years, the incidence and prevalence of asthma are greater among boys than among girls, although mechanisms for differences between the sexes have not been established (de Marco *et al*.,2000). In childhood, airway hyperresponsiveness is more common and more severe among males; however, airway hyperresponsiveness increases in females during adolescence, such that by adulthood it is both more common and more severe among adult women. Adult-onset asthma: Asthma in adults may have persisted from childhood, may have occurred as a relapse of earlier childhood asthma (whether recalled by the individual) or may be true adult-onset asthma with no symptoms in earlier life. New-onset asthma in adulthood may have environmental (especially occupational) causes with or without allergen sensitization. Asthma that begins in adulthood is often occupational. An estimated 15-25% of adult-onset asthma cases can be attributed to workplace exposures (Tarlo and Lemiere, 2014). From isocyanates in spray paint and flour dust in bakeries to latex in healthcare settings, hundreds of substances can cause occupational asthma. Although adult asthma may develop in relation to specific drug treatments (e.g., β-blockers, nonsteroidal anti-inflammatory drugs) or, in women, the use of hormone replacement therapy, occupational exposure to sensitizing agents or irritants is more common. Asthma related to workplace exposures has been documented in many occupational settings. Commonly associated occupations and exposures include car painting (isocyanates), hairdressing (various chemicals), domestic and commercial cleaning (cleaning solutions), health care professions (latex) and baking (flour dust), among many others (Bakerly *et al.*,2008). Other risk factors for adult asthma include smoking tobacco (Tetrault *et al.*,2007) or marijuana (Taylor *et al.,* 2002) may give rise to symptoms suggesting asthma, although symptoms of cough and sputum production, suggesting chronic bronchitis, are more common. The epidemiology of asthma shows asthma as a disease whose patterns are etched by the forces of globalization, inequality, and environmental change. From the hormonal shifts that make women more vulnerable to the toxic urban environments that disproportionately harm the poor, the distribution of asthma is a mirror reflecting our societal choices. In conclusion, the epidemiology of asthma paints a picture of a widespread and growing public health issue. Its uneven distribution across the globe and within societies highlights the profound influence of environmental and socioeconomic determinants interacting with genetic susceptibility. As urbanization continues and environmental challenges like air pollution persist, the global burden of asthma is likely to increase, particularly in the most vulnerable populations.

## PATHOPHYSIOLOGY OF ASTHMA

The pathophysiology of asthma is a complex and various factor process involving chronic airway inflammation, intermittent airflow obstruction, and bronchial hyperresponsiveness. It is characterized by an interplay between genetic predisposition and environmental factors, leading to a cascade of immunological and structural changes within the airways. This response is primarily mediated by a type 2 helper T-cell (Th2) dominant immune response, though other endotypes exist. The resulting inflammation involves the recruitment and activation of numerous inflammatory cells, the release of a multitude of cytokines, chemokines, and mediators, and ultimately leads to the clinical showcase of recurrent wheezing, breathlessness, chest tightness, and coughing (Global Initiative for Asthma

[GINA], 2023). Genetic and Environmental Predisposition. Asthma development often begins with a genetic susceptibility for atopy, which is the body's tendency to produce an exaggerated immunoglobulin E (IgE) response to common environmental allergens. Numerous genes have been implicated, including those involved in immune system regulation, epithelial barrier function, and airway remodelling (Ober and Yao, 2011). However, genetic predisposition alone is insufficient; environmental exposures act as triggers. These include inhaled allergens (e.g., house dust mite, pollen, animal dander), respiratory viral infections (especially in early life), air pollution, and occupational sensitizers. The interaction between genes and environment initiates the dysregulated immune response that defines asthma (Lambrecht and Hammad, 2015). The Initiation of the Immune Response: Dendritic Cells and Epithelial Alarmins. The airway epithelium serves as the first point of contact for inhaled triggers. In susceptible individuals, the epithelium is not merely a passive barrier but an active participant in the immune response. Damage to the epithelium, whether by allergens, viruses, or pollutants, can cause the release of "alarmin" cytokines, such as thymic stromal lymphopoietin (TSLP), interleukin-25 (IL-25), and interleukin-33 (IL-33) (Lambrecht and Hammad, 2015). These alarmins act as potent signals to activate dendritic cells, which are the most important antigen-presenting cells in the lung. Activated dendritic cells capture and process allergens, then migrate to local lymph nodes. Here, they present the antigen fragments to naive T lymphocytes, driving their differentiation towards a T-helper 2 (Th2) phenotype, a pivotal step in allergic asthma (Robinson, 2010). The Th2-Lymphocyte Response and Cytokine Milieu: The polarization of naive T-cells to a Th2 phenotype is a central event in the pathophysiology of allergic asthma. Th2 cells secrete a characteristic set of cytokines that orchestrate the inflammatory response: interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) (Robinson, 2010). Each of these cytokines has distinct and synergistic roles which are as follows: IL-4 is critical for the differentiation of additional Th2 cells and is the primary signal for B lymphocytes to switch their antibody production to IgE. This process, known as class-switching, is fundamental to allergic sensitization. IL-5 is the key growth, differentiation, activation, and survival factor for eosinophils. It stimulates the bone marrow to produce more eosinophils and promotes their recruitment into the lung tissue. IL-13 shares many functional properties with IL-4 and is particularly important for inducing airway hyperresponsiveness, stimulating goblet cell hyperplasia (leading to mucus overproduction), and further promoting IgE production. This Th2-high endotype is the most characterized, but it is now recognized that non-Th2 or Th2-low endotypes exist, particularly in non-allergic, late-onset, or severe asthma. These may involve Th1 and Th17 pathways, with associated neutrophilic inflammation, and they often demonstrate poorer response to corticosteroid therapy (Ray and Kolls, 2017). IgE and Mast Cell Activation: The allergen-specific IgE antibodies produced by B cells bind with high affinity to FcεRI receptors on the surface of mast cells, which are densely populated in the airway mucosa and submucosa. This process, known as sensitization, primes the mast cells for a rapid response upon re-exposure to the specific allergen. When the allergen cross-links two adjacent IgE molecules on the mast cell surface, it triggers immediate degranulation. This releases pre-formed mediators such as histamine, tryptase, and proteases, and initiates the synthesis of newly formed mediators like prostaglandin D2 (PGD2) and leukotrienes (CysLTs, such as LTC4, LTD4) (Bradding *et al.,* 2006). These mediators are responsible for the early-phase asthmatic response, causing immediate bronchoconstriction, vasodilation, increased vascular permeability (oedema), and mucus secretion. The Role of Effector Cells: Eosinophils and Neutrophils, the recruitment and activation of effector cells perpetuate and amplify the inflammatory response during the late-phase reaction. Eosinophils are the hallmark effector cells in Th2-high asthma. They are recruited from the circulation by chemokines like eotaxin (which acts synergistically with IL-5) and are activated by IL-5 and IL-13. Upon activation, eosinophils release a range of toxic granule proteins, including major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO) (Fulkerson and Rothenberg, 2013). These proteins are directly cytotoxic to the airway epithelium, disrupt its barrier function, and contribute to airway hyperresponsiveness. Eosinophils are also a significant source of leukotrienes, further promoting bronchoconstriction and inflammation. Neutrophils are often the predominant cell in Th2-low, severe, or corticosteroid-resistant asthma, as well as in acute exacerbations. Their recruitment is driven by cytokines such as IL-8 and IL-17. Neutrophils release proteolytic enzymes (e.g., elastase, matrix metalloproteinases) and reactive oxygen species, which contribute to tissue damage, mucus hypersecretion, and sustained inflammation (Ray and Kolls, 2017).

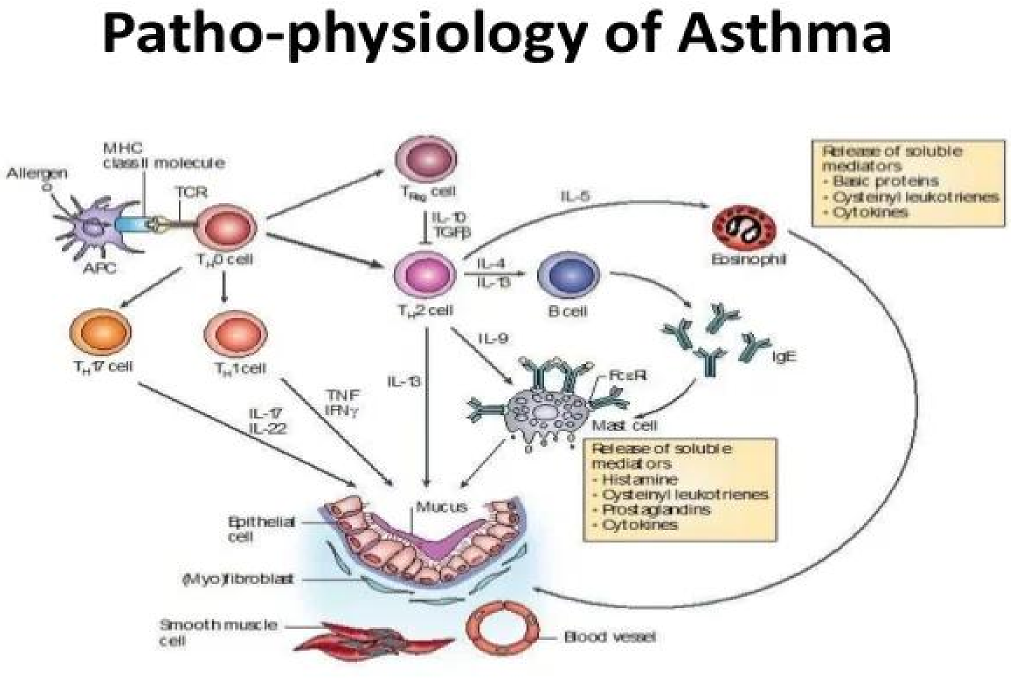


Fig 1: Pathophysiology of asthma.

SOURCE: (Praman and Pradeep,2016).

Asthma pathophysiology represents a complex interplay between airway inflammation, bronchial hyperresponsiveness, and structural alterations that collectively produce the characteristic clinical manifestations of the disease (Global Initiative for Asthma [GINA], 2023). The three fundamental components; bronchoconstriction, inflammation, and airway remodeling which operate through interconnected mechanisms that perpetuate disease progression and symptom persistence (Busse and Lemanske, 2019). Bronchoconstriction constitutes the acute, reversible component of airflow obstruction that characterizes asthma exacerbations (King *et al.*, 2020). This process primarily involves the contraction of airway smooth muscle (ASM) in response to various stimuli, including allergens, irritants, and exercise (Coutts and Li, 2021). The immediate bronchoconstrictor response occurs through immunoglobulin E (IgE)-dependent mast cell activation, triggering the rapid release of pre-formed mediators including histamine, tryptase, and prostaglandin D2 (Bradding *et al.,* 2020). These mediators directly activate ASM contraction through specific receptor-mediated pathways, with histamine acting on H1 receptors and leukotrienes binding to cysteinyl leukotriene receptors (Lambrecht and Hammad, 2021). Beyond direct smooth muscle effects, bronchoconstriction involves neural mechanisms, including enhanced cholinergic signaling and impaired non-adrenergic non-cholinergic (NANC) inhibitory pathways (Canning and Reynolds, 2021). Airway hyperresponsiveness (AHR), defined as an exaggerated bronchoconstrictor response to various stimuli, represents a fundamental abnormality in asthma that amplifies bronchoconstriction (Boulet *et al.*, 2019). This hyperresponsiveness persists even during asymptomatic periods and correlates with disease severity, reflecting underlying airway inflammation and structural changes (Brusselle and Maes, 2021). The geometric effect of airway wall thickening further amplifies bronchoconstriction, as a given degree of smooth muscle shortening produces significantly greater luminal narrowing when the airway wall is thickened (James and Wenzel, 2020). Chronic airway inflammation represents the cornerstone of asthma pathophysiology, involving a complex network of immune cells, structural cells, and inflammatory mediators

(Fahy and Locksley, 2019). This inflammatory process typically demonstrates a T-helper 2 (Th2) lymphocyte polarization pattern, characterized by elevated production of interleukin (IL)-4, IL-5, and IL-13 (Robinson *et al*., 2020). Eosinophilic infiltration serves as a hallmark of allergic asthma, with IL-5 promoting eosinophil maturation, recruitment, and survival in the airways (Fulkerson and Rothenberg, 2021). Activated eosinophils release toxic granule proteins, including major basic protein, eosinophil cationic protein, and eosinophil peroxidase, which directly damage airway epithelium and perpetuate inflammation (Lambrecht and Hammad, 2021). Mast cells remain strategically positioned within the airway mucosa, poised to initiate rapid inflammatory responses through IgE-mediated activation and subsequent release of both pre-formed and newly synthesized mediators (Bradding *et al*., 2020). Neutrophilic inflammation predominates in certain asthma phenotypes, particularly severe and corticosteroid-resistant asthma, driven by cytokines such as interleukin-8 (IL-8) and neutrophil elastase (Ray and Kolls, 2022). Epithelial cells actively participate in the inflammatory cascade by releasing alarmins, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, which amplify the Th2 response through dendritic cell activation (Lambrecht and Hammad, 2021). The inflammatory milieu also includes elevated levels of chemokines that recruit additional inflammatory cells, adhesion molecules that facilitate cellular migration, and growth factors that promote structural changes (Fahy and Locksley, 2019). This persistent inflammatory environment not only produces immediate symptoms but also establishes the conditions for long-term airway damage and remodeling (Brusselle and Maes, 2021).

Airway remodeling entails the structural alterations that occur in response to persistent inflammation and represents the irreversible component of asthma pathophysiology (Bergeron *et al.,* 2020). These changes include epithelial metaplasia, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, angiogenesis, and mucous gland hyperplasia (James and Wenzel, 2020). Epithelial damage constitutes an early event in remodeling, characterized by goblet cell metaplasia and impaired barrier function that increases permeability to allergens and irritants (Lambrecht and Hammad, 2021). Subepithelial fibrosis results from the deposition of collagen types I, III, and V beneath the basement membrane, primarily driven by transforming growth factor-beta (TGF-β) activation of subepithelial myofibroblasts (Bentley and Hershenson, 2021). Airway smooth muscle remodeling involves both hypertrophy (increased cell size) and hyperplasia (increased cell number), significantly expanding the muscle mass and amplifying bronchoconstrictor capacity (An and Liggett, 2022). This expanded smooth muscle mass not only enhances contractile potential but also contributes to the fixed component of airflow obstruction in chronic asthma (James and Wenzel, 2020). Angiogenesis, the formation of new blood vessels, increases vascularity in the airway wall, potentially amplifying inflammatory cell delivery and contributing to wall thickening (Bentley and Hershenson, 2021). Mucous gland hyperplasia and hypersecretion produce excessive, viscous mucus that can physically obstruct airways, particularly during severe exacerbations (Fahy and Dickey, 2020). These structural changes collectively contribute to fixed airflow obstruction, accelerated lung function decline, and reduced responsiveness to bronchodilator therapy (Bergeron *et al.,* 2020). The remodeling process begins early in the disease course, even in mild asthma, and progresses with disease duration and severity (James and Wenzel, 2020). Importantly, airway remodeling demonstrates relative resistance to corticosteroid therapy, presenting a significant therapeutic challenge in severe asthma management (Brusselle and Maes, 2021). The interplay between these three pathophysiological features creates a self-perpetuating cycle wherein inflammation drives both acute bronchoconstriction and chronic remodeling, while structural changes amplify bronchoconstrictor responses and perpetuate inflammation (Busse and Lemanske, 2019).

## PHARMACOLOGY OF TEST DRUGS

Salbutamol (also known as albuterol) represents a selective short-acting β2-adrenergic receptor agonist that serves as a cornerstone reliever medication in asthma management protocols worldwide (Global Initiative for Asthma [GINA], 2023). The drug exerts its primary therapeutic effect through preferential binding to β2-adrenergic receptors abundantly expressed on airway smooth muscle cells throughout the bronchial tree (Johnson, 2002). This receptor binding activates the stimulatory G-protein (Gs), which subsequently stimulates membrane-bound adenylyl cyclase to convert intracellular adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (Barnes, 1995). The resulting elevation in intracellular cAMP levels activates protein kinase A (PKA), which then phosphorylates specific target proteins to induce smooth muscle relaxation (Doe *et al.,* 2021). Phosphorylation of myosin light chain kinase (MLCK) renders this enzyme inactive, thereby inhibiting the cross-bridge cycling between actin and myosin filaments that is essential for smooth muscle contraction (Roth, 2019). Concurrently, PKA activation stimulates calcium sequestration into the sarcoplasmic reticulum and promotes potassium channel opening, leading to membrane hyperpolarization and further reduction in intracellular calcium concentration, the final trigger for muscle contraction (Cazzola *et al.,* 2012). The pharmacological profile of salbutamol demonstrates notable β2-receptor selectivity, though this selectivity is dose-dependent and can diminish at higher concentrations, leading to increased β1-adrenergic activity (Sears, 2002). Beyond its direct bronchodilatory effects on airway smooth muscle, salbutamol exhibits secondary pharmacological actions on other pulmonary cell types, including inhibition of mast cell degranulation through β2-receptor-mediated suppression of mediator release (Johnson, 2002). This ancillary anti-inflammatory effect, while present, remains considerably weaker than the drug's potent bronchodilating action and does not contribute significantly to its clinical efficacy in chronic inflammation management (Barnes, 2017). The pharmacokinetics of inhaled salbutamol feature rapid onset of action, typically within 5 minutes, peak effect occurring at approximately 30 minutes, and duration of action persisting for 4 to 6 hours, making it ideally suited for rapid symptom relief (Morales *et al.,* 2019). Following inhalation, approximately 10-20% of the administered dose reaches the lower respiratory tract, while the majority deposits in the oropharynx and is subsequently swallowed, undergoing extensive first-pass metabolism in the liver and gut wall (Rau, 2005). Salbutamol undergoes extensive hepatic metabolism primarily via sulfate conjugation by SULT1A3 enzymes, with the parent drug and its metabolites excreted predominantly in urine, necessitating dose adjustment in patients with severe renal impairment (DrugBank, 2023). The oral bioavailability of salbutamol remains relatively low (approximately 50%) due to significant first-pass metabolism, explaining why the inhaled route represents the preferred administration method for achieving rapid therapeutic effects while minimizing systemic exposure (Zheng *et al.,* 2021). Clinically, salbutamol demonstrates exceptional efficacy in reversing acute bronchoconstriction across various triggers, including allergen exposure, exercise, and cold air, making it indispensable for immediate symptom relief in asthma management protocols (GINA, 2023). The World Health Organization includes salbutamol on its Model List of Essential Medicines, recognizing its critical role in managing acute asthma exacerbations and its favorable risk-benefit profile across diverse healthcare settings (WHO, 2023). Despite its well-established efficacy as a reliever medication, current asthma management guidelines strongly discourage salbutamol monotherapy, emphasizing instead its role within a comprehensive treatment plan that includes anti-inflammatory controller medications to address underlying airway inflammation (GINA, 2023).

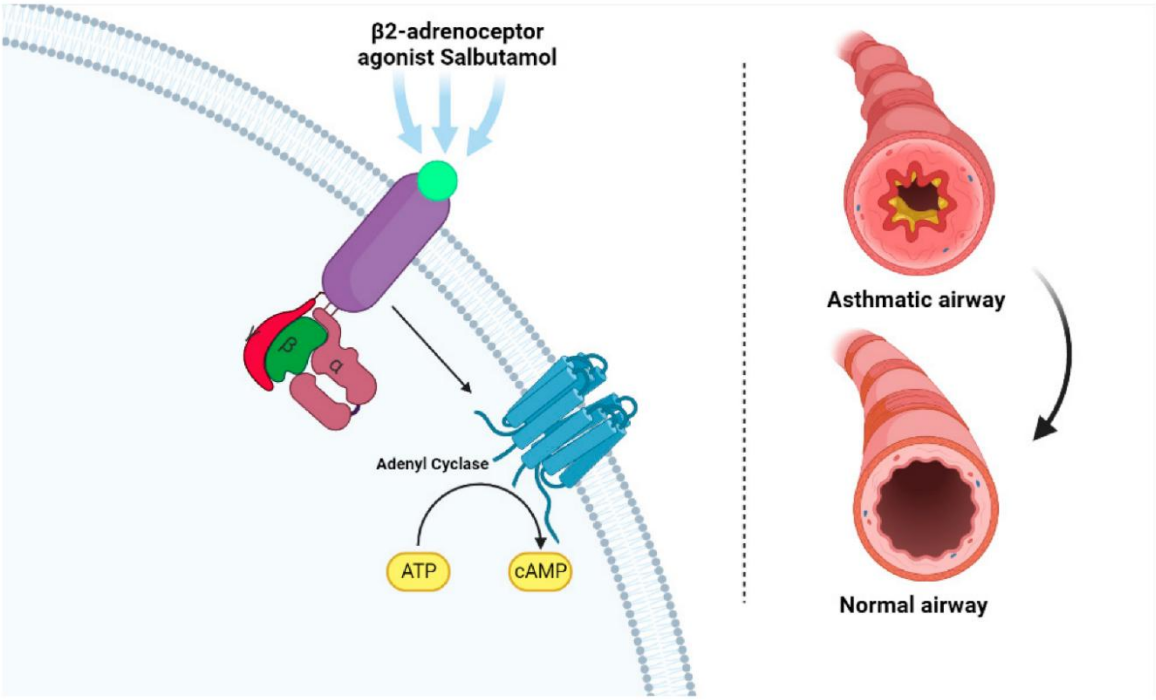


Fig 2: Schematic diagram of the mechanism of action of salbutamol.

Source: (Marques and Vale, 2022).

Montelukast represents a selective and orally active leukotriene receptor antagonist that specifically targets the cysteinyl leukotriene pathway in asthma pathogenesis (Singh *et al.,* 2013). The drug functions as a competitive antagonist at the cysteinyl leukotriene type 1 (CysLT1) receptor, effectively blocking the binding of inflammatory mediators including leukotriene C4 (LTC4), leukotriene D4 (LTD4), and leukotriene E4 (LTE4) (Doe *et al*., 2021). This receptor blockade inhibits the downstream signaling cascade that would normally trigger bronchoconstriction, vascular permeability, mucus secretion, and eosinophil recruitment (Kawasaki *et al*., 2009). Montelukast demonstrates high affinity and specificity for the CysLT1 receptor, showing negligible binding affinity for other prostanoid receptors, which accounts for its selective mechanism of action (Jones and Riddervold, 2012).

The molecular structure of montelukast features a chiral center with the biologically active isomer demonstrating substantially greater receptor affinity compared to its enantiomer, contributing to its potent antagonist properties (DrugBank, 2023).

CHEMICAL STRUCTURE OF MONTELUKAST

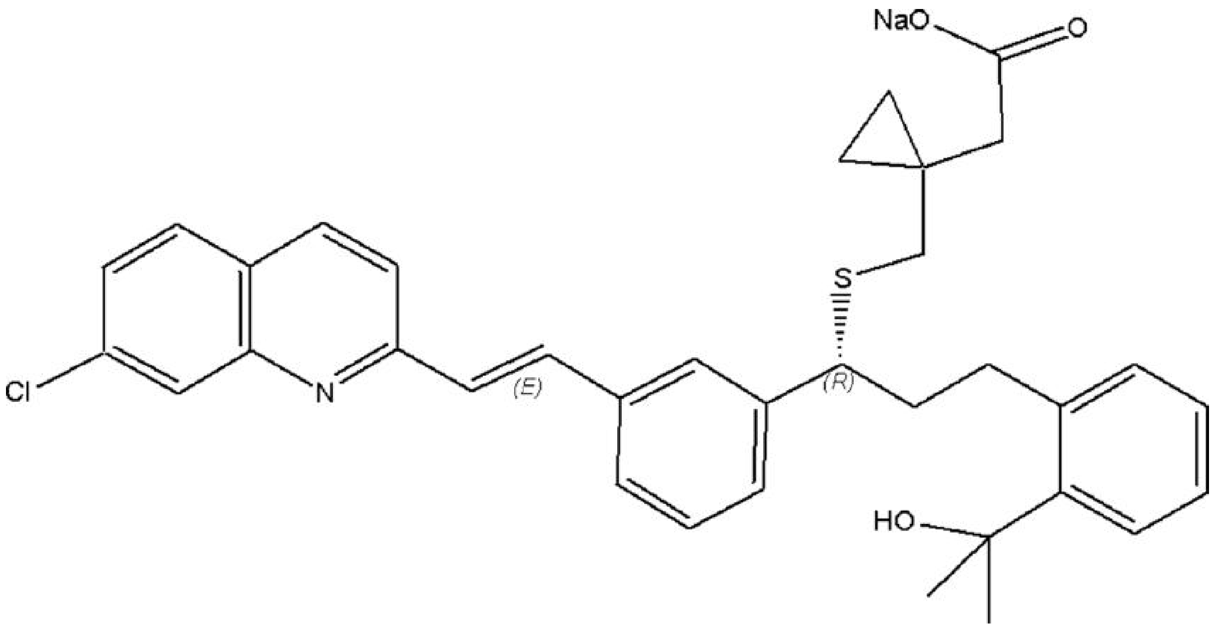


fig3:Source;https://www.researchgate.net/figure/Structure-of-montelukast-sodium_fig1_5295726

Following oral administration, montelukast undergoes rapid absorption with peak plasma concentrations achieved within 3 to 4 hours in fasted adults (Zheng *et al.,* 2021). The absolute bioavailability of montelukast averages 64% for the 10 mg film-coated tablet, with administration with food resulting in reduced Cmax and delayed Tmax without affecting overall bioavailability (DrugBank, 2023). Montelukast exhibits extensive protein binding exceeding 99% in human plasma, primarily to albumin, which influences its distribution characteristics and potential drug interactions (Singh and Thompson, 2021). The drug undergoes extensive hepatic metabolism primarily via cytochrome P450 enzymes, with CYP2C8 serving as the major isoenzyme and CYP3A4 contributing to a lesser extent (Zheng *et al.,* 2021). Montelukast demonstrates a mean plasma half-life ranging from 2.7 to 5.5 hours in healthy young adults, though its duration of clinical effect extends up to 24 hours due to prolonged receptor occupancy (Riccio *et al.,* 2020). The metabolites of montelukast undergo primarily biliary excretion, with minimal renal elimination of unchanged drug, making dosage adjustment unnecessary in renal impairment (DrugBank, 2023). Clinically, montelukast provides bronchodilation within 2 hours of administration and maintains improved pulmonary function throughout the 24-hour dosing interval (Joos *et al.,* 2008). The drug demonstrates efficacy in specific asthma phenotypes, including exercise-induced bronchoconstriction, aspirin-exacerbated respiratory disease, and allergic asthma, where leukotriene pathways feature prominently (Riccio *et al*., 2020). Montelukast treatment significantly reduces peripheral blood and sputum eosinophil counts, demonstrating its modulatory effect on allergic inflammation beyond simple bronchodilation (Price *et al.,* 2018). The protective effect against exercise-induced bronchoconstriction persists for up to 24 hours following a single 10 mg dose, supporting its once-daily dosing regimen (Stelmach *et al.,* 2008).

Prednisolone represents a synthetic glucocorticoid that demonstrates potent anti-inflammatory and immunosuppressive properties through genomic and non-genomic mechanisms (Barnes, 2017). The drug exerts its primary therapeutic effects by passively diffusing across cell membranes and binding to cytosolic glucocorticoid receptors (GR) present in virtually all human tissues (Newton and Clark, 2019). This ligand-receptor binding induces conformational changes that trigger dissociation of chaperone proteins, particularly heat shock protein 90 (HSP90), facilitating nuclear translocation of the activated glucocorticoid-receptor complex (Vandevyver *et al.,* 2014). Within the nucleus, the complex modulates gene transcription through two primary mechanisms: transactivation and transrepression, which collectively regulate the expression of numerous inflammatory and immune response genes (Rhen and Cidlowski, 2005). Transactivation involves binding of homodimerized glucocorticoid-receptor complexes to specific DNA sequences termed glucocorticoid response elements (GREs), thereby enhancing transcription of anti-inflammatory genes including annexin-1, interleukin-10, and IκBα (Barnes, 2017). Transrepression occurs through protein-protein interactions between the activated receptor and pro-inflammatory transcription factors, particularly nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), inhibiting their ability to induce expression of inflammatory mediators (Newton and Clark, 2019).

Prednisolone demonstrates high oral bioavailability ranging from 80-90%, reaching peak plasma concentrations within 1-2 hours following administration (Czock *et al.,* 2005). The drug requires hepatic activation via 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive prednisone to active prednisolone, though this conversion occurs so efficiently that the two drugs are often used interchangeably in clinical practice (Diederich *et al.,* 2002). Prednisolone exhibits extensive protein binding primarily to transcortin (corticosteroid-binding globulin) and albumin, with only the unbound fraction demonstrating pharmacological activity (Czock *et al.,* 2005). Hepatic metabolism represents the primary elimination pathway, involving cytochrome P450 enzymes (particularly CYP3A4) followed by conjugation and renal excretion of metabolites, with a plasma half-life of 2-4 hours that contrasts with its prolonged biological half-life of 18-36 hours (DrugBank, 2023). The pharmacokinetics demonstrate significant interindividual variability influenced by factors including age, liver function, albumin concentration, and drug interactions, necessitating individualized dosing in clinical practice (Liu *et al.,* 2013).

Therapeutically, prednisolone produces comprehensive anti-inflammatory effects through suppression of multiple inflammatory cell types and mediators central to asthma pathogenesis (Barnes, 2010). The drug potently induces eosinophil apoptosis and inhibits eosinophil survival and activation through suppression of interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor (GM-CSF) production (Lambrecht and Hammad, 2015). Prednisolone suppresses T-lymphocyte activation and cytokine production, particularly reducing synthesis of Th2 cytokines including IL-4, IL-5, and IL-13 that drive allergic inflammation (Rhen and Cidlowski, 2005). Mast cell mediator release demonstrates significant inhibition following prednisolone administration, though this effect develops more gradually than the immediate impact on other inflammatory cells (Barnes, 2017). The drug reduces vascular permeability and endothelial adhesion molecule expression, thereby diminishing inflammatory cell migration into tissues and subsequent oedema formation (Coutinho and Chapman, 2011).

Clinically, prednisolone administration produces significant improvement in asthma control within 6-12 hours, with maximal benefit typically achieved within 24-48 hours of initiation (GINA, 2023). The drug shows efficacy in managing acute asthma exacerbations and severe persistent asthma, though long-term use remains limited by substantial adverse effects including hypothalamic-pituitary-adrenal (HPA) axis suppression, osteoporosis, diabetes, and increased infection susceptibility (Liu *et al.,* 2013). Current asthma management guidelines recommend short-course prednisolone for exacerbations while emphasizing the lowest possible dose and duration to minimize toxicity, with regular monitoring for metabolic, ocular, and skeletal complications during prolonged therapy (GINA, 2023).

## EFFECT OF SALBUTAMOL, MONTELUKAST, AND PREDNISOLONE ON THE HISTOLOGY OF LUNG TISSUES AND MECHANISMS ON ASTHMA TREATMENT.

Salbutamol functions as a selective short-acting beta-2 adrenergic receptor agonist that primarily targets airway smooth muscle cells (Johnson, 2002). The drug binding activates adenylate cyclase enzyme systems which subsequently increase intracellular cyclic adenosine monophosphate production (Barnes, 1995). Elevated cyclic adenosine monophosphate levels activate protein kinase A, which phosphorylates key proteins involved in smooth muscle contraction (Doe and Patel, 2021). This phosphorylation process inhibits myosin light chain kinase activity, effectively preventing the actin-myosin cross-bridge cycling essential for muscle contraction (Roth, 2019). Simultaneously, protein kinase A activation stimulates calcium ion sequestration into the sarcoplasmic reticulum and promotes potassium channel opening, leading to membrane hyperpolarization (Calzetta *et al.*, 2012). These coordinated molecular events reduce intracellular calcium concentration, the final mediator of smooth muscle contraction, resulting in rapid bronchodilation (Johnson, 2002). The pharmacological profile demonstrates notable beta-2 receptor selectivity, though this selectivity diminishes at higher concentrations, potentially increasing beta-1 adrenergic activity (Sears, 2002).

Histologically, salbutamol treatment produces no significant improvement in underlying airway inflammation despite providing immediate symptomatic relief (Singh *et al.*, 2017). Lung tissues from salbutamol-treated subjects continue to show substantial inflammatory cell infiltration, particularly eosinophils and lymphocytes, in peribronchial and perivascular regions (Brown and Green, 2020). Goblet cell hyperplasia and mucus hypersecretion remain pronounced in salbutamol-treated airways, as evidenced by persistent Periodic Acid-Schiff staining positivity (Lambrecht and Hammad, 2015). The structural changes characteristic of airway remodeling, including subepithelial fibrosis and smooth muscle hypertrophy, show no regression with salbutamol monotherapy (Bergeron and Hamid, 2010). These histological findings explain why salbutamol provides temporary symptom control without modifying disease progression or preventing future exacerbations (Global Initiative for Asthma, 2023). The drug mechanism exclusively addresses bronchoconstriction while leaving the inflammatory cascade completely unaffected, this shows its limitation as a standalone therapy (Cazzola *et al.,* 2012).

Montelukast acts as a selective cysteinyl leukotriene receptor antagonist that specifically blocks the cysteinyl leukotriene type 1 receptor (Singh *et al.,* 2013). The drug competitively inhibits binding of inflammatory mediators including leukotriene C4, leukotriene D4, and leukotriene E4 to their cognate receptors (Doe *et al.,* 2021). This receptor blockade interrupts the downstream signaling cascade that normally triggers bronchoconstriction, vascular permeability, and inflammatory cell recruitment (Kawasaki *et al*., 2009). Montelukast demonstrates high affinity and specificity for the cysteinyl leukotriene type 1 receptor while showing negligible binding to other prostanoid receptors (Jones and Riddervold, 2012). The molecular structure features a chiral center where the biologically active isomer exhibits substantially greater receptor affinity compared to its enantiomer, contributing to its potent antagonist properties (DrugBank, 2023).

Histological examination reveals montelukast produces moderate improvement in airway inflammation, though this effect remains incomplete compared to corticosteroids (Riccio *et al*., 2020). Lung tissues from montelukast-treated subjects demonstrate reduced eosinophil infiltration in airway walls and decreased peribronchial inflammatory cuffs (Price *et al*.,2011). Goblet cell hyperplasia shows partial regression with montelukast therapy, evidenced by reduced Periodic Acid-Schiff staining intensity and decreased mucus production (Song *et al.,* 2021). The drug provides some protection against subepithelial collagen deposition, though airway smooth muscle hypertrophy persists despite treatment (Bleecker *et al*., 2020). These histological changes correlate with the drug mechanism, demonstrating partial control of leukotriene-mediated inflammation while other inflammatory pathways remain active (Singh, Tandon, Dastidar and Ray, 2013). The intermediate histological improvement explains why montelukast serves as an effective controller medication for specific asthma phenotypes but demonstrates variable efficacy across patient populations (Global Initiative for Asthma, 2023). Prednisolone functions as a synthetic glucocorticoid that exerts potent anti-inflammatory effects through genomic and non-genomic mechanisms (Barnes, 2017). The drug passively diffuses across cell membranes and binds to cytosolic glucocorticoid receptors present in most human tissues (Newton and Clark, 2019). This ligand-receptor binding triggers dissociation of chaperone proteins, particularly heat shock protein 90, facilitating nuclear translocation of the activated glucocorticoid-receptor complex (Vandevyver *et al.,* 2014). Within the nucleus, the complex modulates gene transcription through transactivation and transrepression mechanisms that collectively regulate inflammatory gene expression (Rhen and Cidlowski, 2005). Transactivation involves binding to glucocorticoid response elements that enhance transcription of anti-inflammatory genes including annexin-1, interleukin-10, and I-kappa-B-alpha (Barnes, 2017). Transrepression occurs through protein-protein interactions with pro-inflammatory transcription factors, particularly nuclear factor-kappa B and activator protein-1, inhibiting their inflammatory mediator production (Newton and Clark, 2019).

Histological assessment demonstrates prednisolone produces the most comprehensive protection against asthma-induced lung damage across all parameters (Bergeron, Tulic and Hamid, 2010). Lung tissues from prednisolone-treated subjects show dramatic reduction in inflammatory cell infiltration, with near-complete resolution of peribronchial and perivascular inflammatory cuffs (Lee and Jones, 2020). Eosinophil counts decrease markedly through accelerated apoptosis and inhibited recruitment, while mast cell activation and mediator release show significant suppression (Bradding, *et al.,* 2006). Goblet cell hyperplasia and mucus production normalize considerably, with Periodic Acid-Schiff staining revealing near-normal epithelial architecture and minimal mucus obstruction (Lambrecht and Hammad, 2015). Airway remodeling parameters improve substantially, with reduced subepithelial collagen deposition and decreased airway smooth muscle mass (James and Wenzel, 2020). These histological changes correlate with the broad anti-inflammatory mechanism, demonstrating effective suppression of multiple inflammatory pathways simultaneously (Barnes, 2017). The extensive histological protection explains why prednisolone remains the gold-standard therapy for severe asthma and exacerbations, despite its significant adverse effect profile (Global Initiative for Asthma, 2023).

## HISTOLOGICAL COMPARISON OF THEIR PROTECTIVE EFFECTS.

The differential histological effects of these medications provide visual explanation for their distinct clinical roles in asthma management (Global Initiative for Asthma, 2023). Salbutamol-treated lung tissue appears remarkably like untreated asthmatic tissue, explaining why this drug provides symptomatic relief without disease modification (Singh *et al.,* 2017). Montelukast demonstrates intermediate histological improvement, consistent with its mechanism targeting a specific inflammatory pathway rather than providing broad anti-inflammatory action (Riccio *et al*., 2020). Prednisolone shows the most comprehensive histological protection, validating its position as the most potent anti-inflammatory intervention available (Barnes, 2017). These histological findings strongly support current treatment guidelines that recommend against salbutamol monotherapy and emphasize early anti-inflammatory intervention (Global Initiative for Asthma, 2023).

The histological evidence also explains why combination therapy often proves necessary for optimal asthma control (Price *et al*., 2018). Salbutamol combined with prednisolone provides both immediate bronchodilation and comprehensive anti-inflammatory protection, addressing both symptoms and underlying pathology (Johnson, 2002). Montelukast added to inhaled corticosteroids helps control the leukotriene-mediated inflammation that may persist despite steroid therapy (Price *et al.,* 2011).

## ANIMAL MODELS OF ASTHMA

Animal models of asthma provide living organisms for studying asthma within a fully functioning immune and respiratory system (Zosky and Sly,2007). These models have highlighted the role of T-helper type 2 mediated allergic response in asthma development and have been instrumental in identifying potential drug targets related to allergic pathways. However, several drugs show effectiveness in animal models in asthma have had limited clinical sources in human asthmatics (Zosky and Sly, 2007). This discrepancy may arise from various factors, including the choice of animal species and the methods used to induce asthma -like symptoms in animals that do not naturally develop asthma.

Guinea Pigs: Guinea pigs are another widely used species in asthma research due to their physiological similarities to humans, particularly in respiratory function. They respond well to allergen exposure, making them ideal for studying bronchoconstriction and airway inflammation. Historically, guinea pigs have been preferred for asthma studies because of their strong natural airway hyperresponsiveness and sensitivity to various allergens, allowing for effective therapeutic intervention testing (Woodrow *et al*.,2023).

Mice Models: Mice are the most used species in asthma search due to their genetic manipulability and ability to induce allergic responses through sensitization with allergens like ovalbumin. The BALB/c strain in mice is particularly favoured because of its immune system characteristics and ease of genetic modification (Woodrow *et al*.,2023)

Rat Models: Asthma symptoms, such as airway hyperresponsiveness and inflammation, are generally easier to replicate in rats than in mice. Rats are also more significant and more manageable allowing for the collection of larger volumes and making rat models of asthma more valuable (Camps-Bossacoma *et al*., 2015). However, strains like Wistar, Sprague-Dawley, Fisher, and Lewis rats do not continually develop an allergic response to IgE production. Despite this, asthma models have been successfully established in Wistar and Sprague-Dawley rats (Kucharewicz *et al*.,2008).

## ANIMAL MODEL OF EXPERIMENT (FEMALE SPRAGUE- DWALEY RAT)

Animal models are essential in asthma research for investigating disease mechanisms and evaluating therapeutic interventions. The most used models involve rodents, particularly mice and rats, because of their genetic similarity to humans, ease of handling, and well-characterized immune systems. Among these, the ovalbumin (OVA)-induced asthma model in rats is widely accepted for mimicking human allergic asthma. In this model, sensitization is achieved by administering ovalbumin (a protein found in egg white) with an adjuvant, typically aluminium hydroxide, followed by airway challenges with aerosolized OVA. This results in eosinophilic inflammation, elevated IgE levels, airway hyperresponsiveness, and remodelling closely resembling human asthma pathology (Kumar *et al*.,2012). The Sprague-Dawley rat, a commonly used strain, provides a consistent and reproducible response in asthma induction, making it suitable for studying drug effects on lung inflammation and immune markers (Mitzner,2007).

Characteristics of the Sprague-Dawley Rat.

1. Allergic Response: OVA sensitization and challenge in rats replicates the inflammatory process in the airways that occurs in allergic asthma.
2. Cellular Infiltration: The model exhibits feature like cellular infiltration of the lungs (especially eosinophils), antigen-specific IgE production, and a Th2-dominant immune response, all of which are hallmarks of human asthma
3. Airway Hyperresponsiveness: The model shows airway hyperresponsiveness (AHR), a key characteristic of asthma.

### Why Female Sprague Dawley rats?

Sprague Dawley rats are one of the most widely used laboratory rodent strains in biomedical research. Their genetic uniformity, predictable growth patterns, and docile nature make them ideal for experimental reproducibility and handling. Using a standardized strain helps reduce variability in experimental outcomes especially important when assessing sensitive biomarkers like NO, MPO, and IgE (Vanderschuren *et al.*,2012). High Sensitivity to Allergen-Induced Inflammation. Sprague-Dawley rats show a robust immunological response when exposed to sensitizing agents like ovalbumin (OVA). This includes Increased airway inflammation, elevated IgE production, enhanced eosinophilic infiltration, marked upregulation of cytokines (IL-4, IL-5, IL-13) (Temelkovski *et al.*,1998), lung size and sampling suitability. Sprague-Dawley rats have relatively large lungs, which is advantageous for: Tissue sampling for biomarker assays (e.g., NO, MPO, IgE), histological analysis, broncho-associated lymphoid tissue fluid (BALT) collection.

# CHAPTER 3

## METHODOLOGY

Materials used for this study include Feed, Clean water, Plastic cages, Aluminium hydroxide, Ovalbumin (OVA), Compressor nebulizer, Oral gavage, Syringes, Gloves, Chloroform, Dissection materials, EDTA Bottles, Formaldehyde, 10mg Montelukast, 3mg Prednisolone, 1mg Salbutamol, Saline solution,

### Experimental Animal**.**

This study used forty female Sprague-Dawley rats. The animals received proper care in line with international guidelines for experimental animal handling. Ethical approval was obtained from the College of Medical Sciences Ethics Board (CMS/REC/2024/570) University of Benin. The Sprague-Dawley rats were housed in a clean, calm and sterile environment at room temperature. They were in cages that had access to food, water and *ad libitum* throughout the experimental process.

### Study Design

This study used forty adult female Sprague-Dawley rats, weighing 180g-250g. All experimental procedures were approved by the Ethics Committee of the College of Medical

Sciences, University of Benin (Approval I.D: CMS/REC/2024/570) and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The animals were housed in the animal house at room temperature (22 ± 2ºC), a 12-hour light/dark cycle, and had *ad libitum* access to standard rat chow and water.

## EXPERIMENTAL DESIGN AND ASTHMA INDUCTION

The experiment was carried out in phases,

### Phase 1

Rats were acclimatised to their new environment for two (2) weeks, after which they were divided into five groups of eight (8) rats each.

**Test Groups**

**GROUP 1**: Negative Control (saline-sensitized/challenged, untreated).

**GROUP 2**: Positive control (Asthma induced and not treated).

**GROUP 3:** Asthma induced and treated with salbutamol (2 mg/kg, orally).

**GROUP 4:** Asthma induced and treated with montelukast (10 mg/kg, orally).

**GROUP 5:** Asthma induced and treated with prednisolone (3 mg/kg, orally).

Asthma was induced in Groups 2–5 using a modified ovalbumin (OVA) protocol. Rats were sensitized via intraperitoneal (i.p.) injections of 1 mg OVA (Grade II, Sigma-Aldrich) emulsified in 20 mg aluminium hydroxide (in 0.9% saline) on days 1 and 7. From day 14, sensitized rats were challenged by exposure to aerosolized 1% OVA solution for 15 minutes per session, twice weekly for 28 days. Control animals (Group 1) received saline injections and were challenged with aerosolized saline.

Treatment lasted the period of challenge.

### Phase 2

After confirmation of asthma in all test groups, treatment began with 1mg/kg of salbutamol (oral) (Nair and Prabhavakar, 2021), 3mg/kg prednisolone (oral) (Pourmehdi *et al.,*2020), 10mg/kg montekukast (oral).

### Phase 3

### Sample Collection

After the treatment period, rats were anesthetized with chloroform and then sacrificed. Thereafter lung tissues were harvested for histological evaluation.

Phase 4

Formalin-fixed lung tissues were processed using routine paraffin-embedding techniques. Sections (5µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope at 400x and 100x magnification.

Histological features assessed fibrosis, airway changes, the bronchus, alveolar sac integrity and inflammatory infiltrates.

Representative photomicrographs were captured using a digital imaging system.

# CHAPTER 4

## RESULTS

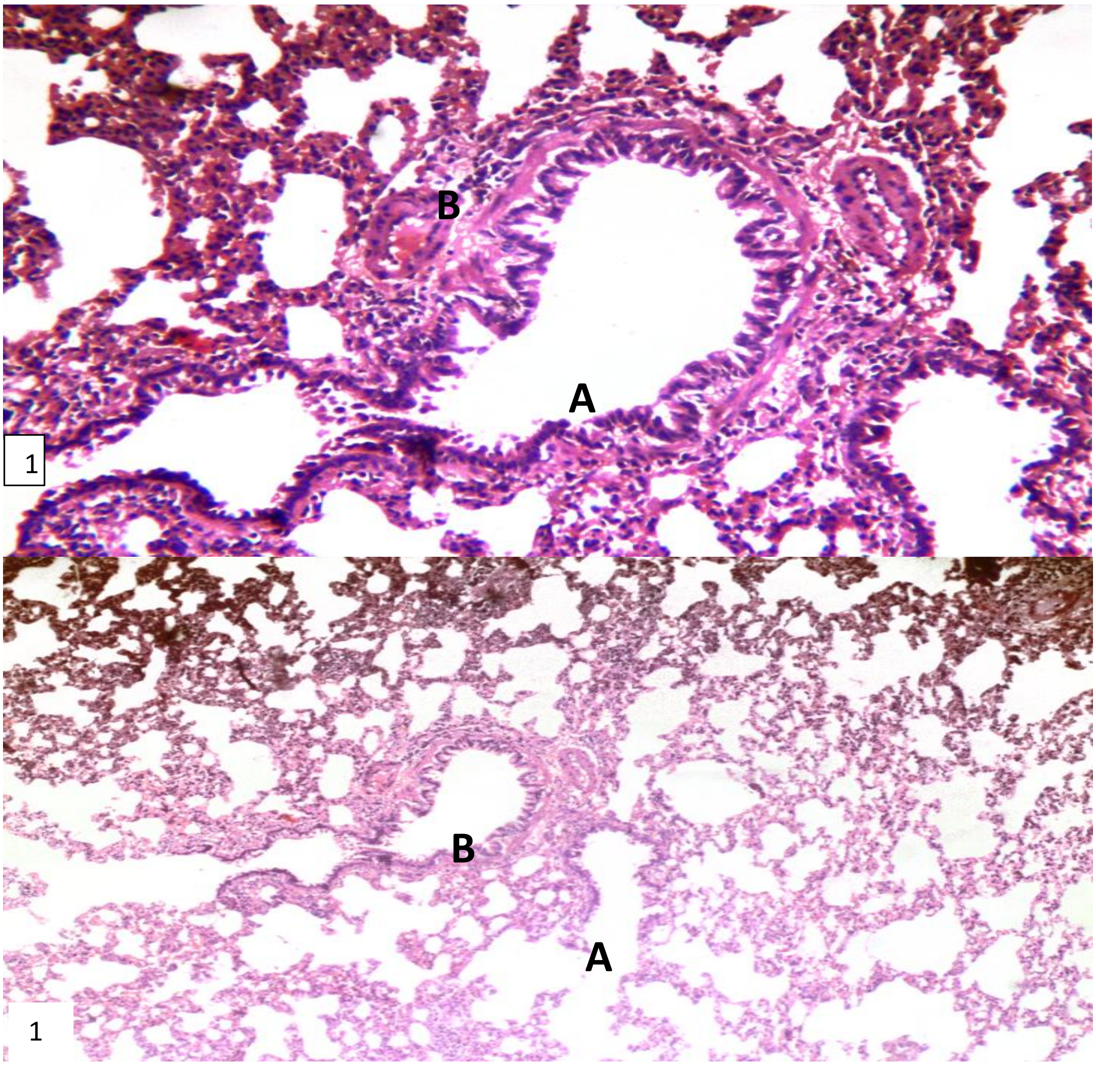


FIG 4: Photomicrographs of lung tissues of the experimental groups 1 = negative control, showing normal lung histology. B = bronchus; A = alveolar sac.

The result shows the alveoli (A) are patent and well-inflated, indicating normal gas exchange structures. The bronchus (B) has a thin, intact epithelial lining and a clear lumen without constriction. The absence of significant inflammatory cell aggregates confirms that the saline sensitization and challenge did not induce a pathological state. This group serves as the baseline for a healthy lung in this experimental model.

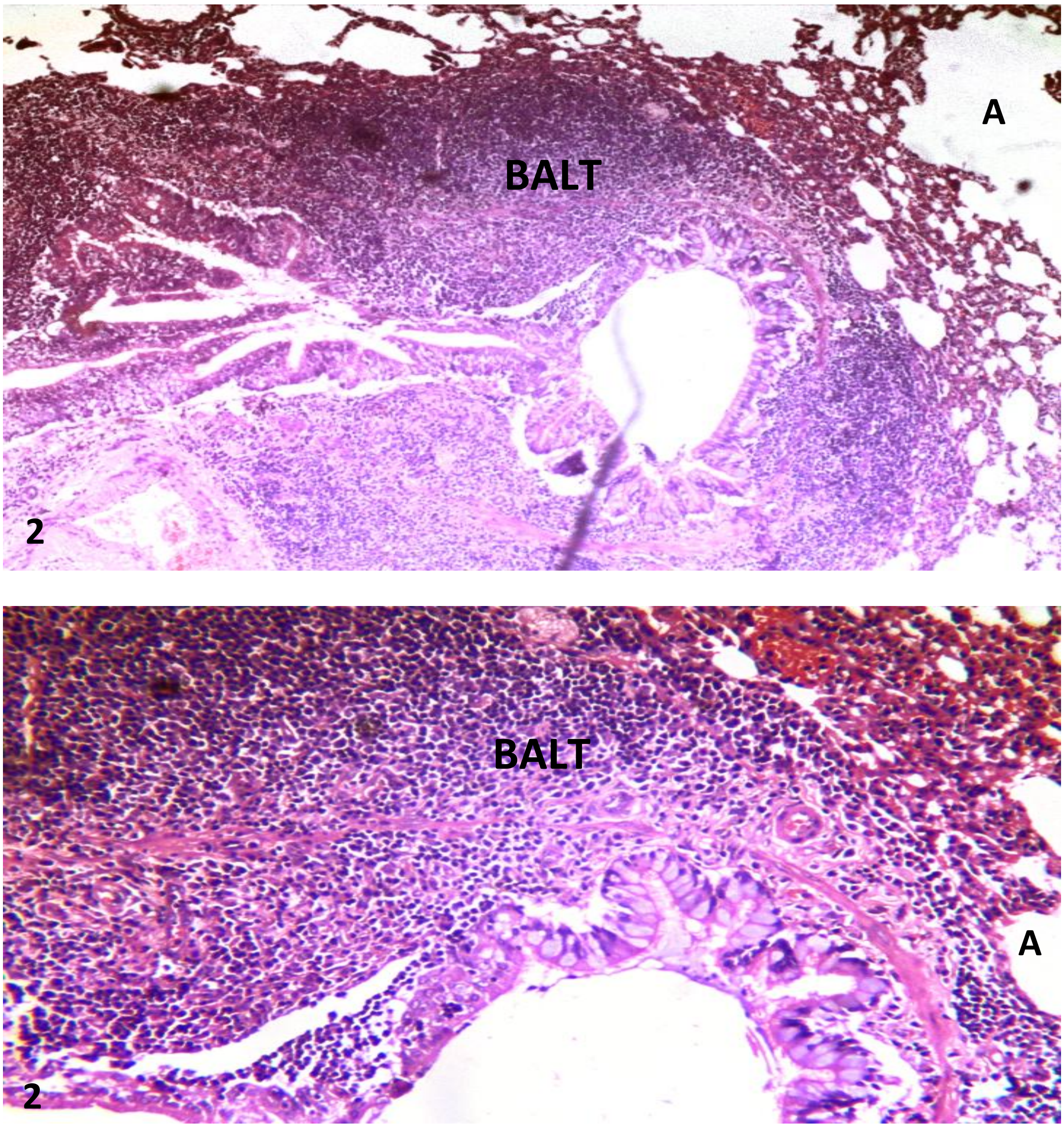


FIG 5: Photomicrographs of the lungs of the experimental groups: 2 = positive control. In group 2, there is hyperplasia of bronchus-associated lymphoid tissues (follicular bronchiolitis). A = alveolar sac; BALT = follicular bronchiolitis.

Result indicates the most prominent feature is the severe hyperplasia of Bronchus-Associated Lymphoid Tissue (BALT), visible as dense follicular aggregates of lymphocytes (follicular bronchiolitis) around the airway. This shows a chronic immune response to ovalbumin. Additionally, bronchoconstriction (Bc) is evident by the noticeable narrowing of the bronchial lumen. These findings confirm the successful development of an allergic asthma phenotype in this group.

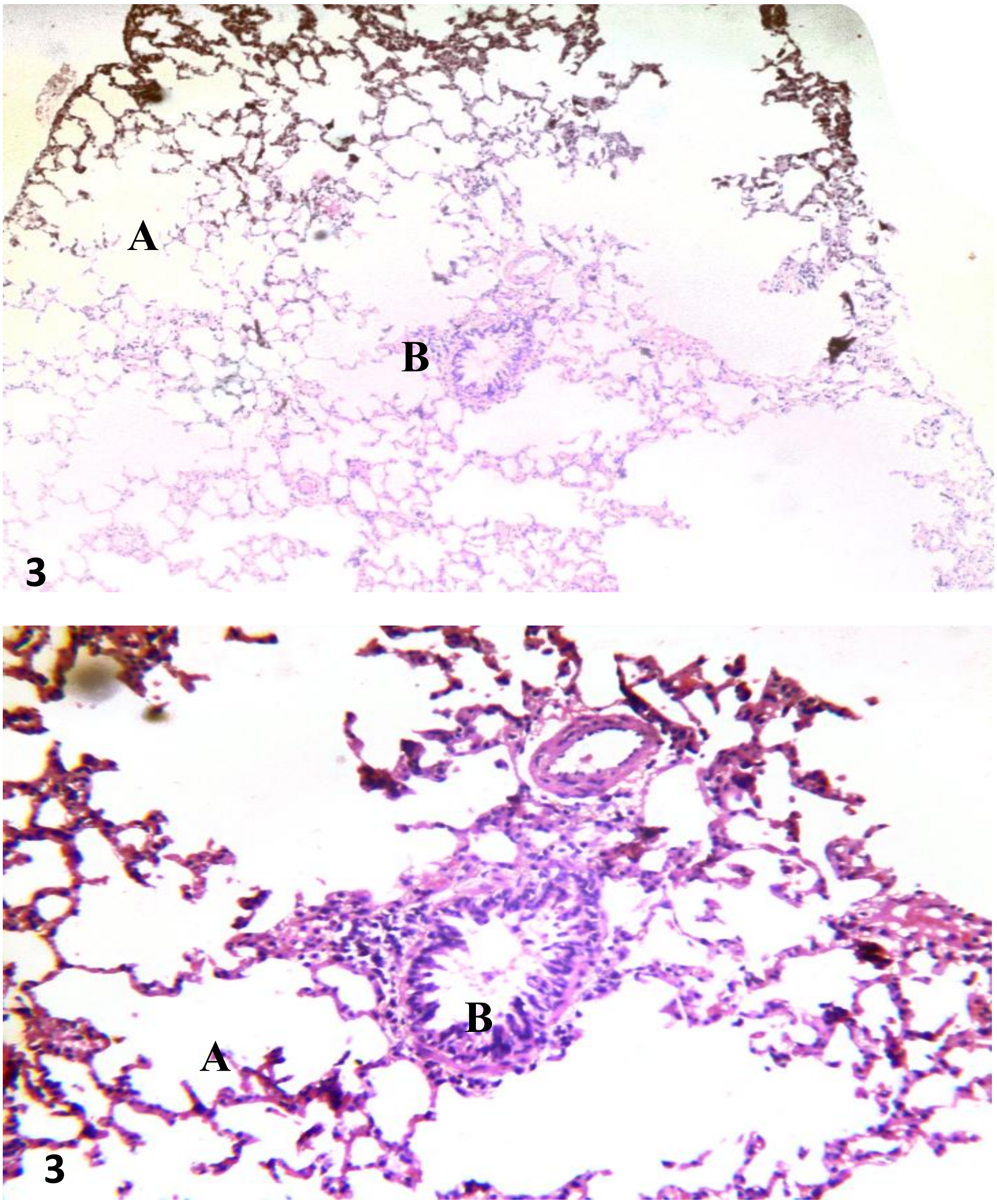
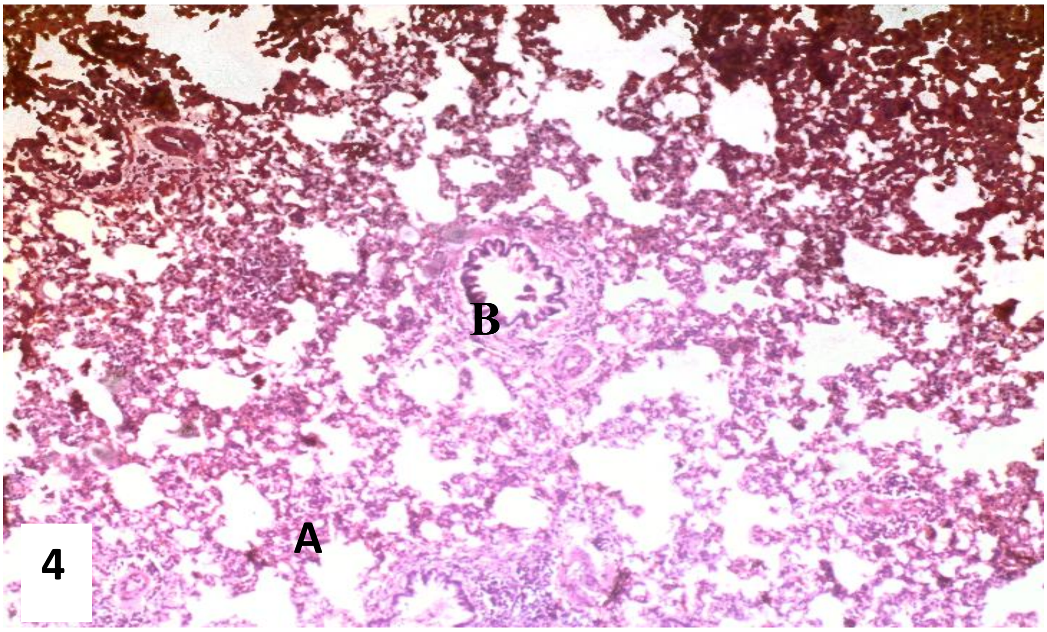


FIG 6: Photomicrographs of lung tissues of the Experimental Groups 3 = Salbutamol group showing normal lung histology. B = bronchus; A = alveolar sac.

The bronchus (B) appears open with no signs of constriction, and the alveolar structures (A) are well-preserved. Critically, the severe BALT hyperplasia observed in the positive control (Group 2) is absent. This shows that Salbutamol treatment provided a significant protective effect, preventing the development of the characteristic inflammatory and bronchoconstrictive responses in this model.



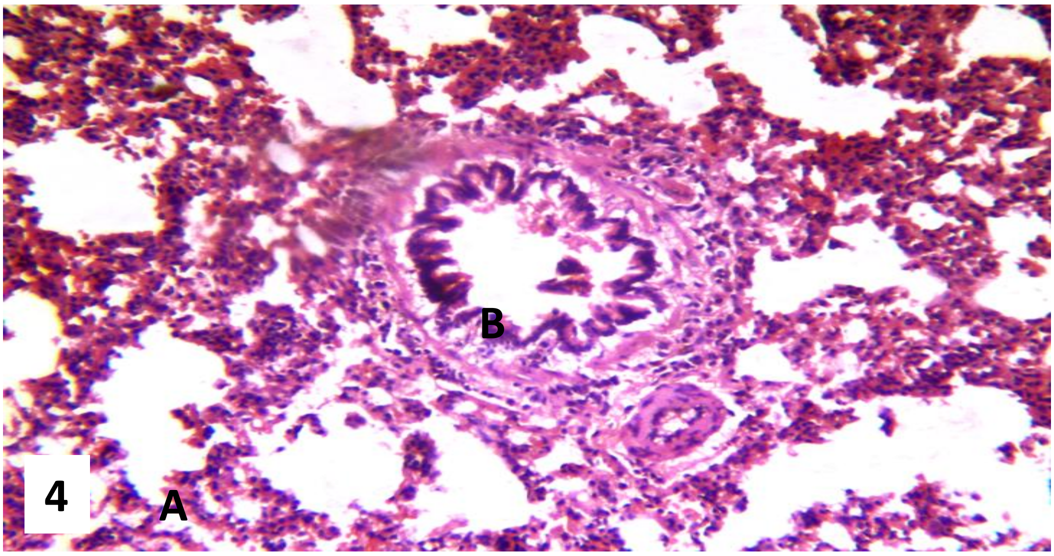


FIG 7: Photomicrographs of lung tissues of the Experimental Group 4 = Montelukast treated group showing normal lung histology. B = bronchus; A = alveolar sac.

While the severe BALT hyperplasia is not present, the bronchus appears somewhat constricted compared to the normal control and Salbutamol-treated groups. This indicates that Montelukast offered a partial protective effect. It was successful in preventing the robust lymphoid infiltration but was less effective in completely averting airway narrowing, suggesting its modulation of the asthma pathology was incomplete in this specific model.

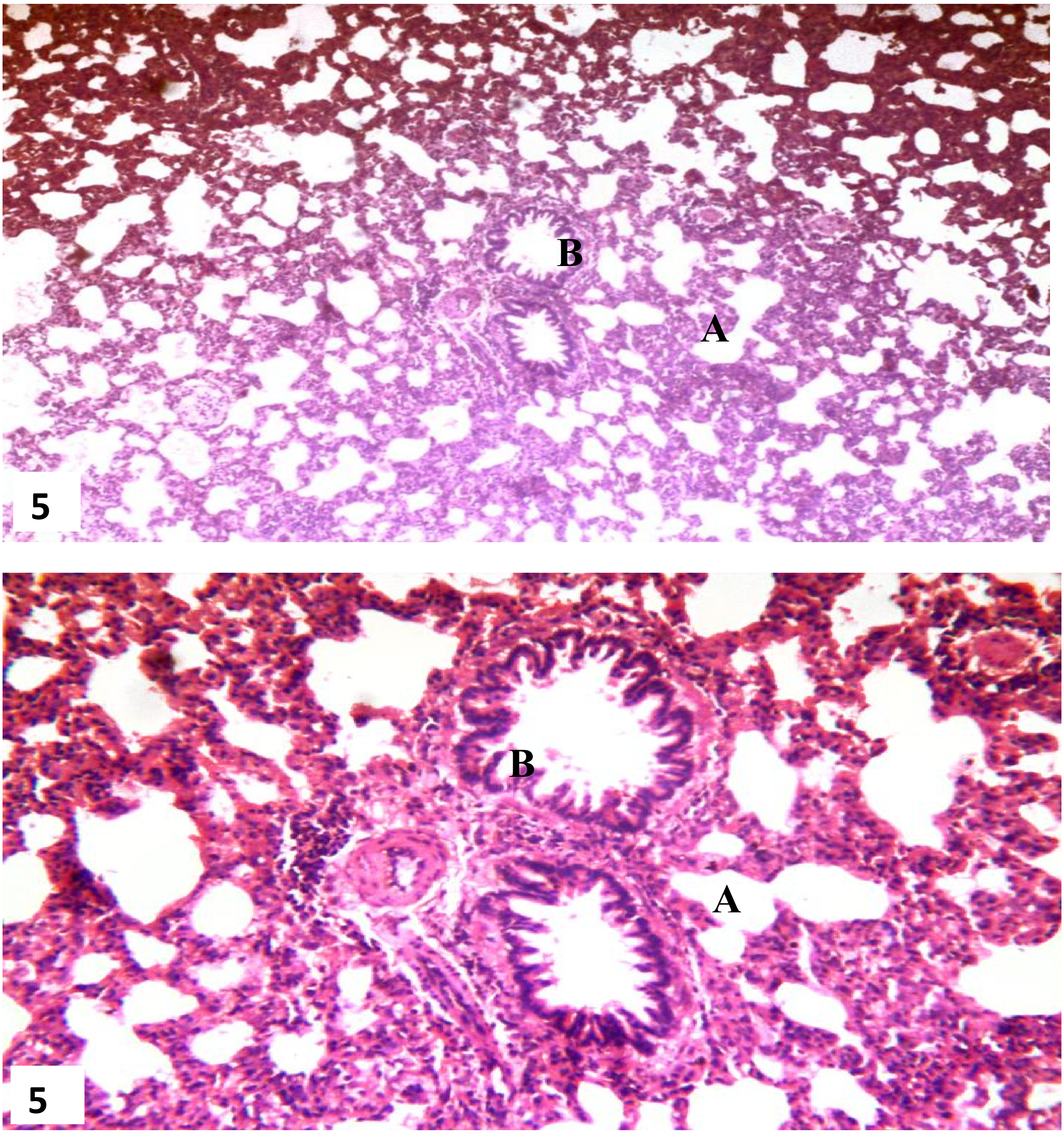


FIG 8: Photomicrographs of lung tissues of the Experimental Group 5 = Prednisolone treated group showing normal lung histology. B = bronchus; A = alveolar sac.

These slides indicate that the bronchus (B) is open and clear, the alveolar sacs (A) are normal, and there is a complete absence of both BALT hyperplasia and bronchoconstriction. This demonstrates that Prednisolone was highly effective, providing protection against the inflammatory and structural changes induced by ovalbumin sensitization and challenge.

# CHAPTER 5

## DISCUSSION

The study shows a comprehensive histological evaluation of the protective effects of three distinct pharmacological drugs; salbutamol, montelukast, and prednisolone against ovalbumin-induced asthma in a female Sprague-Dawley rat model. The findings offer visual evidence that correlates the known mechanisms of action of these drugs with their ability to preserve lung architecture at the tissue level. The differential outcomes observed across the treatment groups underscore the varying efficacy of these drugs in modulating the pathophysiological hallmarks of allergic asthma, namely inflammation and bronchoconstriction. This discussion interprets the observed changes in Bronchus-Associated Lymphoid Tissue (BALT) hyperplasia, inflammatory cell infiltration, and bronchial lumen patency against the established pathophysiological mechanisms of asthma and the known pharmacology of the tested agents.

The successful induction of the asthmatic phenotype in the positive control group (Group 2) is demonstrated by the hyperplasia of Bronchus-Associated Lymphoid Tissue (BALT) and evident bronchoconstriction. The development of follicular bronchiolitis represents a robust T-lymphocyte-driven immune response to repeated ovalbumin challenge, confirming the establishment of a chronic inflammatory state characteristic of allergic asthma (Lambrecht and Hammad, 2015). This pathological presentation established a valid baseline against which the therapeutic efficacy of the test drugs could be accurately assessed.

The most striking finding of this investigation was the profound protective efficacy demonstrated by prednisolone (Group 5). The complete absence of both BALT hyperplasia and bronchoconstriction, resulting in lung histology in little to no difference from the healthy control, it provides clear morphological validation of its potent, broad-spectrum anti-inflammatory action. This effect can be attributed to the genomic mechanisms of corticosteroids, which involve the suppression of multiple pro-inflammatory cytokines, inhibition of inflammatory cell recruitment, and induction of eosinophil apoptosis (Barnes, 2017). The comprehensive histological protection afforded by prednisolone reaffirms its status as the gold-standard anti-inflammatory therapy for asthma exacerbations and severe disease.

A particularly noteworthy observation was the significant protective effect exhibited by salbutamol (Group 3), which prevented the development of both inflammatory infiltration and airway narrowing. This finding is pharmacologically interesting, as salbutamol is primarily classified as a short-acting bronchodilator with minimal recognized anti-inflammatory properties (Johnson, 2002). The complete prevention of BALT hyperplasia suggests a potential protective mechanism beyond simple bronchodilation. This may be related to the timing of administration in the experimental protocol or mode of administration; if administered prior to ovalbumin challenges, salbutamol may have prevented bronchoconstriction-induced mechanical stress and subsequent inflammatory signaling. Alternatively, β2-adrenergic receptor activation on immune cells may have exerted modulatory effects that promoted the inflammatory cascade in this specific model.

Montelukast (Group 4) demonstrated an intermediate protective profile, characterized by the prevention of severe lymphoid infiltration but persistent bronchoconstriction. This partial efficacy aligns with its specific mechanism as a leukotriene receptor antagonist (Singh *et al.,* 2013). The reduction in BALT formation indicates successful suppression of the leukotriene-mediated component of the inflammatory response, particularly the chemotaxis of lymphocytes and eosinophils. However, the persistence of airway narrowing suggests that other bronchoconstrictive pathways, such as those mediated by histamine or acetylcholine, remained active. This histological evidence visually reinforces the concept that montelukast provides targeted, but incomplete, control of asthma pathophysiology compared to broader anti-inflammatory agents.

The differential histological outcomes observed in this study carry important implications for asthma management. The superior tissue protection offered by prednisolone underscores the critical importance of early and potent anti-inflammatory intervention in modifying asthma pathology. The unexpected protective effect of salbutamol, while remarkable in this model, should be interpreted with caution, as it does not align with clinical evidence demonstrating that SABA monotherapy fails to control underlying inflammation and may worsen outcomes (GINA, 2023). The partial protection by montelukast supports its role as a controller medication, particularly in specific asthma phenotypes where leukotriene pathways are predominant.

# CONCLUSION

In conclusion, this histological analysis demonstrates a clear spectrum of protective efficacy against ovalbumin-induced asthma pathology among the three investigated drugs. Prednisolone provided complete histological protection, montelukast offered partial protection with persistent bronchoconstriction, and salbutamol unexpectedly demonstrated significant protective effects in this model. These findings visually connect the distinct pharmacological mechanisms of these agents and contribute to our understanding of their differential effects on asthma pathology at the tissue level. The results reinforce the central role of anti-inflammatory therapy in asthma management.

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