EFFECTS OF SALBUTAMOL, MONTELUKAST AND PREDNISOLONE AND THEIR COMBINATION ON LUNG TISSUE HISTOLOGY IN OVALBUMIN INDUCED ASTHMA IN FEMALE SPRAGUE DAWLEY RATS

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A PROJECT WORK WRITTEN AND SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work on "EFFECTS OF SALBUTAMOL, MONTELUKAST AND PREDNISOLONE AND THEIR COMBINATION ON LUNG TISSUE HISTOLOGY IN OVALBUMIN INDUCED ASTHMA IN FEMALE SPRAGUE DAWLEY RATS"was carried out by
OMOLEGELE OSEMEGA MIRACLE, with the matriculation number BMS2101661, in partial fulfillment for the award of a Bachelor of Science Degree (B.Sc) in the department of physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State,Nigeria.

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(EXTERNAL EXAMINER) **DATE**

DEDICATION

This work is dedicated to Almighty God, whose endless grace and guidance have sustained me throughout this journey.Also to my parents MR and Mrs Mathew Omolegele thank you for your continuous support

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor **DR. F.O EBOJELE** my family and friends for their support and encouragement throughout this project.

I wholeheartedly dedicate this
Project to my loving parents, Mr. and Mrs. Martins Omolegele, for their unwavering support, sacrifices, and encouragement that have shaped my path.

To my amazing siblings Excellent, Immaculate, and Winner thank you for your love, patience, and constant motivation.To my dedicated lecturers and mentors, thank you for your knowledge and inspiration.

To my dear friends who stood by me, your support means so much.
And most importantly, I dedicate this work to myself, Omolegele Miracle, for the resilience, commitment, and hard work I put into seeing this through. This is a reminder that I am capable of achieving anything I set my mind to.

TABLE OF CONTENT

ABSTRACT

Asthma is a chronic inflammatory airway disorder marked by bronchoconstriction, mucus hypersecretion, and infiltration of inflammatory cells. While current therapies such as salbutamol, montelukast, and prednisolone are effective in alleviating symptoms, their combined effects on lung tissue integrity remain unclear. This study evaluated the effects of salbutamol, montelukast, prednisolone, and their combinations

on lung histology in ovalbumin (OVA)-induced asthmatic female Sprague-Dawley rats. Fifty-six adult female Sprague-Dawley rats were divided into five groups (n = 8): negative control, positive control (OVA-induced, untreated), salbutamol + prednisolone, salbutamol + montelukast, and prednisolone + montelukast. Asthma was induced through OVA sensitisationand aerosol challenge for four weeks. After confirming the development of asthma, treatment was administered orally for four weeks. Lung tissues were excised, fixed in 10% formalin, processed, and stained with hematoxylin and eosin (H&E) for histopathological evaluation under light microscopy. The negative control group showed normal lung architecture with intact bronchi and alveolar sacs. The OVA-induced positive control exhibited hyperplasia of bronchus-associated lymphoid tissue (BALT) and features of follicular bronchiolitis. The salbutamol/prednisolone and prednisolone/montelukast groups showed preserved lung structure with minimal inflammation and normal bronchi and alveolar spaces. In contrast, the salbutamol/montelukast group displayed marked bronchoconstriction and severe BALT hyperplasia, exceeding that seen in the untreated asthmatic group.

Conclusion:

Combination therapy with prednisolone and either salbutamol or montelukast effectively preserved lung histoarchitecture in OVA-induced asthmatic rats, indicating potent anti-inflammatory protection. However, the salbutamol/montelukast combination failed to reverse asthmatic histopathology, suggesting reduced therapeutic synergy or potential antagonistic interaction. These findings underscore the importance of drug selection in optimising asthma management and preventing structural lung damage.

INTRODUCTION

1.1 BACKGROUND OF STUDY

Asthma is a chronic inflammatory disorder of the airways characterized by reversible airway obstruction, bronchial hyperresponsiveness, and airway remodeling. It is a common respiratory condition affecting both children and adults, with increasing prevalence worldwide (Hall, 2021). Asthma results from a complex interplay of genetic predisposition and environmental triggers that stimulate abnormal immune responses in the airways. Physiologically, asthma involves inflammation of the airway epithelium, constriction of bronchial smooth muscle, increased mucus secretion, and infiltration by inflammatory cells, especially eosinophils, mast cells, and Th2 lymphocytes (Guyton and Hall, 2021). These changes lead to airway narrowing, manifesting clinically as wheezing, coughing, shortness of breath, and chest tightness, particularly during the night or early morning.

Asthmatic airway histology often reveals goblet cell hyperplasia, thickened basement membrane, smooth muscle hypertrophy, and inflammatory cell infiltration, features that contribute to the process known as airway remodeling (Barrett *et al.*, 2019).

Effective asthma management involves pharmacologic strategies aimed at reducing inflammation and relieving bronchoconstriction. These include short-acting β2-agonists like salbutamol, glucocorticoids like prednisolone, and leukotriene receptor antagonists like montelukast, each targeting different aspects of the disease mechanism (West *et al*., 2020).

The pathophysiology of asthma is multifactorial, involving immune-mediated inflammation predominantly driven by T-helper 2 (Th2) lymphocytes, eosinophils, mast cells, and the release of cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13). These immune responses lead to goblet cell hyperplasia, excess mucus secretion, airway smooth muscle constriction, and epithelial damage (Kumar et al., 2022). Over time, repeated inflammatory insults may result in airway remodeling, a structural alteration of the airway that contributes to persistent symptoms and reduced response to therapy.

In asthma management, several drug classes are used to control symptoms and reduce inflammation. Salbutamol, a short-acting β2-adrenergic agonist, acts as a bronchodilator; Montelukast is a leukotriene receptor antagonist that helps reduce bronchoconstriction and inflammation; and Prednisolone, a glucocorticoid, suppresses immune-mediated airway inflammation. While these drugs are widely used in clinical settings either singly or in combination, their individual and combined effects on lung histopathology in animal models remain an area of active research.

This project aims to investigate the effects of salbutamol, montelukast, prednisolone, and their combinations on lung tissue histology in ovalbumin-induced asthma in female Sprague-Dawley rats. Through histological analysis, the study seeks to provide insight into how these treatments influence inflammatory cell infiltration, mucus production, epithelial damage, and structural changes in the lungs which are crucial features of asthma pathology.

Asthma continues to be a growing public health challenge worldwide, with increasing morbidity despite the availability of various medications. Understanding its underlying pathophysiology and how different drugs affect airway remodeling is essential in developing improved therapeutic

strategies. Animal models provide a valuable platform to study the cellular and structural changes in asthma and evaluate treatment effects under controlled conditions. Ovalbumin-induced asthma models mimic human asthma features, such as airway inflammation, hyperresponsiveness, and remodeling. Evaluating the histological outcomes of standard antiasthmatic medications such as salbutamol, montelukast, and prednisolone in this model can contribute to a better understanding of their roles, limitations, and potential synergistic effects on lung tissue repair and inflammation control.Animal models, particularly ovalbumin (OVA)-induced asthma in rats, have been widely used in experimental physiology and pharmacology to investigate the pathophysiological changes in asthma and assess the efficacy of pharmacological interventions. The female Sprague-Dawley rat is commonly utilized due to its responsiveness to allergen sensitization and consistent histopathological features that mimic human asthma (Barrett *et al.*, 2019).

1.2 Justification of the Study

Despite the availability of asthma medications, many patients continue to experience poor control and progressive airway remodeling. By investigating the histological outcomes of commonly used asthma drugs both alone and in combination in an animal model, this study may help uncover their relative effectiveness on lung tissue recovery. This will provide foundational data that could be relevant for advancing drug formulation, improving treatment protocols, and reducing long-term complications in asthma patients. Additionally, using female Sprague-Dawley rats allows exploration of potential gender-related physiological responses in asthma treatment, which is often under-represented in preclinical research.

1.3 Aim of the Study

To investigate the histological effects of salbutamol, montelukast, prednisolone, and their combinations on lung tissue in ovalbumin-induced asthma using female Sprague-Dawley rats.

1.4 Research Questions

1. What are the individual histological effects of salbutamol, montelukast, and prednisolone on lung tissue in ovalbumin-induced asthmatic rats?
2. What are the combined histological effects of salbutamol, montelukast, and prednisolone in treating ovalbumin-induced asthma?
3. How does each treatment affect key histopathological markers such as inflammatory cell infiltration, epithelial damage, mucus hypersecretion, and airway remodeling?

1.5 Specific Objectives

1. To determine the individual histological effects of salbutamol, montelukast, and prednisolone on lung tissue in ovalbumin-induced asthmatic rats.
2. To evaluate the combined histological effects of salbutamol, montelukast, and prednisolone in the treatment of ovalbumin-induced asthma.
3. To assess the impact of each treatment on histopathological markers, including inflammatory cell infiltration, epithelial damage, mucus hypersecretion, and airway remodeling.

CHAPTER 2

ASTHMA

Asthma is a chronic inflammatory disorder of the airways characterized by reversible airway obstruction, bronchial hyperresponsiveness, and airway remodeling. It is a common respiratory condition affecting both children and adults, with increasing prevalence worldwide (Hall, 2021). Asthma results from a complex interplay of genetic predisposition and environmental triggers that stimulate abnormal immune responses in the airways. Physiologically, asthma involves inflammation of the airway epithelium, constriction of bronchial smooth muscle, increased mucus secretion, and infiltration by inflammatory cells, especially eosinophils, mast cells, and Th2 lymphocytes (Guyton and Hall, 2021).

Epidemiology of Asthma

Asthma is one of the most common chronic respiratory diseases globally, affecting individuals across all age groups. Its global burden is estimated at over 334 million people, with approximately 345,000 deaths annually (approximately one in every 150 deaths). Cost of which occur in low and middle-income countries (GINA, 2024; WHO, 2023).

According to the Global Burden of Disease (GBD) Study, asthma affects over 300 million people, and this number may rise to 400 million by 2025 if current trends persist (GBD 2019 Risk Factors Collaborators, 2020). High-income countries tend to have higher prevalence rates, while mortality is often worse in low- and middle-income countries, largely due to underdiagnosis and limited treatment access (GINA, 2024; WHO, 2023). Prevalence varies from 1% to 18% between countries, often higher in developed nations due to environmental and lifestyle factors. Most deaths (over 80%) occur in low and middle-income countries, where underdiagnosis and lack of access to treatment remain major public health concerns. Asthma can develop at any age. Childhood asthma is more common in boys, while adult asthma is more prevalent in women, likely due to hormonal and anatomical differences (Asher et al., 2021).

Asthma is a global health concern, but the burden varies significantly across countries due to differences in healthcare systems, environmental exposures, diagnostic practices, socioeconomic status, and genetic predisposition. Asthma prevalence varies widely: United Kingdom, Australia, and New Zealand report rates over 15%, while Asian and African countries report <5% though underreporting in resource-limited settings likely contributes to this disparity (To *et al.*, 2012). Urban environments are associated with higher prevalence due to air pollution, indoor allergens, sedentary lifestyles, and dietary changes (von Mutius, 2007).

A closer look at the United Kingdom, Russia, and Nigeria reveals these disparities in asthma prevalence, control, and outcomes.

Asthma in the United Kingdom (UK)

The United Kingdom has one of the highest asthma prevalence rates globally. According to the British Lung Foundation, approximately 8 million people in the UK have been diagnosed with asthma, representing about 12% of the population (British Lung Foundation, 2022). The disease is more prevalent in women and children, and the UK also reports high asthma mortality compared to other high-income countries.

Environmental factors such as air pollution, especially in urban centers like London, indoor allergen exposure, and lifestyle habits such as smoking, contribute to the high burden. The National Health Service (NHS) spends over £1 billion annually managing asthma, yet poor medication adherence and inconsistent follow-up remain barriers to achieving optimal control (Shah *et al.,* 2020).

Alarmingly, data from Asthma + Lung UK show that three people die from asthma every day in the UK, and up to two-thirds of these deaths are preventable with better care and self-management education (Asthma + Lung UK, 2023). These statistics highlight the ongoing need for improved asthma education, personalized care plans, and environmental health reforms.

Asthma in Russia

Russia has a lower reported prevalence of asthma compared to Western Europe, estimated at around 6–7% of the adult population (Chuchalin *et al.,* 2016). However, this figure is likely underestimated due to underdiagnosis and the lack of standardized national surveillance programs. In Russia, environmental exposures such as industrial air pollution, tobacco use, and cold climate-induced respiratory infections exacerbate asthma symptoms, especially during winter months. Access to modern asthma medications like inhaled corticosteroids can be limited in some regions, and patient education remains insufficient, particularly in rural areas (Chuchalin *et al.,* 2016).

Moreover, asthma mortality in Russia is higher than in many European countries, largely due to inadequate primary care infrastructure and late presentation of cases. Despite efforts to integrate asthma care into national respiratory disease programs, gaps persist in diagnosis, access to care, and long-term disease control.

Asthma in Nigeria

In contrast to the UK and Russia, asthma in Nigeria represents a growing yet under-recognized public health concern. The prevalence of asthma in Nigeria is estimated to be 7–10%, depending on the region and study population (Desalu *et al.,* 2012). Urban centers such as Lagos, Abuja,

and Port Harcourt report higher rates compared to rural areas, attributed to air pollution, vehicular emissions, poor indoor air quality, and exposure to biomass fuels (Adeloye et al., 2013). Children and young adults are particularly affected, and the lack of diagnostic tools, including spirometry, leads to frequent underdiagnosis and mismanagement. Many cases are mistaken for chronic bronchitis or pneumonia, and patients often present in emergency settings with acute exacerbations (Desalu *et al*., 2012).

A 2017–2018 national survey found:

- Physician-diagnosed asthma: 2.5%
- Clinical asthma: 6.4%
- Wheezing in the past year: 9.0%

This suggests nearly 13 million Nigerians may be affected (Adeloye *et al.,* 2013). Regional studies show:

- Ilorin adults: probable asthma at 15.2% (Desalu *et al.,* 2012)
- Niger Delta children: wheeze prevalence 5.4%, with rural children reporting higher rates than urban peers
- Kwara State: urban asthma prevalence ~3.3% vs rural ~1.5%; urban dwellers had 5.6x higher odds of asthma symptoms.

In Nigeria, healthcare access is unequal, and many patients cannot afford inhaled medications. The cost of controller medications, poor awareness of disease triggers, and limited asthma education all contribute to poor disease control and frequent hospitalizations.

Classification of Asthma

The classification of asthma is critical in understanding the pathophysiology, guiding pharmacological treatment, and evaluating prognosis. Asthma is not the same in every person. It is commonly classified based on severity, control, and phenotype.

Classification by Severity

Asthma severity is defined as the intrinsic intensity of the disease process, assessed before the initiation of long-term controller therapy (GINA, 2024). The Global Initiative for Asthma outlines four major categories:

- *Intermittent Asthma: Characterized by symptoms occurring ≤2 days per week, ≤2 nighttime awakenings per month, and normal pulmonary function (FEV_1 ≥80%) between episodes. There is no interference with normal activity (GINA, 2024).*

- *Mild Persistent Asthma: Symptoms are present >2 days/week (but not daily), with 3–4 nighttime symptoms/month, FEV_1 ≥80% predicted, and minor activity limitation (GINA, 2024).*

- Moderate Persistent Asthma: Involves daily symptoms, nighttime awakenings more than once a week, FEV_1 between 60–80% of predicted value, and some limitation of physical activity (GINA, 2024).

- Severe Persistent Asthma: Symptoms occur throughout the day, frequent nighttime awakenings (7 times/week), and significant airflow limitation with FEV_1 <60%, resulting in extreme interference with normal activity (GINA, 2024).

Classification by Level of Control

Asthma control is assessed by the extent to which the manifestations of asthma are minimized by treatment and the goals of therapy are met (GINA, 2024). Asthma may be:

- Well-Controlled: Defined by symptoms ≤2 days/week, nighttime symptoms ≤2/month, no activity limitations, and minimal need for rescue bronchodilators (GINA, 2024).
- Partly Controlled: Includes more frequent daytime symptoms (>2/week), some activity limitation, and occasional nighttime symptoms (GINA, 2024).
- Uncontrolled: Characterized by frequent symptoms, regular use of rescue medication, limited activity, and reduced lung function despite treatment (GINA, 2024).

Classification by Phenotype

The heterogeneity of asthma has led to the recognition of distinct phenotypes, which describe observable characteristics linked to underlying pathophysiological mechanisms (Wenzel, 2012). Common phenotypes include:

- Allergic (Atopic) Asthma: Often begins in childhood and is associated with a history of atopy, elevated serum IgE, and eosinophilic airway inflammation (Wenzel, 2012).
- Non-Allergic Asthma: Typically develops in adults without allergen sensitization. Inflammatory profiles may be neutrophilic, eosinophilic, or paucigranulocytic (Wenzel, 2012).
- Late-Onset Asthma: Occurs predominantly in non-atopic adults and tends to be more severe and less responsive to standard corticosteroid therapy (Wenzel, 2012).
- Asthma with Fixed Airflow Limitation: Results from long-standing disease with airway remodeling, leading to partially irreversible obstruction (Wenzel, 2012).
- Obesity-Associated Asthma: Presents with prominent respiratory symptoms and reduced response to inhaled corticosteroids, with less eosinophilic inflammation (Wenzel, 2012).

Types of Asthma

Asthma is a complex and heterogeneous respiratory condition with multiple phenotypes and endotypes, each with distinct triggers, presentations, and treatment responses (GINA, 2024). The classification of asthma into various types allows for more targeted and personalized management strategies, improving disease control and outcomes (Wenzel, 2012; Pavord *et al.,* 2018).

1. Allergic (Extrinsic) Asthma

Allergic asthma is the most common phenotype, particularly in children and young adults. It is associated with a personal or family history of atopy, elevated serum IgE levels, and positive skin prick tests to environmental allergens such as dust mites, pollen, pet dander, and mold (Lötvall et al., 2011). The Global Initiative for Asthma (GINA, 2024) identifies this type as typically responsive to inhaled corticosteroids and allergen avoidance strategies.

2. Non-Allergic (Intrinsic) Asthma

Non-allergic asthma lacks an identifiable allergic trigger and tends to present in adulthood, more commonly among women (Wenzel, 2006). It is often more severe and less responsive to standard therapy. It is thought to involve non-IgE mediated inflammatory pathways, such as neutrophilic inflammation (Simpson *et al*., 2006).

3. Exercise-Induced Bronchoconstriction (EIB)

EIB, formerly termed exercise-induced asthma, occurs when physical activity triggers transient airway narrowing. Symptoms include wheezing, coughing, and dyspnea during or after exercise, particularly in cold, dry environments (Parsons *et al*., 2013). Pre-treatment with short-acting β2-agonists and regular controller therapy can be effective (GINA, 2024).

4. Occupational Asthma

This form of asthma results from exposure to specific agents in the workplace, such as isocyanates, flour, or cleaning chemicals (Tarlo and Lemiere, 2014). It is characterized by symptom improvement on weekends or holidays and may take months or years to develop. Timely identification and avoidance of the triggering occupational exposure are crucial.

5. Cough Variant Asthma

Cough-variant asthma is a subtype in which chronic dry cough is the predominant or only symptom. It is more common in children and often misdiagnosed as chronic bronchitis or post-viral cough (Corrao *et al.,* 2008). Pulmonary function tests or methacholine challenge tests aid in diagnosis.

6. Aspirin-Exacerbated Respiratory Disease (AERD)

Also known as Samter's Triad, AERD is a severe asthma phenotype involving asthma, chronic rhinosinusitis with nasal polyposis, and hypersensitivity to aspirin or other NSAIDs. It is thought to be mediated by overproduction of leukotrienes (Stevenson and Szczeklik, 2006). Treatment includes aspirin desensitization and leukotriene-modifying agents.

7. Nocturnal Asthma

Nocturnal asthma is characterized by worsening of symptoms at night, often disrupting sleep. It may be related to circadian variations in bronchial tone, increased exposure to allergens during sleep, or comorbid conditions like gastroesophageal reflux disease (GE reflux) (Kelly and Sorkness, 2008).

8. Severe Asthma

Severe asthma is defined as asthma that remains uncontrolled despite adherence to optimized high-dose inhaled corticosteroid and long-acting β2-agonist therapy, and after addressing modifiable risk factors and comorbidities (Chung et al., 2014). These patients often require biologic therapies targeting IL-5, IL-4/13, or IgE (Pavord *et al.,* 2018).

9. Pediatric (Childhood-Onset) Asthma

Asthma frequently begins in childhood, with early onset linked to viral respiratory infections and genetic predisposition. Boys are more commonly affected in early childhood, but this reverses in adolescence due to hormonal influences (Asher *et al.*, 2021).

Pathophysiology of Asthma:

Asthma is a chronic inflammatory disorder of the airways characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation (GINA, 2024). The pathophysiology of asthma involves a complex interplay between immune cells, inflammatory mediators, structural cells, and environmental factors that result in both acute and chronic changes in the airway.

1. Airway Inflammation

The hallmark of asthma is chronic inflammation of the airways, which contributes to symptoms such as wheezing, coughing, chest tightness, and shortness of breath (Holgate, 2012). The inflammatory response is primarily mediated by Type 2 helper T (Th2) cells, which release cytokines such as:

- Interleukin-4 (IL-4): Stimulates B cells to produce immunoglobulin E (IgE).
- Interleukin-5 (IL-5): Promotes eosinophil growth, activation, and survival.
- Interleukin-13 (IL-13): Contributes to mucus hypersecretion and airway hyperresponsiveness.

Eosinophilic infiltration is a typical feature in allergic asthma, while neutrophilic inflammation may be more prominent in severe or non-allergic asthma (Simpson *et al.*, 2006).

2. Airway Hyperresponsiveness (AHR)

Airway hyperresponsiveness refers to an exaggerated bronchoconstrictor response to various stimuli such as allergens, exercise, cold air, and pollutants. It is a functional consequence of chronic inflammation and remodeling (Kelly and Busse, 2008). Structural changes in the airway wall, including smooth muscle hypertrophy and epithelial damage, enhance sensitivity to bronchoconstrictors.

3. Bronchoconstriction

Bronchoconstriction is the acute narrowing of the airway lumen due to the contraction of airway smooth muscle. It is triggered by:

- Allergen exposure (via IgE-mediated mast cell degranulation).
- Irritants (e.g., smoke, pollution).
- Physical exertion or cold air.

Mast cell activation leads to the release of histamine, prostaglandins, leukotrienes, and other mediators that cause immediate bronchospasm (Barnes, 2008).

4. Airway Remodeling

Chronic asthma can lead to structural changes in the airway wall, a process termed airway remodeling. These changes contribute to persistent airflow limitation and reduced response to therapy and include:

- Goblet cell hyperplasia → Mucus hypersecretion.
- Subepithelial fibrosis → Thickening of the basement membrane.
- Smooth muscle hypertrophy and hyperplasia.
- Angiogenesis (increased airway vascularity).

Airway remodeling is associated with disease progression and may occur early in the disease course, especially in poorly controlled asthma (Bousquet *et al*., 2000).

5. Mucus Hypersecretion

Increased mucus production and impaired mucociliary clearance contribute to airway obstruction. IL-13 promotes goblet cell metaplasia and excess mucus secretion, which may lead to mucus plugging, especially during severe exacerbations (Fahy and Dickey, 2010).

6. Neural Mechanisms

The autonomic nervous system, particularly cholinergic pathways, also plays a role in asthma pathophysiology. Vagal stimulation increases bronchial smooth muscle tone and mucus secretion, while β2-adrenergic receptors mediate bronchodilation. Dysfunctional β2-receptor signaling can exacerbate bronchoconstriction (Barnes, 2008).

7. Immunoglobulin E (IgE) and Mast Cells

In allergic asthma, IgE binds to mast cells, which degranulate upon allergen exposure. This early-phase reaction results in bronchoconstriction, while the late-phase response characterized by the recruitment of eosinophils and other inflammatory cells sustains the inflammatory process and contributes to long-term damage (Holgate, 2012).

Asthma Medications And Mechanisms of Action

Salbutamol (SABA)

Salbutamol (albuterol in North America) is a widely used short-acting β2-adrenergic receptor agonist (SABA) primarily employed to relieve bronchospasm associated with asthma and chronic obstructive pulmonary disease (COPD). Its rapid onset and targeted mechanism of action have made it a cornerstone in emergency management of airway constriction (Barnes, 1995). It is primarily employed for the rapid relief of bronchospasm due to its fast onset of action and selective mechanism targeting airway smooth muscle [GINA, 2024].

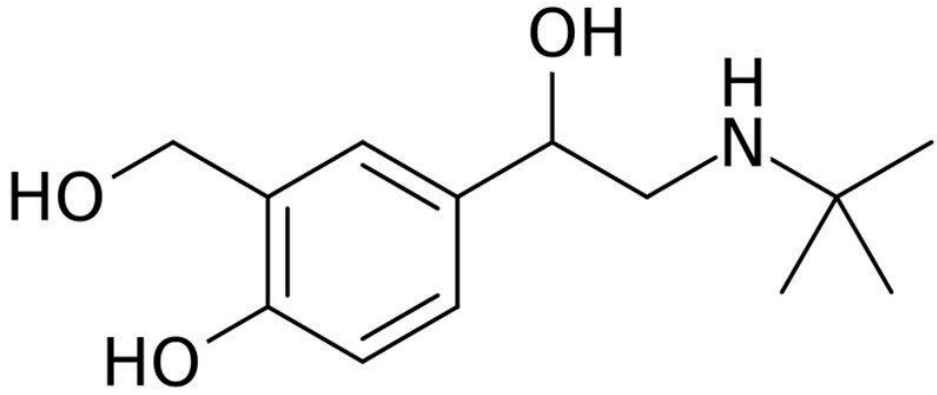


Figure 1: CHEMICAL STRUCTURE OF SALBUTAMOL

SOURCE: ResearchGate

At the cellular level, salbutamol exerts its effects by selectively binding to β2-adrenergic receptors expressed on airway smooth muscle cells. These receptors belong to the G-protein-coupled receptor (GPCR) family and mediate downstream signaling through the stimulation of adenylyl cyclase, resulting in increased intracellular cyclic adenosine monophosphate (cAMP) levels (Insel, 2004; Johnson, 2006). Elevated cAMP activates protein kinase A (PKA), which phosphorylates targets that decrease intracellular calcium concentrations, culminating in smooth muscle relaxation and bronchodilation (Daaka, 1997; Roffel et al., 1990). This cascade typically produces bronchodilation within 5 to 10 minutes post-inhalation, making salbutamol highly effective as a rescue therapy during acute exacerbations (Cazzola et al., 2000). The primary therapeutic benefit of salbutamol arises from its bronchodilator action, which reduces airway resistance and improves expiratory airflow. Improvements in forced expiratory volume in one second (FEV_1) are well documented following administration, reflecting enhanced ventilation (O'Donnell et al., 2012). Additionally, salbutamol facilitates mucociliary clearance by stimulating ciliary beat frequency, aiding mucus removal and airway patency (Fahy and Dickey, 2010). These effects collectively contribute to alleviating airway obstruction during asthma attacks and exercise-induced bronchospasm (Anderson, 2005). Though salbutamol is selective for β2 receptors, at elevated doses or with chronic administration, cross-activation of β1-adrenergic receptors in the heart may occur, leading to cardiovascular side effects including tachycardia and palpitations (Zinner *et al.,* 1987; Salpeter et al., 2002). β2 receptor stimulation in skeletal muscle can cause tremors, and peripheral vasodilation induced by β2 activation may reduce diastolic blood pressure slightly (Cazzola *et al*., 1996). Such systemic effects warrant caution when prescribing salbutamol to patients with underlying cardiovascular disorders (British Thoracic Society, 2014).

Salbutamol is rapidly absorbed through the pulmonary route, reaching peak plasma concentrations shortly after inhalation. Oral absorption is slower and less efficient. The drug undergoes hepatic metabolism primarily through sulfation pathways, with renal excretion of both unchanged drug and metabolites (Daley-Yates, 1990; Chrystyn et al., 1992). The plasma half-life ranges from approximately 3.8 to 6 hours, with bronchodilatory effects lasting up to 6 hours after inhalation (Fanta, 2009).

Tolerance and Clinical Considerations

Chronic use of β2 agonists like salbutamol can induce receptor desensitization and downregulation, mediated by phosphorylation via G-protein-coupled receptor kinases (Liggett, 2001; Lohse et al., 2008). This phenomenon reduces drug efficacy over time and highlights the importance of combination therapy with inhaled corticosteroids to maintain asthma control and reduce exacerbations (Global Initiative for Asthma, 2024).

Montelukast

Montelukast is an oral leukotriene receptor antagonist (LTRA) used in the long-term management of asthma and allergic rhinitis [Israel et al., 1996]. It is especially effective in reducing inflammation, bronchoconstriction, and mucus production mediated by leukotrienes in the respiratory tract [Drazen et al., 1999].

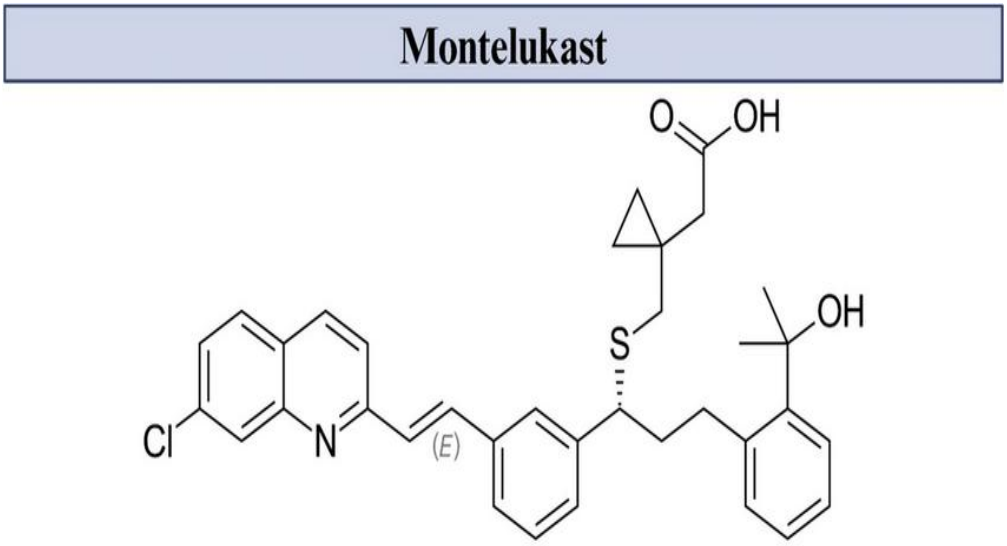


Fig. 1. Chemical Structure of the Montelukast

Scientific Figure on ResearchGate

Mechanism of Action

Montelukast selectively blocks the cysteinyl leukotriene receptor type 1 (CysLT1) found on airway smooth muscle cells, eosinophils, macrophages, and other inflammatory cells [Bisgaard, 2000]. Leukotrienes—particularly LTC_4, LTD_4, and LTE_4—are potent inflammatory mediators derived from arachidonic acid via the 5-lipoxygenase pathway [Funk, 2001]. They bind to CysLT1 receptors and cause bronchoconstriction, increased vascular permeability, mucus secretion, and eosinophil recruitment [Henderson, 1994]. Montelukast, by antagonizing CysLT1, prevents these downstream effects, leading to reduced airway inflammation and hyperresponsiveness [Holgate, 2000].Montelukast improves asthma symptoms by reducing bronchial inflammation and preventing exercise-induced bronchoconstriction [Reiss et al., 1997]. Unlike β2-agonists, which act on airway smooth muscle, montelukast targets the inflammatory cascade, making it useful as a controller drug rather than a reliever [Foster and Barnes, 1998]. Studies show that montelukast significantly improves FEV_1, reduces nocturnal symptoms, and decreases the need for rescue inhaler use in mild-to-moderate persistent asthma [Murray et al., 1999].

In allergic rhinitis, montelukast reduces nasal congestion, rhinorrhea, and sneezing by inhibiting leukotriene-mediated inflammation in nasal mucosa [Philip et al., 2004].

Montelukast is generally well tolerated, but recent findings have raised concerns about possible neuropsychiatric effects, particularly in children and adolescents [FDA, 2020]. These may include mood changes, sleep disturbances, and rarely, suicidal ideation—potentially linked to montelukast's ability to cross the blood-brain barrier and interfere with leukotriene signaling in the brain [Philip et al., 2004; Baldacci et al., 2020]. Montelukast is rapidly absorbed after oral administration, with peak plasma concentrations reached in 2–4 hours [Zubairi et al., 2007]. It has high plasma protein binding (>99%) and undergoes extensive hepatic metabolism via cytochrome P450 enzymes, especially CYP3A4 and CYP2C9 [Zhou et al., 2009]. Its elimination half-life is 2.7 to 5.5 hours, and it is excreted mainly in bile [Klahre et al., 1998].

Montelukast is effective as an adjunct to inhaled corticosteroids in asthma management, especially in patients with aspirin-sensitive asthma or exercise-induced bronchospasm [Dahlen et al., 2002]. It is not as effective as inhaled corticosteroids for controlling chronic inflammation but is preferred in patients who cannot tolerate inhalers or prefer oral medications [Busse et al., 2001].

PREDNISOLONE

Prednisolone is a synthetic glucocorticoid widely used for its potent anti-inflammatory and immunosuppressive effects, particularly in conditions like asthma, autoimmune diseases, and allergic reactions (Barnes, 2006). It mimics the effects of endogenous cortisol but with greater potency and longer duration of action (Rhen and Cidlowski, 2005).

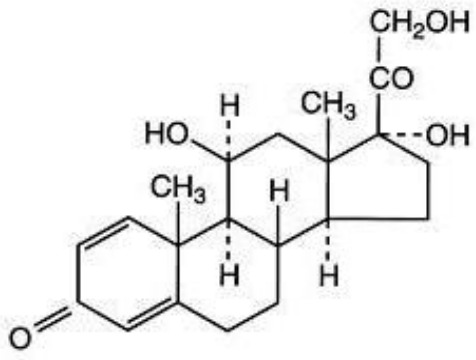


Fig. 3: Structure of Prednisolone

Figure source: ResearchGate

Mechanism of Action: Cellular Physiology

Prednisolone exerts its effects by diffusing through the cell membrane and binding to cytoplasmic glucocorticoid receptors (GRs), forming a hormone-receptor complex that translocates into the nucleus (Oakley and Cidlowski, 2013). This complex binds to glucocorticoid response elements (GREs) in DNA to regulate gene transcription, either upregulating anti-inflammatory proteins like lipocortin-1 or repressing pro-inflammatory genes like NF-κB and AP-1 (Newton, 2000; Barnes, 2006).

This results in decreased synthesis of inflammatory mediators including cytokines (IL-1, IL-6, TNF-α), prostaglandins, and leukotrienes (Coutinho and Chapman, 2011). Prednisolone also suppresses immune cell proliferation, reduces capillary permeability, and stabilizes lysosomal membranes (Rhen and Cidlowski, 2005).

In asthma, prednisolone reduces bronchial inflammation, decreases mucosal edema, and lowers airway hyperresponsiveness (Barnes, 2011). It increases β2-adrenergic receptor expression, thereby enhancing the efficacy of bronchodilators like salbutamol (Adcock and Caramori, 2001). It also decreases eosinophil infiltration and mucus secretion in the airways, improving overall lung function (Gina, 2023).

Orally administered prednisolone is used in acute asthma exacerbations, typically providing rapid relief within 4–6 hours (Rowe et al., 2001).

Systemic Physiology and Endocrine Effects

Glucocorticoids like prednisolone affect nearly every system in the body. On the endocrine level, prednisolone suppresses the hypothalamic-pituitary-adrenal (HPA) axis through negative feedback, leading to reduced ACTH secretion and potential adrenal suppression with long-term use (Charmandari et al., 2005).

In the cardiovascular system, it can increase vascular tone by enhancing catecholamine sensitivity and inhibiting nitric oxide production (Walker, 2007). Metabolically, prednisolone promotes gluconeogenesis, proteolysis, and lipolysis, while impairing glucose uptake—contributing to hyperglycemia and insulin resistance (Rhen and Cidlowski, 2005).

Side Effects and Long-Term Impact

Prolonged use of prednisolone is associated with multiple side effects such as Cushingoid features (moon face, central obesity), osteoporosis, immunosuppression, muscle wasting, peptic ulcers, and psychiatric disturbances (Barnes, 2006; Schäcke et al., 2002). The risk increases with higher doses and longer duration of therapy.

Because of its systemic effects, clinicians often prefer inhaled corticosteroids (e.g., beclomethasone) for long-term asthma control and reserve oral prednisolone for acute exacerbations (GINA, 2023).

Pharmacokinetics and Clearance

Prednisolone is rapidly absorbed after oral administration, reaching peak plasma levels in 1–2 hours (Derendorf et al., 1993). It is metabolized in the liver primarily by reduction and conjugation and excreted by the kidneys (Rhen and Cidlowski, 2005). Its plasma half-life is approximately 2–4 hours, but its biological effects may last up to 36 hours due to genomic regulation (Czock et al., 2005).

Effects on Lung Histology: OVA-Induced Female Sprague–Dawley Rat Model

The ovalbumin (OVA)-induced asthma model in female Sprague–Dawley rats is a well-established experimental paradigm for investigating allergic asthma, owing to its strong histopathological similarity to the human condition (Temelkovski et al., 1998; Kumar et al., 2021). In this model, rats are first sensitized to OVA via intraperitoneal injection, typically in combination with an adjuvant such as aluminum hydroxide, followed by repeated aerosol or intranasal OVA challenges. This protocol elicits a robust Th2-driven inflammatory response that closely mirrors the immunopathology of human allergic asthma.

Histologically, OVA-sensitized and challenged female Sprague–Dawley rats exhibit hallmark features of asthma, including prominent eosinophilic infiltration within the peribronchial and perivascular regions, airway wall thickening, and goblet cell hyperplasia within the bronchial epithelium, leading to mucus hypersecretion and plugging (Korsgren et al., 1997). Structural remodeling is evident, with subepithelial fibrosis due to excessive collagen deposition beneath the basement membrane, and peribronchial edema, reflecting increased vascular permeability. These changes not only impair airflow but also perpetuate chronic inflammation.

The reproducibility and pathophysiological relevance of the OVA-induced asthma model make it a valuable tool for evaluating the efficacy of pharmacological interventions aimed at attenuating airway inflammation, reversing structural remodeling, and restoring normal lung architecture (Kumar et al., 2021). By replicating both the inflammatory and remodeling components of asthma, this model enables detailed investigation into drug mechanisms and potential therapeutic targets for chronic airway diseases.

Salbutamol Histological Effects on Lung Tissue

Short-Term Effects

In acute use, salbutamol improves airflow, reduces airway resistance, and leads to temporary dilation of bronchi and bronchioles. Histologically, lung tissue may exhibit reduced peribronchial inflammation and minimal mucus plugging (Sears et al., 1990). Capillary perfusion may also improve due to relaxation of surrounding smooth muscle.

Long-Term or High-Dose Exposure

Long-term use or high doses of β2-agonists like salbutamol have been linked with tolerance, receptor desensitization, and oxidative damage in lung tissues (Lipworth, 1996). Some studies in animal models have shown increased inflammatory infiltrates, epithelial cell hyperplasia, and extracellular matrix remodeling (Amrani *et al.,* 2004).

Excessive stimulation of β2-receptors may also contribute to mitochondrial dysfunction and reactive oxygen species (ROS) production, potentially leading to lung injury or fibrosis in extreme cases (Rennard *et al.,* 2007).

Clinical Implications

The beneficial effects of salbutamol make it a cornerstone in managing acute bronchospasm and chronic airway diseases. However, inappropriate use, especially in absence of corticosteroids, may exacerbate underlying inflammation (Global Initiative for Asthma [GINA], 2024).

Combining salbutamol with inhaled corticosteroids reduces these risks and helps maintain histological integrity of the lung parenchyma (Barnes, 2011). Thus, treatment guidelines emphasize its use as a reliever rather than a controller medication.

Prednisolone Histological Effects on Lung Tissue

Short-Term Use

During acute inflammation, prednisolone reduces infiltration of neutrophils, eosinophils, and lymphocytes into the lung parenchyma and bronchiolar walls. This results in:

- Decreased mucus hypersecretion
- Restoration of normal epithelial lining
- Reduced edema and congestion (Jeffery, 1992)

The architecture of the lung tissue is preserved, and airway resistance is lowered.

Long-Term or High-Dose Use

Chronic exposure to systemic corticosteroids like prednisolone can impair tissue repair and regeneration. Histological changes observed with long-term use may include:

- Thinning of alveolar walls due to reduced fibroblast activity
- Atrophy of bronchial epithelium
- Suppressed mucosal immunity, predisposing to infections
- Delayed wound healing and fibrosis in some models (Cottin *et al*., 2004)

Clinical Implications

Prednisolone remains one of the most effective agents for controlling severe inflammatory responses in the lung. It is used in:

- Acute asthma exacerbations
- Severe COPD flare-ups
- Hypersensitivity pneumonitis
- Sarcoidosis
- Autoimmune interstitial lung disease

However, clinicians must balance its benefits with its side-effect profile, including increased risk of pneumonia, osteoporosis, and adrenal suppression (Ramakrishnan et al., 2021).

Inhaled corticosteroids (ICS) are often preferred for long-term control due to their localized action and reduced systemic toxicity. Decrease fibronectin, basement membrane thickness, and airway wall hypertrophy (Leung *et al.,* 2005).

Effects of Montelukast on Lung Tissue

Montelukast is an orally administered anti-asthmatic drug classified as a leukotriene receptor antagonist (LTRA). It is particularly useful in patients with persistent asthma or aspirin-exacerbated respiratory disease (AERD) (Israel *et al.,* 1996). Leukotrienes are lipid mediators derived from arachidonic acid that contribute significantly to airway inflammation and hyperresponsiveness. Montelukast blocks the CysLT1 receptor, thereby mitigating these effects (Peters-Golden and Henderson, 2007).

Short-Term Effects

Histological studies have shown that short-term montelukast administration results in decreased peribronchial eosinophilic infiltration, reduced goblet cell hyperplasia, and minimized mucus production (Salvi et al., 1999). The epithelial lining appears less disrupted, and there is improved mucociliary clearance.

Long-Term Use

Chronic inflammation in asthma may lead to airway remodeling, including thickening of the basement membrane and fibrosis. Montelukast has been shown to inhibit key remodeling processes such as:

- Subepithelial fibrosis
- Smooth muscle hypertrophy
- Goblet cell metaplasia

Animal and human studies suggest that long-term use of montelukast preserves normal lung architecture by downregulating matrix metalloproteinases (MMPs) and transforming growth factor-beta (TGF-β) (Zhou *et al.,* 2010).

Clinical Implications

Montelukast is particularly effective in:

- Exercise-induced bronchoconstriction
- Nocturnal asthma
- Aspirin-sensitive asthma
- Reducing corticosteroid dependency

Unlike β2-agonists, montelukast targets the inflammatory component of asthma, leading to sustained protection and better histological outcomes (Reiss *et al.,* 1997). It is generally well-tolerated, but rare side effects such as neuropsychiatric symptoms (e.g., mood changes, agitation) have been reported, especially in pediatric populations (FDA, 2020)

Chapter 3

RESEARCH AND METHODOLOGY

3.1 Materials

- Plastic cages
- Feed
- Clean drinking water
- Feeding plates
- Drinking plates
- Weighing balance

- Gloves
- Lab coats
- Chloroform
- Salbutamol
- Montelukast
- Prednisolone
- Dissection instruments
- Universal bottles
- Syringes
- Cotton wool
- Formalin
- Permanent markers
- Saline solution

3.2 Experimental Animals

This study used forty (40) female Sprague Dawley rats, all of which received proper care in accordance with international guidelines for experimental animal handling. The animals underwent a two-week acclimatization period to minimize stress and allow for physiological adaptation to the laboratory environment. During the acclimation period, the rats were housed in plastic cages under standard laboratory conditions, maintained at a room temperature of 22°C, with unrestricted access to feed and clean drinking water throughout the study. All procedures were carried out in compliance with internationally accepted principles for laboratory animal care.

3.3 Study Design

This experimental study was conducted over seven (7) weeks, divided into three main phases: acclimatization/sensitization, asthma induction, and drug treatment. The forty (40 rat) were randomly assigned into eight (8) groups of ten rats each as follows:

- Group 1 – Negative control: No asthma induction, no treatment.
- Group 2 – Positive control: Asthma induced, no treatment.
- Group 3 – Salbutamol + Prednisolone: Asthma induced and treated with a combination of Salbutamol and Prednisolone.
- Group 4 –.Salbutamol + Montelukast: Asthma induced and treated with a combination of Salbutamol and Montelukast.
- Group 5 –Salbutamol + Prednisolone: Asthma induced and treated with a combination of Salbutamol and Prednisolone.

3.4 Experimental Protocols

The experimental study was carried out over a period of seven (7) weeks and divided into four sequential phases: acclimatization/sensitization, asthma induction, drug treatment, and terminal procedures.

Phase 1 – Acclimatization/Sensitization (Weeks 1–2)

All forty)rats were allowed a two-week acclimatization period under standard laboratory conditions (temperature 22 ± 2°C12-hour light/dark cycle) in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). This period allowed the animals to adapt to the environment and minimized stress-related physiological changes (Ganong, 2016).

During acclimatization, rats were provided standard feed and clean drinking water ad libitum. Sensitization in Groups 2–8 was achieved via intraperitoneal injection of ovalbumin (OVA) mixed with aluminium hydroxide [$Al(OH)_3$] as adjuvant, a method consistent with established allergic asthma models (Kumar *et al.,* 2021).

Phase 2 – Asthma Induction (Week 3)

At the beginning of week 3, asthma was induced in all groups except the negative control (Group 1). Each rat received an intraperitoneal injection of 1 mg OVA dissolved in 1.0 ml sterile saline solution along with aluminium hydroxide to enhance immunogenicity. This dosage has been shown to reliably elicit an allergic airway response in rodents (West, 2012).

Across all induced groups, a total of 56 mg OVA was used. The negative control group received only 1.0 ml of sterile saline intraperitoneally. Successful induction was confirmed via physical signs including nasal flaring, audible wheezing, and labored respiration within 24–48 hours post-challenge.

Phase 3 – Drug Treatment (Weeks 4–7)

Following induction, rats in treatment groups received their allocated drugs orally once daily for four consecutive weeks:

- Salbutamol: 2 mg/ml (bronchodilator)
- Montelukast: 10 mg/ml (leukotriene receptor antagonist)
- Prednisolone: 3 mg/ml (glucocorticoid anti-inflammatory)

Combination therapy groups received two agents administered in quick succession according to their group assignment. Doses were selected based on literature demonstrating effective bronchodilatory and anti-inflammatory activity in rat models of asthma (Barnes, 2013). All treatments were administered at the same time each day to maintain consistency in pharmacokinetic exposure.

Phase 4 – Terminal Procedures (End of Week 7)

At the conclusion of the treatment period, animals were anaesthetized with chloroform vapor and sacrificed by cervical dislocation. Blood was collected via cardiac puncture for serum preparation. Lungs were carefully removed, rinsed in saline to remove excess blood, and prepared for histological examination by fixation in 10% neutral buffered formalin for 48 hours (Junqueira and Carneiro, 2015).

3.5 Histological Examination

Lungs were rinsed with saline to remove blood, fixed in 10% neutral buffered formalin and dehydrated in graded ethanol, cleared with xylene, embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin (HandE) for microscopic evaluation (Junqueira and Carneiro, 2015).

Histological assessment included:

- Airway epithelial integrity
- Goblet cell hyperplasia and mucus secretion
- Inflammatory cell infiltration
- Smooth muscle hypertrophy

3.6 Blood Sampling and Serum Isolation

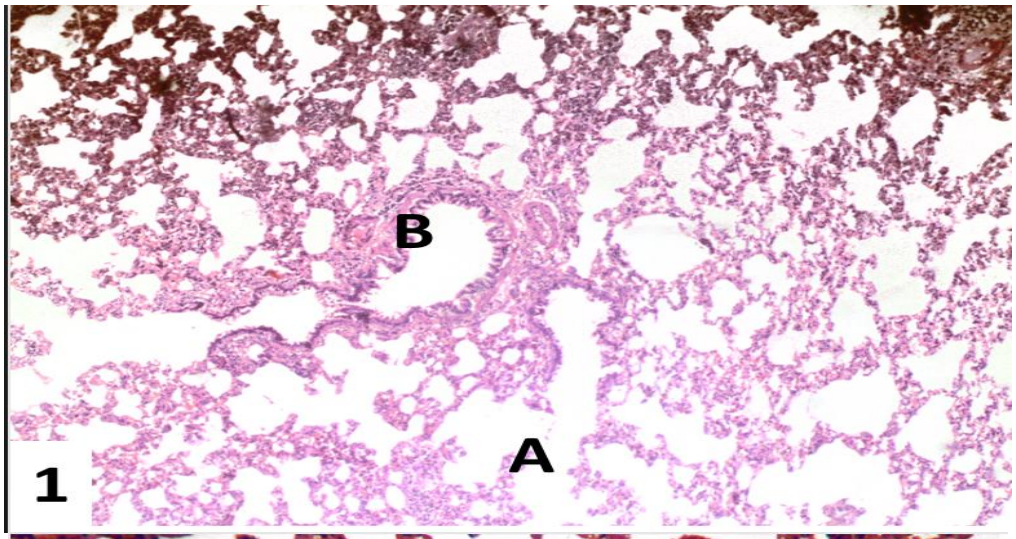
At the end of the experimental period, animals were anaesthetized with chloroform. Blood was collected via cardiac puncture into plain universal bottles. Samples were allowed to clot at room temperature before centrifugation at 5,000 rpm for 10 minutes. Serum was separated and stored at –20°C until analysis.

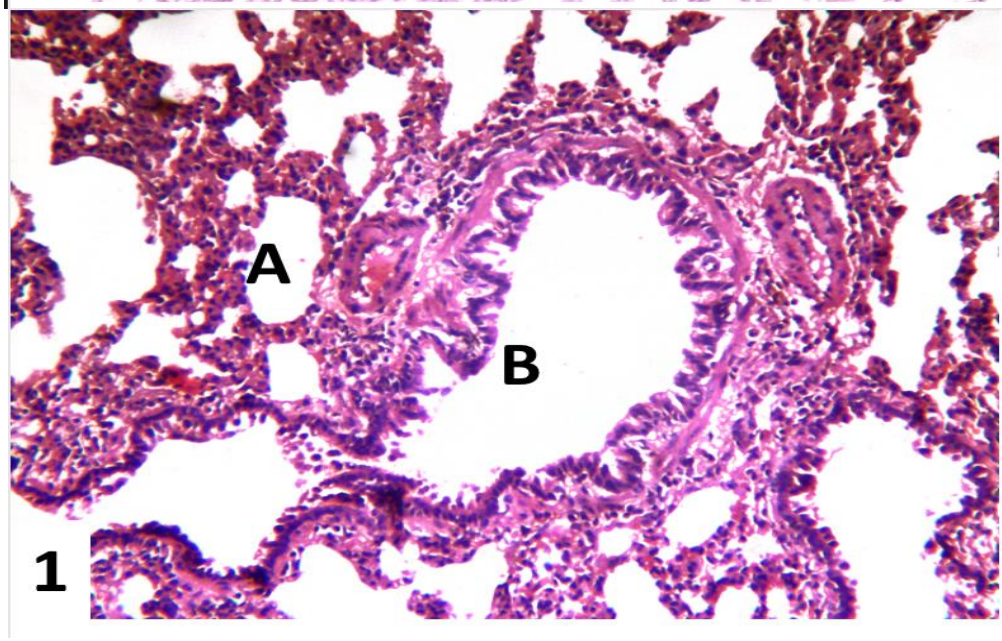
3.7 HISTOLOGICAL EVALUATION

Formalin-fixed lung tissues were processed using standard paraffin-embedding techniques. Tissue sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for general histological examination. The slides were observed under a light microscope at 400× magnification to evaluate alveolar architecture, bronchial epithelium integrity, vascular congestion, inflammatory cell infiltration, mucosal thickening, and fibrotic changes within the pulmonary parenchyma. Representative photomicrographs of each group were captured using a digital imaging system for documentation and comparative analysis

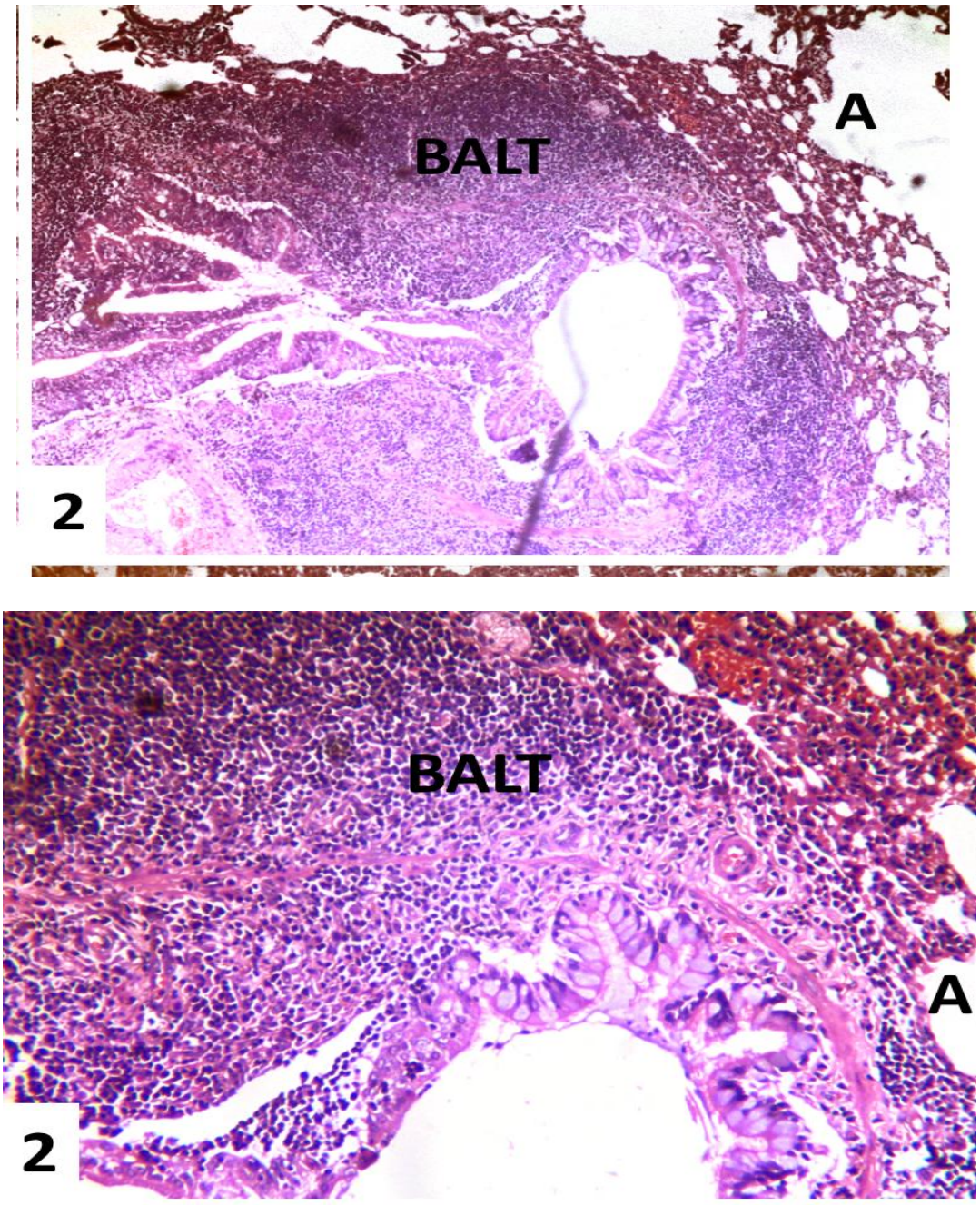
CHAPTER FOUR

RESULT

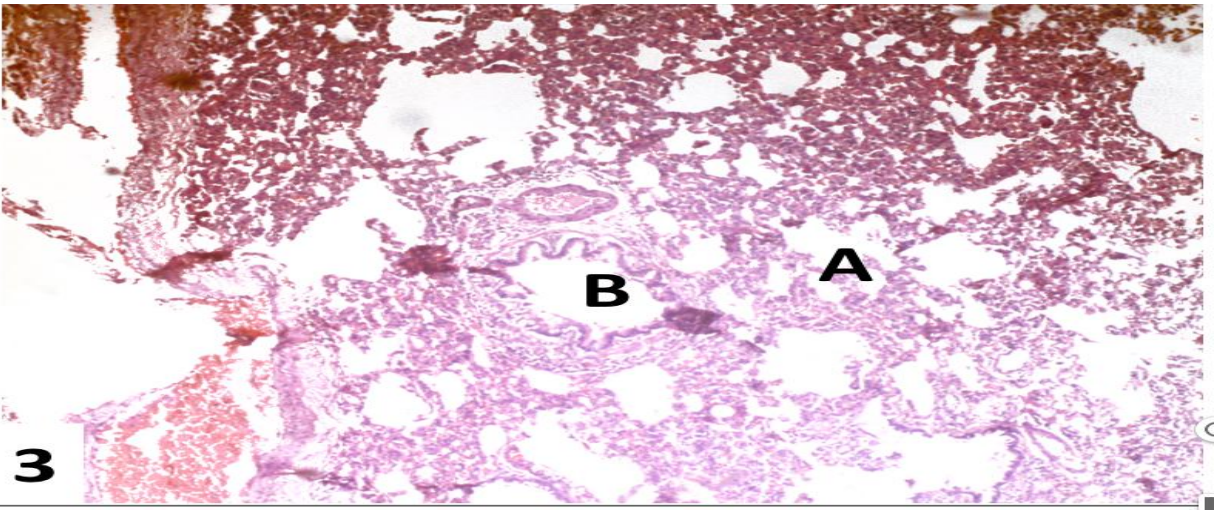


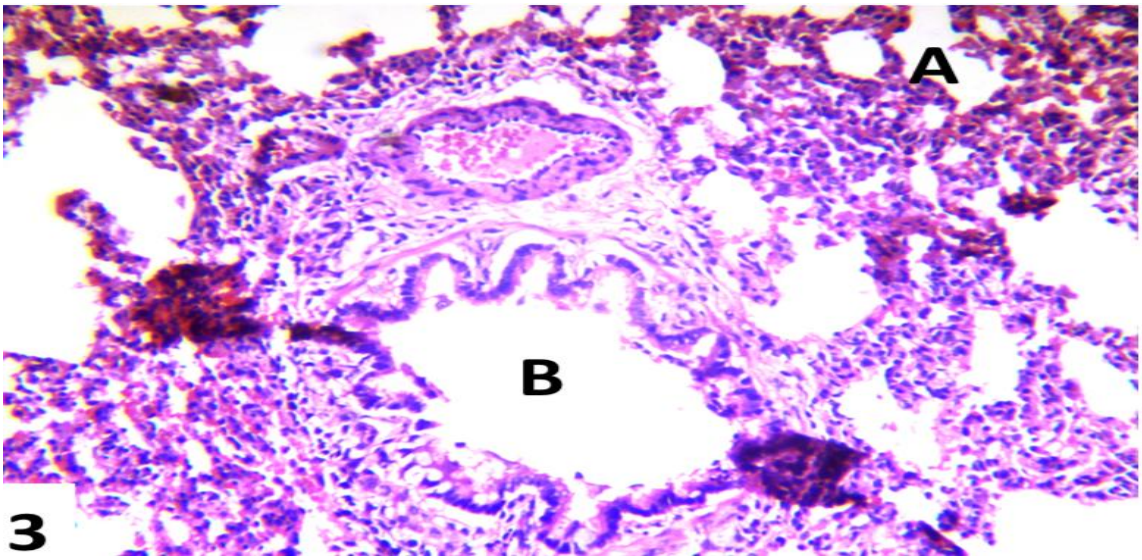


Photomicrographs of the lungs (group 1) of the negative control showing normal lung histology: bronchus (B) and alveolar sac (A)

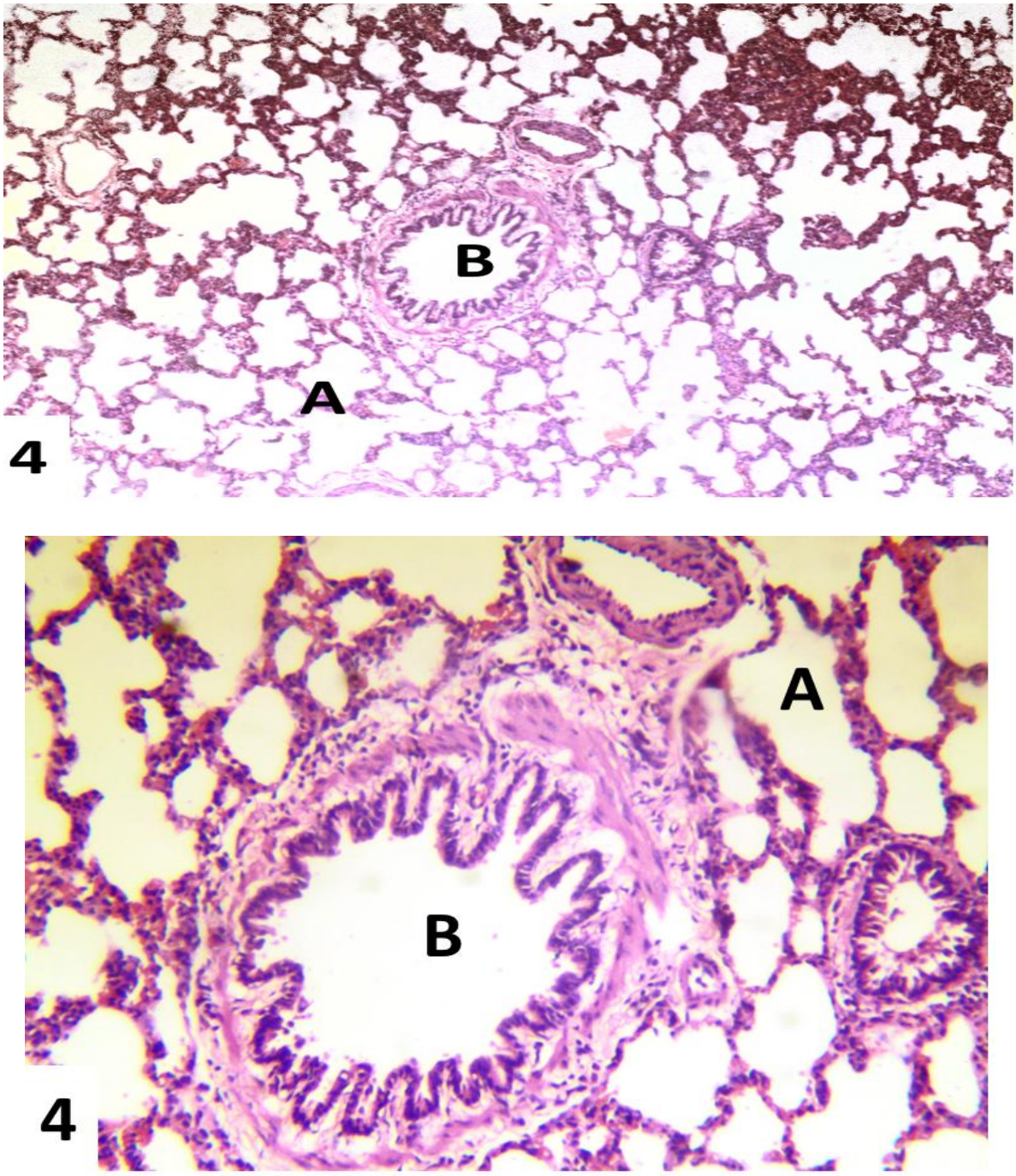


Photomicrographs of the lungs (group 2) of the positive control hyperplasia of the bronchus-associated lymphoid tissues (BALT) (follicular bronchiolitis)

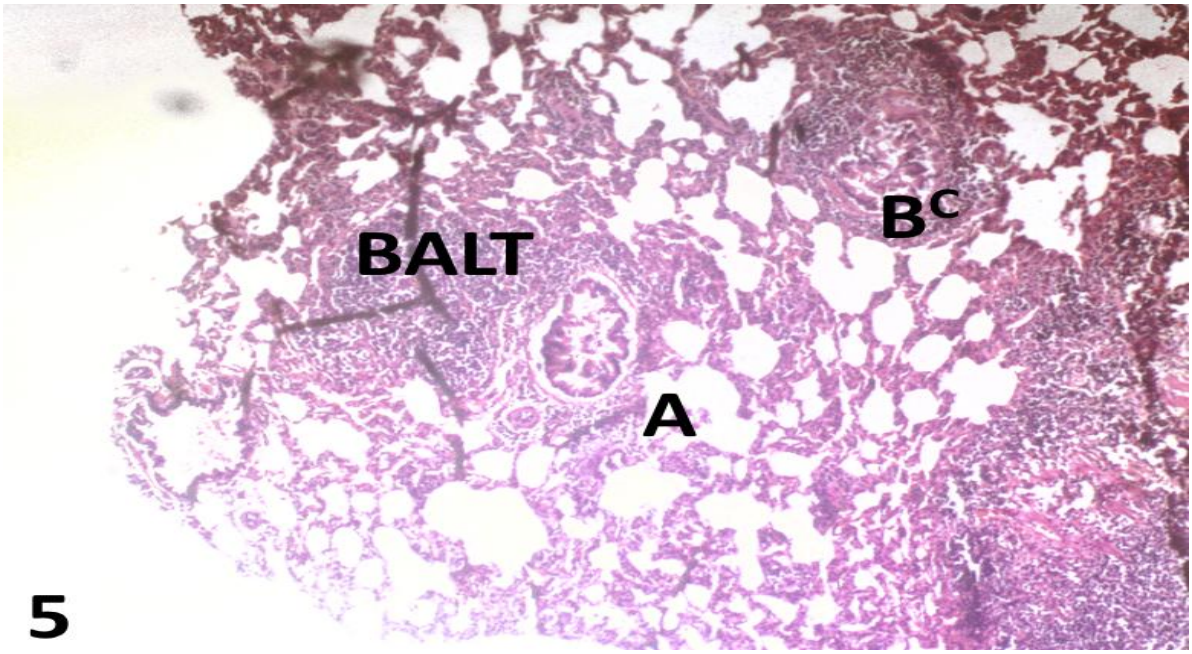


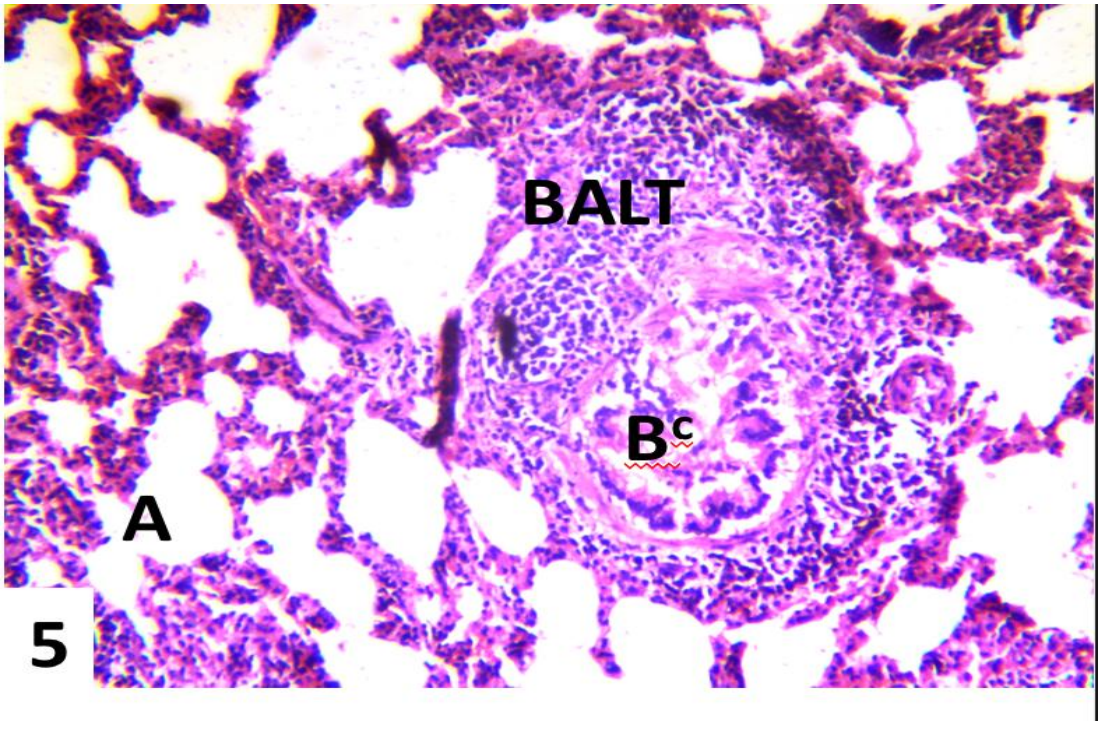


Photomicrographs of the lungs (group 3) of thesalbutamol/prednisolone showing a normal lung histology:bronchus (B) and alveolar sac (A)



Photomicrographs of the lungs (group 4) of theprednisolone/montelukast showing a normal lung histology:bronchus (B) and alveolar sac (A)





Photomicrographs of the lungs (group 5) of thesalbutamol/montelukast showing Broncho-constriction ($B^{c)}$, alveolar sacs (A), and hyperplasia of bronchus-associated lymphoid tissues (follicular bronchiolitis), which is more severe compared with group

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Histological examination of lung tissues across the experimental groups revealed distinct morphological changes consistent with the biochemical findings. The lungs of the negative control group (Group 1) showed normal architecture characterized by intact bronchi (B) and alveolar sacs (A) with no observable inflammatory infiltrates or structural distortions, confirming the healthy baseline of the animals.

In contrast, the positive control group (Group 2) exhibited marked hyperplasia of bronchus-associated lymphoid tissue (BALT), indicating follicular bronchiolitis and airway inflammation. This suggests successful induction of asthma-like pathology in the model group. The thickened peribronchial wall and lymphoid cell aggregation around the airways signify an immune response typical of allergic airway inflammation.

The groups treated with salbutamol, montelukast, or prednisolone individually (Groups 3–5) displayed near-normal histological architecture with minimal lymphoid infiltration. This indicates that these drugs increased airway inflammation and preserved tissue integrity, consistent with their known bronchodilatory and anti-inflammatory actions.

The combination-treated groups showed varied effects. The montelukast + prednisolone group (Group 4) maintained normal bronchial and alveolar structures similar to the controls, suggesting a synergistic anti-inflammatory effect. However, the salbutamol + prednisolone group (Group 5) showed evidence of both BALT hyperplasia and bronchoconstriction, indicating partial protection with residual inflammation and airway narrowing. This aligns with the biochemical data showing reduced antioxidant enzyme activity and increased oxidative stress markers in this group, suggesting that combined salbutamol and prednisolone treatment may not fully reverse airway inflammation or oxidative damage.

Overall, the histological findings confirm that montelukast and prednisolone, individually or in combination, effectively preserved lung tissue morphology, while the combination of salbutamol and prednisolone was less protective. The presence of BALT hyperplasia and broncho-constriction in Groups 2 and 5 shows persistent airway inflammation despite treatment, emphasizing the complex interplay between β_2-agonists and corticosteroids in oxidative stress modulation.

CONCLUSION

The histological findings shows that salbutamol, montelukast, and prednisolone exert varying degrees of protection on lung tissue structure in asthma-induced rats. Individual treatments with these drugs preserved normal bronchial and alveolar structures, reflecting effective reduction of inflammation and tissue damage. But, the combination of salbutamol and prednisolone showed persistent features of bronchus-associated lymphoid tissue hyperplasia and bronchoconstriction, indicating incomplete resolution of airway inflammation. These results suggest that while corticosteroids and leukotriene antagonists provide strong anti-inflammatory protection, the combined use of salbutamol and prednisolone may not fully mitigate oxidative or inflammatory stress in lung tissues. Overall, montelukast and prednisolone, either alone or together, appear to offer better histological protection in asthma management compared to salbutamol-based combinations.

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