EFFECT OF THE COMBINATIONS OF SALBUTAMOL, MONTELUKAST AND PREDNISOLONE ON LUNG OXIDANT AND ANTIOXIDANT ENZYME ACTIVITIES IN OVALBUMIN-INDUCED FEMALE SPRAGUE DAWLEY RATS

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EFFECT OF THE COMBINATIONS OF SALBUTAMOL, MONTELUKAST, PREDNISOLONE ON LUNG TISSUE OXIDANT AND ANTIOXIDANT ENZYME ACTIVITIES IN OVALBUMIN-INDUCED FEMALE SPRAGUE DAWLEY RATS

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CERTIFICATION

This is to certify that this project work on "**EFFECT OF THE COMBINATIONS OF SALBUTAMOL MONTELUKAST AND PREDNISOLONE ON LUNG TISSUE OXIDANT AND ANTIOXIDANT ENZYME ACTIVITIES IN OVALBUMIN-INDUCED SPRAGUE DAWLEY RATS**" was carried out by **LAWANI STEPHANIE EKPEME**, with the matriculation number **BMS2101644**; in partial fulfilment for the Award of Bachelor of Sciences (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 work to God Almighty, whose wisdom, grace, and love have made this project successful.

To my beloved parents, Mr and Mrs Lawani, your support, love and encouragement have been the cornerstone of my journey. Your financial assistance, moral guidance and prayers have sustained me and I pray that God continues to bless you.

To my dear siblings, Goodluck and Queen Lawani, thank you for your constant love and support.

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ABSTRACT

Salbutamol, montelukast, and prednisolone are widely used in the management of respiratory disorders. Despite their therapeutic benefits, their effects on pulmonary oxidative stress and antioxidant defenses, particularly when used in combination, remain unclear. This study evaluated the influence of these agents on oxidative stress markers and total protein concentration in lung tissue. Experimental animals were divided into five groups (n = 8 group): negative control, positive control, salbutamol, montelukast, prednisolone, salbutamol/prednisolone, salbutamol/montelukast, and prednisolone/montelukast. Lung tissue homogenates were analyzed for total protein concentration, antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)—and oxidative stress markers, including hydrogen peroxide (H_2O_2) and nitric oxide (NO). Data were expressed as mean ± SEM and analyzed using one-way ANOVA with significance set at $p < 0.05$. Total protein concentration significantly increased only in the salbutamol/prednisolone-treated group compared with the negative control ($p < 0.05$). This group also exhibited significant decreases in SOD, CAT, and GPx activities relative to both negative and positive controls ($p < 0.05$), indicating reduced antioxidant capacity. In contrast, salbutamol/montelukast and prednisolone/montelukast treatments did not alter protein concentration or most antioxidant enzymes compared with the negative control, although CAT and GPx were decreased relative to the positive control ($p < 0.05$). Hydrogen peroxide levels were significantly elevated in salbutamol/montelukast and prednisolone/montelukast groups compared with the negative control ($p < 0.05$), while NO levels did not differ significantly among groups. However, compared with the positive control, NO concentration increased in salbutamol/prednisolone and salbutamol/montelukast groups ($p < 0.05$). In conclusion, combination therapy with salbutamol and prednisolone reduces pulmonary antioxidant enzyme activity while increasing total protein, suggesting mild oxidative stress. Montelukast-containing combinations primarily modulate hydrogen peroxide without major effects on protein content or overall antioxidant capacity. These findings indicate that drug combinations exert differential effects on lung oxidative homeostasis, highlighting the need for careful evaluation of pulmonary redox status during combination therapy.

CHAPTER ONE

1.0 INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation. The condition leads to episodes of wheezing, breathlessness, chest tightness, and coughing, especially at night or in the early morning (Global Initiative for Asthma [GINA], 2024). Asthma has been recognized since ancient times. The term "asthma" originates from the Greek word “azo”, meaning “to pant” or “breathe hard,” and was first used by Hippocrates around 400 BC to describe respiratory distress. Over the centuries, various physicians, including Galen in the 2nd century AD, associated asthma with airway obstruction and environmental factors. However, it was not until the 20th century that asthma came to be fully understood as a chronic inflammatory condition of the airways, leading to modern treatments such as inhaled corticosteroids and bronchodilators (Pearce *et al.*, 1998). Asthma affects an estimated 262 million people worldwide and caused approximately 455,000 deaths in 2019, according to the

World Health Organization (WHO, 2021). It is one of the most common chronic diseases in children, but it also affects adults. The prevalence varies by country and is generally higher in high-income nations due to better awareness and diagnosis. However, low- and middle-income countries bear a greater burden of severe and poorly controlled asthma (WHO, 2021). Asthma is a major public health concern due to its global prevalence, economic burden, and impact on quality of life. It affects individuals' ability to perform daily activities, attend school or work, and can lead to frequent hospital visits or even death if not properly managed. In children, asthma is one of the leading causes of school absenteeism, while in adults, it can reduce productivity and work performance. Economically, asthma contributes to high healthcare costs through emergency care, hospitalizations, and long-term medication use (Global Asthma Network, 2018). Asthma is caused by a combination of genetic predisposition and environmental exposures. Individuals with a family history of asthma, eczema, or allergic rhinitis are at higher risk. Environmental factors such as exposure to allergens like dust mites, pollen, and mold, as well as air pollution, tobacco smoke, viral respiratory infections, and occupational irritants, can trigger asthma development and exacerbate symptoms. Additional risk factors include obesity, early childhood exposure to antibiotics, and living in urban environments with high pollution levels (Dharmage, Perret, and Custovic, 2019). The pathophysiology of asthma involves chronic inflammation of the airways, which leads to airway hyperresponsiveness, intermittent airflow obstruction, and structural changes in the bronchial walls. Inflammatory cells such as eosinophils, mast cells, T lymphocytes, and neutrophils are activated in response to triggers, releasing chemical mediators like histamine and leukotrienes. This results in bronchoconstriction, mucus hypersecretion, edema, and smooth muscle hypertrophy, all of which contribute to the characteristic symptoms of asthma such as wheezing, coughing, and shortness of breath (Holgate, 2012). Asthma symptoms can vary in intensity and frequency but commonly include wheezing, shortness of breath, chest tightness, and coughing, particularly at night or in the early morning. These symptoms are typically episodic and may worsen with exposure to triggers such as allergens, cold air, exercise, or respiratory infections. In some individuals, symptoms may be mild and infrequent, while in others they may be severe and persistent, significantly impairing daily activities (National Heart, Lung, and Blood Institute [NHLBI], 2020). Asthma can be classified based on its triggers, clinical presentation, or severity. One major type is allergic (extrinsic) asthma, which is triggered by allergens such as pollen, dust mites, or pet dander and often coexists with other allergic conditions like eczema or allergic rhinitis. In contrast, non-allergic (intrinsic) asthma is not associated with allergens and is typically triggered by factors such as stress, exercise, cold air, or respiratory infections. Other types of asthma include exercise-induced asthma, which presents symptoms during or after physical activity, especially in cold or dry environments; occupational asthma, which is caused by exposure to irritants in the workplace such as fumes or chemicals; cough-variant asthma, in which chronic coughing is the primary or sole symptom; and severe asthma, which is a more difficult to control form that persists despite high-dose treatment and may require specialized therapies (Wenzel, 2012). Diagnosis is based on clinical history and confirmed through lung function tests such as spirometry, peak expiratory flow measurement, and bronchodilator responsiveness. These help assess the degree of airflow obstruction and its reversibility (NHLBI, 2020). Management of asthma follows a stepwise approach depending on symptom severity and level of control. Inhaled corticosteroids (ICS) remain the cornerstone of long-term control. For patients with moderate to severe asthma, combination therapies may include long-acting beta-2 agonists (LABAs), leukotriene receptor antagonists, or oral corticosteroids. Quick-relief medications such as short-acting beta-2 agonists (SABAs) are used for acute symptom relief. Non-pharmacologic strategies such as allergen avoidance, smoking cessation, weight management, and patient education are also essential (GINA, 2024). Asthma medications are broadly classified into long-term control and quick-relief medications. Long-term control medications are taken daily to prevent symptoms. These include inhaled corticosteroids such as fluticasone and budesonide, which reduce airway inflammation; long-acting beta-2 agonists such as salmeterol, which provide sustained bronchodilation; and leukotriene receptor antagonists such as Montelukast, which block inflammatory mediators responsible for bronchoconstriction and mucus production (Barnes, 2023). Montelukast is particularly useful in allergic asthma and offers an oral alternative for patients who struggle with inhaler use. In more severe cases, oral corticosteroids like Prednisolone are used to quickly suppress inflammation during exacerbations. However, long-term use is limited due to potential systemic side effects. For patients who do not respond adequately to standard therapies, biologic agents such as omalizumab may be prescribed (Pavord *et al*., 2021). Quick-relief medications are used to treat acute symptoms or asthma attacks. The most commonly used rescue drug is Salbutamol (Albuterol), a short-acting beta-2 agonist that works within minutes to relax airway muscles and restore airflow. It is usually administered via inhaler or nebulizer. Frequent reliance on Salbutamol may indicate poorly controlled asthma and a need to reassess long-term management. Other quick-relief options include anticholinergics and systemic corticosteroids like Prednisolone, which are used short-term during severe exacerbations (Barnes, 2023). While these medications are essential for asthma control, they may also influence oxidative stress responses in lung tissues, particularly under chronic or combination drug exposure. Oxidative stress resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defenses plays a key role in asthma pathogenesis by promoting airway inflammation, tissue damage, and bronchial hyperreactivity (Comhair and Erzurum, 2010). Drugs like Salbutamol and Prednisolone have been reported to modulate oxidant and antioxidant enzyme activities, including markers such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). While these effects may support symptom relief, prolonged or excessive activation could disturb redox homeostasis in the lungs. Montelukast has also demonstrated antioxidant properties by reducing lipid peroxidation and enhancing endogenous antioxidant levels (Uzun *et al*., 2012). Therefore, evaluating the combined effects of Salbutamol, Montelukast, and Prednisolone on lung tissue oxidant and antioxidant enzyme activities is crucial, especially in the context of ovalbumin-induced asthma, which closely mimics human allergic asthma in experimental models. Understanding these interactions can offer deeper insights into the redox-modulatory potential of these agents and inform safer, more effective asthma treatment strategies.

1.1 JUSTIFICATION OF THE STUDY

Asthma is a chronic inflammatory disease of the airways that is associated with increased oxidative stress, contributing to epithelial damage, airway remodeling, and disease severity. Reactive oxygen species (ROS), generated during inflammation, overwhelm the lung's natural antioxidant defenses, resulting in oxidative damage to pulmonary tissue. Commonly used asthma medications such as salbutamol, montelukast, and prednisolone not only relieve symptoms through bronchodilation and anti-inflammatory effects but may also influence oxidative and antioxidative processes in the lungs. However, the combined impact of these drugs on the balance between oxidant production and antioxidant defense in the lungs has not been fully elucidated. Investigating this in an ovalbumin-induced asthma model using female Sprague-Dawley rats provides a robust experimental platform for understanding these effects. By evaluating key oxidative stress markers and antioxidant enzyme activities in lung tissue, this study aims to offer deeper insight into how this combination therapy modulates pulmonary redox homeostasis, with potential implications for improving asthma management strategies and limiting oxidative lung injury.

1.2 AIM OF THE STUDY

The aim of this study is to evaluate the effect of the combination of salbutamol, montelukast, and prednisolone on lung tissue oxidant and antioxidant enzyme activities in ovalbumin-induced female Sprague-Dawley rats.

1.3 RESEARCH QUESTIONS

1. What are the effects of the combinations of Salbutamol/Montelukast, Montelukast/Prednisolone, and Prednisolone/Salbutamol on lung tissue oxidant in ovalbumin-induced female Sprague-Dawley rats?
2. What are the effects of the combinations of Salbutamol/Montelukast, Montelukast/Prednisolone, and Prednisolone/Salbutamol on lung tissue antioxidant enzyme in ovalbumin-induced female Sprague-Dawley rats?

1.4 SPECIFIC OBJECTIVES

1. To determine the effect of the combinations of Salbutamol/Montelukast, Montelukast/Prednisolone, and Prednisolone/Salbutamol on lung tissue oxidant in ovalbumin-induced female Sprague-Dawley rats.

2. To determine the effect of the combinations of salbutamol/montelukast, montelukast/prednisolone, and prednisolone/salbutamol on lung tissue antioxidant enzyme in ovalbumin-induced female Sprague-Dawley rats.

CHAPTER TWO

2.0 ASTHMA

Asthma is a chronic, inflammatory disorder of the airways characterized by recurrent episodes of wheezing, shortness of breath, chest tightness, and coughing especially at night or early in the morning. It involves variable and reversible airflow obstruction, increased bronchial responsiveness to a variety of stimuli, and airway inflammation (National Heart, Lung, and Blood Institute [NHLBI], 2022; Global Initiative for Asthma [GINA], 2023). The condition arises from a complex interaction of genetic and environmental factors, leading to immune system activation, mucus overproduction, and bronchial smooth muscle constriction (Holgate, 2012). Over time, persistent inflammation may lead to structural changes in the airway wall, known as airway remodeling, which can result in more permanent airflow limitation (Bousquet *et al.*, 2010). Triggers for asthma symptoms include allergens (such as pollen, dust mites, and animal dander), respiratory infections, physical exertion, cold air, air pollutants, strong odors, and certain medications (Busse and Lemanske, 2001; GINA, 2023). Although asthma cannot be cured, it can be effectively managed with medications, lifestyle changes, and avoidance of known triggers (NHLBI, 2022).

2.1 EPIDEMIOLOGY OF ASTHMA

Asthma is a widespread chronic respiratory disease that affects people of all ages and demographics across the globe. According to the World Health Organization (WHO, 2021) and the Global Initiative for Asthma (GINA, 2023), asthma currently affects over 260 million people worldwide, with projections suggesting that this number could rise to 400 million by 2025 if current trends continue. The global prevalence of asthma varies significantly between countries and regions, ranging from 1% to over 20% (Asher *et al.,* 2020). High-income countries generally report higher prevalence rates, with Australia at approximately 21%, the United Kingdom at 15–18%, the United States at around 8–10%, and New Zealand also reporting high rates (GINA, 2023). In contrast, many low and middle-income countries (LMICs), including African nations, have historically reported lower prevalence. However, these regions are now witnessing a steady rise in asthma cases due to urbanization, environmental changes, and westernized lifestyles (Adeloye *et al*., 2021). In Nigeria, asthma is increasingly becoming a public health concern. Although prevalence estimates vary by study and region, recent data suggest a national asthma prevalence ranging from 5% to 10% (Falade *et al.,* 2012; Adeyola *et al.,* 2013). This translates to an estimated 10 to 20 million Nigerians currently living with asthma. Urban centers such as Lagos, Abuja, and Port Harcourt report higher prevalence rates due to factors such as poor air quality, industrial pollution, traffic-related emissions, and overcrowded living conditions (Nriagu *et al.,* 2016). Studies over the last two decades show a gradual but consistent increase in asthma prevalence across both urban and rural communities in Nigeria. This growth is attributed to increased exposure to indoor and outdoor air pollutants, changing environmental conditions, poor housing, limited health education, and underdiagnosis (Asher *et al.*, 2020; Adeloye *et al.,* 2021). Asthma commonly begins in childhood, with many cases diagnosed before the age of 10 (GINA, 2023). In children, the condition is more prevalent among boys, while in adults, women are more frequently affected (NHLBI, 2022). Among older adults, asthma may coexist with chronic obstructive pulmonary disease (COPD), a situation referred to as asthma-COPD overlap syndrome (ACOS) (GINA, 2023). Ethnic and racial disparities are also evident. For example, in the United States, African American and Hispanic populations experience higher rates of asthma-related complications compared to non-Hispanic Whites due to socioeconomic and healthcare inequalities (CDC, 2023). Environmental and lifestyle factors play a major role in the epidemiology of asthma. Key contributors include urbanization, indoor and outdoor air pollution, allergen exposure (such as dust mites, mold, and cockroach allergens), tobacco smoke, and occupational irritants (WHO, 2021; GINA, 2023). In Nigeria, additional risk factors include widespread use of biomass fuels (wood, charcoal, kerosene) for cooking, poor ventilation, and lack of awareness about asthma triggers and management (Falade *et al*., 2012; Nriagu *et al.,* 2016). Occupational asthma is also notable, especially among individuals exposed to chemical irritants, smoke, or dust in workplaces such as factories and construction sites (GINA, 2023). Despite being manageable with proper treatment, asthma remains a leading cause of preventable morbidity and mortality, contributing to over 400,000 deaths annually worldwide (WHO, 2021). In Nigeria, poor asthma control is linked to frequent hospital admissions, emergency room visits, and reduced productivity (Adeloye *et al*., 2021). Inadequate access to essential asthma medications, such as inhaled corticosteroids and bronchodilators, continues to be a major challenge (GINA, 2023). Over the past few decades, asthma prevalence has increased globally, especially in industrialized nations, though it is now stabilizing or declining in some of those regions (Asher *et al*., 2020). In contrast, prevalence continues to rise in developing countries, including Nigeria, where rapid urbanization, industrialization, and environmental degradation are exposing larger populations to asthma risk factors (Adeloye *et al*., 2021). If these trends persist, the number of asthma patients in Nigeria is expected to grow significantly in the coming years, putting additional strain on the country's healthcare system. Asthma remains a major global and national health issue. In Nigeria, the increasing number of asthma cases, rising prevalence, and limited healthcare access pose significant public health challenges. Tackling this growing burden requires coordinated efforts in early diagnosis, public education, improved access to effective treatment, and policies aimed at reducing environmental risk factors (WHO, 2021; GINA, 2023).

2.2 TYPES AND CLASSIFICATIONS OF ASTHMA

Asthma is a heterogeneous disease that presents with varying symptoms, triggers, and treatment responses. Its classification helps guide diagnosis and personalized management. Asthma can be classified clinically (by cause and triggers), physiologically (by severity and control), and immunologically (by inflammatory mechanisms) (Agache and Akdis, 2019; GINA, 2023).

I. Classification by Cause and Triggers (Phenotypes)

This common approach groups asthma based on identifiable patterns (Verywell Health, 2009; American Lung Association, 2024). Allergic (extrinsic) asthma is the most common in children and young adults, often with a history of eczema or allergic rhinitis. It is triggered by allergens like pollen, dust mites, or pet dander, involves IgE-mediated, eosinophilic inflammation, and typically responds well to corticosteroids and immunotherapy (GINA, 2023; Amboss, 2025). Non-allergic (intrinsic) asthma occurs without known allergens or elevated IgE, commonly in adults, and is triggered by factors such as cold air, infections, and stress. It often involves neutrophilic or mixed inflammation and may be more severe and corticosteroid-resistant (Amboss, 2025). Exercise-induced asthma (EIA/EIB) is triggered by physical activity, especially in cold or dry air, is common in adolescents, and is managed with pre-exercise bronchodilators (Verywell Health, 2009). Occupational asthma is caused by exposure to workplace irritants such as fumes and chemicals; early diagnosis and removing the trigger can lead to symptom improvement (StatPearls, 2024). Aspirin-induced asthma (AIA/NERD) is triggered by NSAIDs and is associated with chronic sinusitis and nasal polyps. It is linked to altered arachidonic acid metabolism and managed by avoiding NSAIDs and using leukotriene modifiers (Wikipedia, 2025). Cough-variant asthma presents mainly as chronic cough without wheezing, and diagnosis is confirmed through medication response and pulmonary testing (GINA, 2023; Amboss, 2025).

II. Classification by Severity and Control

This classification focuses on symptom frequency, intensity, and treatment response (GINA, 2023; Medscape, 2024). Intermittent asthma presents with symptoms less than two times per week and minimal activity interference. Mild persistent asthma has symptoms more than two times per week with minor limitations. Moderate persistent asthma is characterized by daily symptoms and reliever use, along with reduced lung function (FEV_1 60 to 80%). Severe persistent asthma presents with frequent symptoms, severe activity limitation, FEV_1 less than 60%, and often requires high-dose corticosteroids.

III. Classification by Immunologic and Cellular Profiles (Endotypes)

Endotypes are defined by specific molecular and cellular mechanisms (PMC, 2019; Wenzel *et al.*, 2023). Type 2 (T2)-high asthma includes allergic and eosinophilic asthma, is characterized by elevated IL-4, IL-5, IL-13, high eosinophils and IgE, and responds well to corticosteroids and biologics such as mepolizumab and omalizumab (PMC, 2024). Type 2 (T2)-low asthma includes neutrophilic or paucigranulocytic asthma, is more common in older adults, and is less responsive to corticosteroids. Management may involve macrolides or non-Th2 biologics (NCBI PMC, 2023; Dove Press, 2024).

IV. Other Special Types of Asthma

Pediatric asthma may present as episodic viral wheeze or multi-trigger wheeze and is influenced by genetics and environment (GINA, 2023). Adult-onset asthma is often more severe and linked to obesity or occupational factors (Amboss, 2025). Asthma-COPD overlap syndrome (ACOS) is seen in older adults with features of both diseases and requires dual management strategies (Verywell Health, 2009; Amboss, 2025).

2.3 PATHOPHYSIOLOGY OF ASTHMA

The pathophysiology of asthma centers around a complex interaction between immune cells, structural cells of the airway, and environmental triggers. These interactions result in persistent airway inflammation, exaggerated bronchoconstrictive responses, and, over time, structural changes in the airways known as remodeling (Holgate, 2012; GINA, 2023). At the core of asthma is inflammation, which is triggered by various stimuli including allergens, respiratory infections, exercise, cold air, irritants, and air pollutants (Busse and Lemanske, 2001; Holgate, 2012). This inflammation involves a range of immune cells such as mast cells, eosinophils, T-helper lymphocytes (particularly Th2 cells), macrophages, neutrophils, and dendritic cells. When activated, these cells release inflammatory mediators like histamine, leukotrienes, prostaglandins, and a group of cytokines particularly interleukins IL-4, IL-5, and IL-13 that promote the recruitment and activation of additional inflammatory cells (Barnes, 2008; Fahy, 2015). These mediators contribute to swelling of the airway lining, increased mucus secretion, and injury to the airway epithelium (Lambrecht and Hammad, 2015). In allergic asthma, Th2-type immune responses are dominant. IL-4 promotes IgE production by B cells, leading to mast cell sensitization; IL-5 is essential for eosinophil activation and survival; and IL-13 enhances mucus production and airway hyperresponsiveness (Wenzel, 2012; Fahy, 2015). This type 2 (T2) inflammation is usually associated with elevated eosinophil counts and IgE levels and tends to respond well to corticosteroids (Barnes, 2008; GINA, 2023). In contrast, non-allergic asthma may be driven by neutrophilic or mixed granulocytic inflammation, often resulting in a more severe disease course that responds poorly to conventional therapy (Wenzel, 2012; Chung, 2015). Airway hyperresponsiveness (AHR) is another key feature of asthma and refers to an exaggerated narrowing of the airways in response to stimuli that would not affect healthy individuals. This is due to the heightened sensitivity of airway smooth muscle, thickened airway walls, and chronic inflammation (Holgate, 2012; GINA, 2023). The resulting airflow obstruction is typically reversible but may become partially irreversible over time due to airway remodeling (Bousquet *et al.,* 2000). Bronchoconstriction, or tightening of the airway smooth muscles, is a major cause of airflow limitation during an asthma attack. This is compounded by the accumulation of thick mucus and inflammatory cells within the airways, as well as edema of the airway walls (Barnes, 2008). These changes narrow the airway lumen, reduce airflow, and cause the characteristic wheezing and breathlessness seen in asthma patients (GINA, 2023). Over time, persistent inflammation may lead to airway remodeling (a set of structural changes in the airway wall). These include thickening of the basement membrane, increased smooth muscle mass, goblet cell hyperplasia (increased mucus-producing cells), fibrosis, and the formation of new blood vessels (angiogenesis) (Bousquet *et al*., 2000; Holgate, 2012). These changes contribute to fixed airway narrowing and a more severe, less responsive form of asthma. The autonomic nervous system also plays a role in asthma pathophysiology. Activation of the parasympathetic nervous system causes bronchoconstriction through the release of acetylcholine (Barnes, 2008). Sensory nerves within the airways can be activated by irritants or inflammation, further amplifying bronchoconstriction and mucus secretion through neuropeptides (Barnes, 2008; Wenzel, 2012). Finally, asthma is now recognized as a heterogeneous condition with different underlying biological mechanisms, known as endotypes. The most commonly described are T2-high asthma, which is eosinophilic and corticosteroid-responsive, and T2-low asthma, which may involve neutrophilic inflammation and be more resistant to standard treatments (Wenzel, 2012; Fahy, 2015). Understanding these endotypes is crucial for guiding targeted therapies such as anti-IgE, anti-IL-5, and anti-IL-13 biologics in severe asthma (GINA, 2023). The pathophysiology of asthma involves chronic inflammation, airway hyperresponsiveness, episodic bronchoconstriction, excessive mucus production, and long-term structural changes in the airways. These processes interact in a dynamic and often patient-specific way, leading to the varied symptoms and disease patterns observed in asthma patients (Holgate, 2012; GINA, 2023).

2.4 OXIDANTS

Oxidants, also known as reactive oxygen species (ROS), are highly reactive oxygen-derived molecules that are produced naturally in the body during aerobic metabolism, particularly in the mitochondria. They can also be generated in response to external stimuli such as pollution, allergens, drugs, radiation, and infections (Halliwell and Gutteridge, 2015). Major ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and peroxynitrite ($ONOO^-$), all of which can damage essential biomolecules like lipids, proteins, and DNA due to their unpaired electrons and high reactivity (Valko *et al*., 2007). While often viewed as harmful, oxidants also have beneficial roles in low to moderate concentrations. They act as signaling molecules in physiological processes such as apoptosis, proliferation, and immune defense especially in the respiratory burst of phagocytes, which destroy pathogens (Nathan and Ding, 2010). However, excessive production of oxidants or impaired antioxidant defenses leads to oxidative stress, a condition marked by lipid peroxidation, protein oxidation, and DNA damage, contributing to tissue injury and the development of chronic diseases (Pham-Huy *et al*., 2008). In asthma and other respiratory disorders, oxidative stress is a major pathological feature. Activated immune cells like eosinophils and neutrophils release large quantities of ROS into lung tissue, causing epithelial damage, increased mucus production, vascular leakage, and airway hyperresponsiveness (MacNee, 2001; Comhair and Erzurum, 2010). Environmental pollutants such as tobacco smoke and ozone further amplify this oxidative burden. One key marker of oxidative damage is lipid peroxidation, where ROS attack cell membranes to form reactive aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which further disrupt protein and DNA function (Ayala *et al*., 2014). In ovalbumin-induced asthma models, such as those used in Sprague-Dawley rats, elevated ROS levels and lipid peroxidation are commonly observed, highlighting the role of oxidants in chronic inflammation and lung dysfunction

(Rahman and Adcock, 2006). Measuring these oxidant biomarkers is therefore critical for assessing both disease severity and the effects of antioxidant-based therapies.

2.4.1 EFFECT OF ASTHMA ON LUNG TISSUE OXIDANT ACTIVITIES

Oxidant activities in lung tissue play a central role in the pathogenesis and progression of asthma, particularly in experimental models such as ovalbumin-induced asthma in rats. Oxidants, primarily reactive oxygen species (ROS) like superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH), are generated both endogenously through cellular metabolism and exogenously via exposure to pollutants, allergens, or tobacco smoke (Rahman and Adcock, 2006). In asthma, the infiltration of inflammatory cells such as eosinophils, neutrophils, and macrophages into the airways significantly increases the production of ROS. These oxidants contribute to airway inflammation, epithelial damage, bronchial hyperresponsiveness, and tissue remodeling (Comhair and Erzurum, 2010). In ovalbumin-induced animal models, oxidative stress is typically elevated due to an imbalance between oxidant production and antioxidant defense mechanisms. The overproduction of ROS in the lungs causes lipid peroxidation, DNA damage, and protein modification, leading to impaired lung function and increased disease severity (Lee *et al.*, 2005). Elevated levels of oxidative biomarkers such as malondialdehyde (MDA), 8-isoprostane, and hydrogen peroxide in bronchoalveolar lavage fluid or lung homogenates confirm this oxidative burden in asthmatic models (Nadeem *et al.*, 2003). These biomarkers are not just indicators of oxidative stress but also mediators that further aggravate inflammation by activating transcription factors like nuclear factor-kappa B (NF-κB), which promotes the expression of pro-inflammatory cytokines and chemokines (Rahman, 2002). The effect of heightened oxidant activity is not limited to molecular damage. It also influences the structural integrity of lung tissue. ROS disrupt tight junctions in the airway epithelium, increasing permeability and susceptibility to allergen penetration. Furthermore, oxidants activate matrix metalloproteinases (MMPs), enzymes that degrade extracellular matrix components, contributing to airway remodeling and fibrosis (Cho *et al.*, 2009). This chronic oxidative environment sustains a vicious cycle where inflammation leads to more oxidant production, which in turn amplifies inflammation. Therapeutically, understanding oxidant activity in lung tissue is critical when evaluating the efficacy of drugs like salbutamol, montelukast, and prednisolone. While salbutamol is a bronchodilator, it may paradoxically enhance oxidative stress at high doses by increasing mitochondrial ROS generation in airway cells (Fang *et al.*, 2013). Montelukast and prednisolone, on the other hand, have demonstrated antioxidant properties in various studies by reducing oxidative biomarkers and downregulating oxidative pathways (Nadeem *et al.*, 2008; Yadav and Sah, 2010). Therefore, assessing the level of oxidant activity in lung tissue provides a direct measure of disease progression and drug response in experimental asthma studies.

2.5 ANTIOXIDANTS

Antioxidants are vital molecules that protect the body from oxidative stress by neutralizing reactive oxygen species (ROS) and other free radicals. These ROS, including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are naturally produced during metabolic processes like mitochondrial respiration and immune responses (Halliwell and Gutteridge, 2015). While low levels of ROS play important roles in cell signaling and immune defense, their excessive accumulation can damage lipids, proteins, and DNA, contributing to various diseases (Pham-Huy *et al.*, 2008). To maintain redox balance, the body employs both enzymatic and non-enzymatic antioxidant systems. Key enzymatic antioxidants include superoxide dismutase (SOD), which converts superoxide radicals into hydrogen peroxide; catalase (CAT), which breaks down hydrogen peroxide into water and oxygen; and glutathione peroxidase (GPx), which uses reduced glutathione (GSH) to detoxify peroxides (Birben *et al.*, 2012). Glutathione reductase (GR) further supports this process by regenerating GSH. Non-enzymatic antioxidants such as glutathione, vitamin C, vitamin E, carotenoids, flavonoids, uric acid, and melatonin also contribute by directly scavenging free radicals and supporting enzyme function (Pisoschi and Pop, 2015). These antioxidants help maintain cell membrane integrity, modulate redox-sensitive transcription factors (e.g., NF-κB, AP-1), and regulate inflammatory pathways (Rahman, 2002). In asthma, oxidative stress plays a key role in airway inflammation, mucus overproduction, epithelial damage, and bronchial hyperresponsiveness. Studies in allergen-induced asthma models, such as ovalbumin-sensitized rats, show increased ROS levels and reduced antioxidant enzyme activity, which worsen lung function (Rahman and Adcock, 2006; Comhair and Erzurum, 2010). The lungs are especially vulnerable due to their constant exposure to environmental oxidants and high oxygen levels. Activated immune cells, including eosinophils and macrophages, release ROS during allergic inflammation, further amplifying tissue damage and airway remodeling (MacNee, 2001). A weakened antioxidant defense allows oxidative injury to persist, contributing to the progression of chronic respiratory diseases.

2.5.1 EFFECT OF ASTHMA ON LUNG TISSUE ANTIOXIDANT ENZYME ACTIVITIES

Asthma, particularly when induced by allergens such as ovalbumin in animal models, significantly disrupts the balance between oxidants and antioxidants in the lungs. This imbalance is largely due to a marked reduction in antioxidant enzyme activities. Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are essential components of the body's defense system against oxidative stress. These enzymes act by neutralizing reactive oxygen species (ROS) and preventing the cellular and tissue damage that can result from excessive oxidant production (Rahman and Adcock, 2006; Comhair and Erzurum, 2010). In asthmatic conditions, persistent inflammation and immune cell activation increase the production of ROS, which in turn overwhelms the antioxidant defense system. Several studies in ovalbumin-induced asthmatic rats have reported a significant reduction in the activities of SOD, CAT, and GPx in lung tissues (Nadeem *et al.*, 2003; Lee *et al.*, 2005). This reduction impairs the lungs' ability to scavenge superoxide radicals and hydrogen peroxide, leading to oxidative stress-induced damage, lipid peroxidation, and further aggravation of inflammation and airway hyperresponsiveness. For instance, diminished SOD activity fails to dismutate superoxide anions into hydrogen peroxide, while inadequate CAT and GPx levels hinder the detoxification of hydrogen peroxide into water and oxygen (Rahman, 2002). Moreover, the decline in antioxidant enzyme activities contributes to the deterioration of lung structure and function. Studies have shown that reduced GPx activity correlates with enhanced mucus secretion and airway remodeling, both of which are characteristic features of chronic asthma (Cho *et al.*, 2009). Additionally, the impairment of antioxidant defenses may allow for the activation of redox-sensitive transcription factors like nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which promote the expression of pro-inflammatory cytokines and perpetuate the inflammatory cycle in asthmatic lungs (Rahman and Adcock, 2006). The measurement of antioxidant enzyme activities in lung tissues is a vital tool in asthma research, particularly when evaluating the efficacy of pharmacological interventions. Drugs such as salbutamol, montelukast, and prednisolone have shown varying capacities to restore or enhance antioxidant enzyme activities in asthmatic models. Montelukast, for example, has been reported to improve SOD and GPx activities by inhibiting leukotriene-mediated oxidative responses (Nadeem *et al.*, 2008). Prednisolone, a corticosteroid, exerts broad anti-inflammatory and antioxidant effects by suppressing ROS production and restoring the activity of endogenous antioxidant enzymes (Yadav and Sah, 2010). Understanding the changes in antioxidant enzyme activities during asthma is therefore essential not only for elucidating the disease mechanism but also for assessing the therapeutic potential of drug combinations in mitigating oxidative damage.

2.6 MEDICATIONS USED IN THE MANAGEMENT OF ASTHMA

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation. Medications used in the management of asthma aim to control the disease by addressing two core pathological processes: airway inflammation and bronchoconstriction. Accordingly, asthma medications are broadly classified into controller medications, which are used daily to prevent symptoms and maintain long-term control, and reliever medications, which offer quick symptom relief during acute exacerbations. A third group, add-on therapies or biologics, is reserved for patients with severe asthma who are unresponsive to conventional treatment (National Heart, Lung, and Blood Institute [NHLBI], 2022; Global Initiative for Asthma [GINA], 2023). These therapeutic agents target different aspects of asthma's pathophysiology, including airway smooth muscle constriction, immune cell activation, and oxidative stress.

Bronchodilators play a central role in asthma therapy by relaxing airway smooth muscles and improving airflow. They are subdivided into three main categories: β2-agonists, anticholinergics, and methylxanthines. Short-acting beta-2 agonists (SABAs), such as salbutamol (albuterol) and terbutaline, are the first-line agents for acute symptom relief due to their rapid onset (5–15 minutes) and relatively short duration (4–6 hours). They activate β2-adrenergic receptors on bronchial smooth muscle, resulting in muscle relaxation and bronchodilation (Beasley *et al.,* 2015). While effective in acute management, their overuse can lead to tolerance, decreased responsiveness, and potential systemic effects such as tremors, tachycardia, and possibly increased oxidative stress at higher doses. Long-acting beta-2 agonists (LABAs), such as salmeterol and formoterol, have a prolonged duration of action (12–24 hours). LABAs are used in maintenance therapy but must be combined with inhaled corticosteroids (ICS), as they do not address underlying inflammation and may even worsen asthma outcomes if used alone (Barnes, 2008). Anticholinergics (muscarinic antagonists) block muscarinic receptors in the airways, reducing parasympathetic-mediated bronchoconstriction. Short-acting muscarinic antagonists (SAMAs), such as ipratropium, are often used in acute settings, while long-acting muscarinic antagonists (LAMAs), like tiotropium, are incorporated into maintenance therapy for moderate-to-severe asthma (Bateman *et al*., 2008). Methylxanthines, such as theophylline, work by inhibiting phosphodiesterase, leading to increased cyclic AMP and bronchodilation. They also have mild anti-inflammatory properties. However, their use has declined due to a narrow therapeutic window and significant side effects, including nausea, insomnia, and cardiac arrhythmias (Barnes, 2003).

Inflammation is central to asthma pathogenesis, and targeting this component is crucial for long-term disease control. Inhaled corticosteroids (ICS), including beclomethasone, budesonide, and fluticasone, reduce inflammation by inhibiting pro-inflammatory cytokines, decreasing eosinophil infiltration, and stabilizing the airway epithelium. ICS are the cornerstone of maintenance therapy and significantly reduce exacerbations and symptom severity (Barnes, 2008; GINA, 2023). Systemic corticosteroids, such as prednisolone and methylprednisolone, are used orally or intravenously for acute exacerbations or severe, poorly controlled asthma. They exert broad anti-inflammatory effects and can influence not only cytokine production (e.g., TNF-α, IL-1) but also oxidative stress pathways by modulating antioxidant defenses (Barnes, 2010). Leukotriene receptor antagonists (LTRAs), such as montelukast and zafirlukast, block leukotriene D4 receptors. Leukotrienes are involved in bronchoconstriction, vascular permeability, and eosinophil recruitment. LTRAs are especially useful in patients with aspirin-exacerbated respiratory disease (AERD), exercise-induced asthma, or mild persistent asthma. They also exhibit antioxidant potential by reducing oxidative damage and lipid peroxidation in the lungs (Bisgaard, 2000; Bulat *et al.,* 2021).

Biologic therapies are advanced treatments used for severe asthma with specific immune profiles, often targeting IgE or interleukins associated with eosinophilic or allergic inflammation. Anti-IgE therapy, such as omalizumab, binds free IgE and prevents mast cell activation in allergic asthma (Busse *et al.*, 2001). Anti-IL-5 therapies, such as mepolizumab and reslizumab, target eosinophilic inflammation by blocking IL-5 signaling (Bel *et al.*, 2014). Anti-IL-4/IL-13 agents, such as dupilumab, inhibit type 2 inflammation, benefiting asthma patients with comorbid atopic diseases (Castro *et al.*, 2018).

Other agents include mast cell stabilizers, such as cromolyn sodium, which are now rarely used due to their lower efficacy. Immunotherapy (allergen desensitization) is used in allergic asthma through controlled allergen exposure. Combination inhalers, such as Seretide (fluticasone/salmeterol) and Symbicort (budesonide/formoterol), enhance adherence and treatment efficacy by combining anti-inflammatory and bronchodilatory actions.

While the primary roles of salbutamol, montelukast, and prednisolone are in relieving bronchoconstriction and suppressing inflammation, growing evidence suggests that these drugs also influence oxidative stress pathways in the lungs, a key element in asthma pathophysiology. Oxidative stress results from an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms, contributing to airway remodeling, mucus hypersecretion, and epithelial injury. Salbutamol, though primarily a bronchodilator, can influence redox balance via β2-receptor stimulation, which may enhance mitochondrial activity and ROS generation. In experimental models, prolonged or high-dose exposure has been associated with altered levels of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), potentially as an adaptive response to oxidative injury (Goyal *et al*., 2014). Montelukast, in addition to blocking leukotriene-mediated inflammation, has been shown to reduce lipid peroxidation markers like malondialdehyde (MDA) and enhance antioxidant enzyme activities in lung tissue. Its antioxidative potential may arise from dampening eosinophilic inflammation and downstream oxidative cascades (Bulat *et al*., 2021). Prednisolone exerts potent anti-inflammatory effects by downregulating cytokines such as TNF-α and IL-1β, but also indirectly boosts antioxidant capacity by reducing ROS production from activated immune cells. It may also upregulate antioxidant enzymes, supporting tissue repair and redox homeostasis in the lungs (Barnes, 2008; Kelso *et al*., 2016).

2.7 DRUGS IN TREATMENT OF ASTHMA

2.7.1 SALBUTAMOL

Salbutamol, also known as albuterol (especially in the United States), is a synthetic sympathomimetic amine primarily used as a bronchodilator to relieve symptoms of bronchial asthma and other obstructive airway conditions such as chronic obstructive pulmonary disease (COPD). It belongs to the class of drugs known as short-acting β_2-adrenergic receptor agonists (SABAs) (Ayşin Taşar *et al.,* 2005). Salbutamol is characterized by its selectivity for β_2-adrenoceptors in bronchial smooth muscle, resulting in prompt relaxation of airway muscles and improvement in airflow. It is widely recognized for its rapid onset of action, making it an essential rescue medication for acute asthma attacks and exercise-induced bronchospasm (M. Ayşin Taşar *et al*., 2005). Salbutamol can be administered via inhalation, oral, or intravenous routes, depending on the clinical situation. Inhalation is the preferred route due to its localized effect in the lungs, faster action, and fewer systemic side effects. The drug is included in the World Health Organization's List of Essential Medicines due to its critical role in the management of asthma, especially in emergency settings. Beyond its bronchodilatory function, salbutamol also has extrapulmonary effects, including influence on cardiac and skeletal muscle tissues due to systemic β-adrenergic stimulation, especially at higher doses. These effects may include tachycardia, tremor, hypokalemia, and in rare cases, cardiovascular complications, factors of particular relevance in studies examining its impact on cardiac biomarkers (Ayşin Taşar *et al.,* 2005).

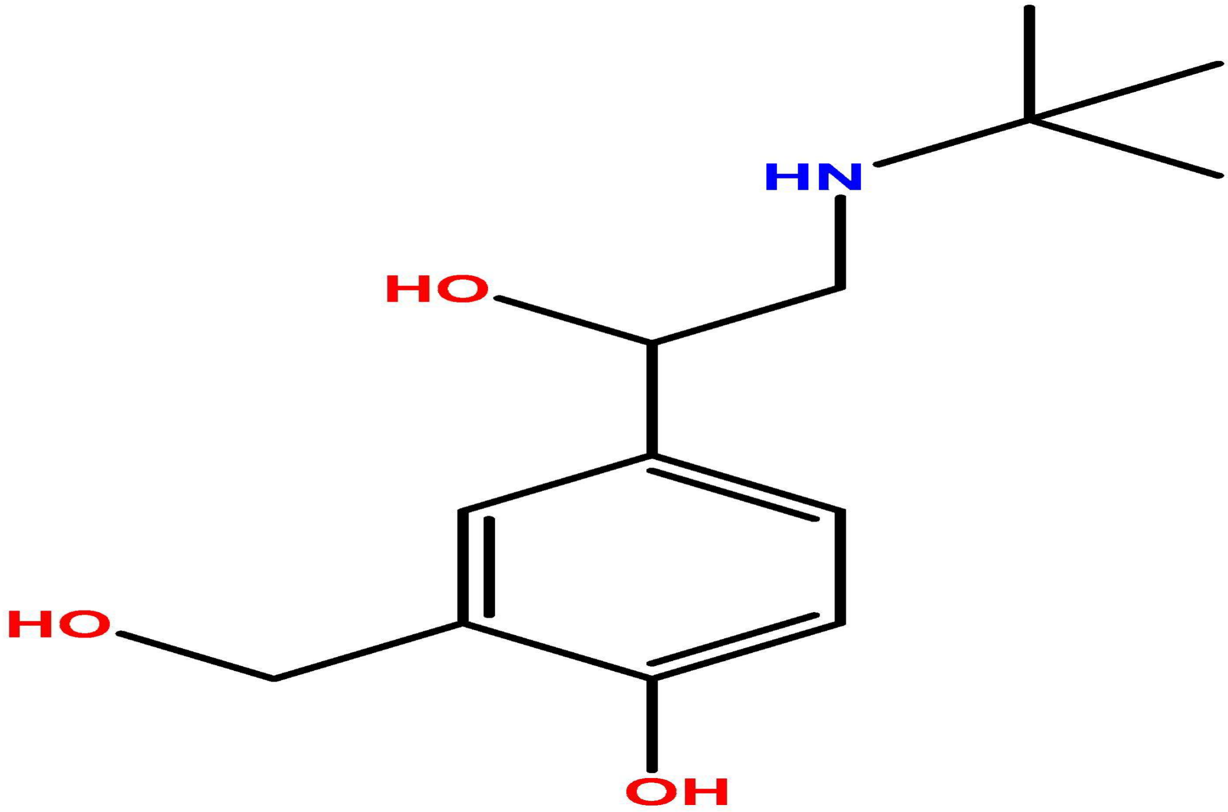

Figure 1: Chemical structure of salbutamol (Marques and Vale, 2022).

2.7.1.1 Mechanism of Action of Salbutamol

Salbutamol exerts its therapeutic effect primarily by stimulating β_2-adrenergic receptors located on the smooth muscle cells of the bronchial tree. Activation of these receptors leads to the stimulation of adenylate cyclase, an enzyme that catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP). The increased intracellular cAMP levels promote relaxation of bronchial smooth muscle, resulting in bronchodilation. Salbutamol enhances mucociliary clearance and reduces airway resistance, further improving airflow. Although salbutamol is β_2-selective, at higher doses or with prolonged use, it may also stimulate β_1-adrenergic receptors, particularly in cardiac tissues, potentially leading to side effects such as tachycardia, palpitations, and increased myocardial oxygen demand (Ayşin Taşar *et al*., 2005).

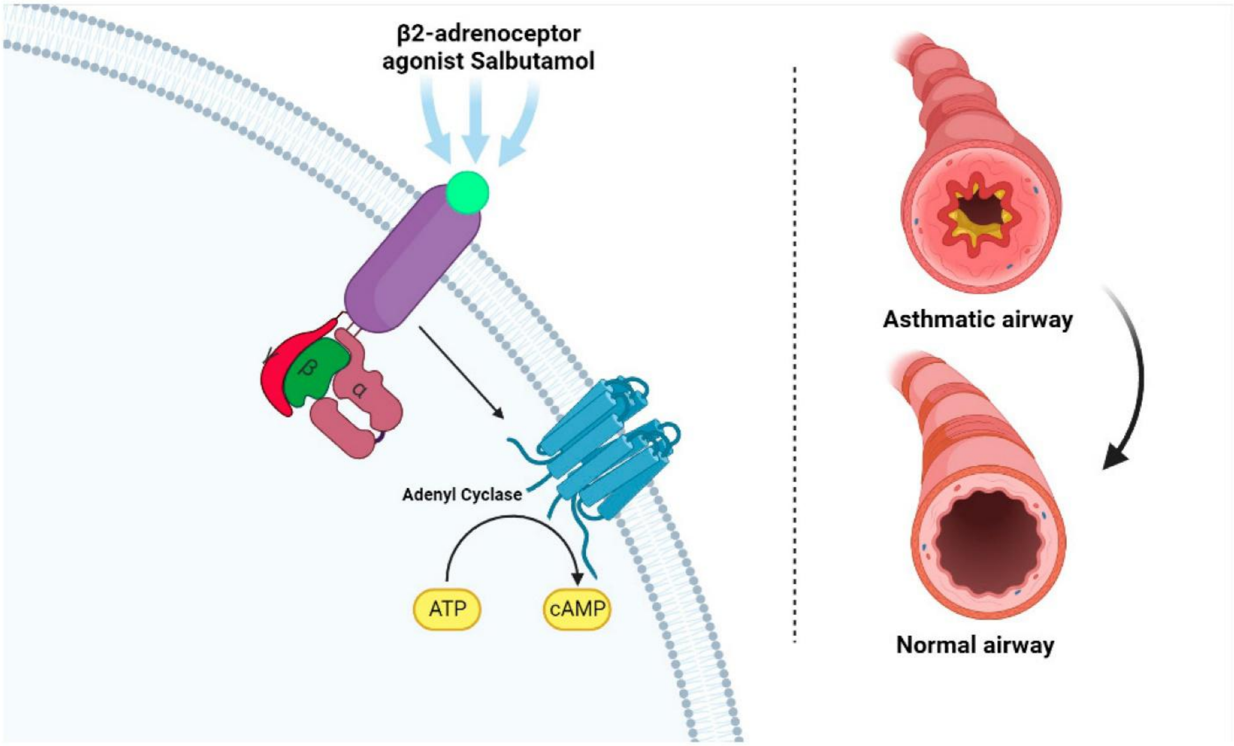

Figure 2: Schematic representation of the mechanism of action of salbutamol (Marques and Vale, 2022).

2.7.1.2 Role of Salbutamol in Asthma

In asthma therapy, salbutamol plays a vital role as a reliever or rescue medication. It is primarily used to quickly reverse acute bronchoconstriction, a hallmark symptom of asthma attacks. Salbutamol's onset of action is rapid (within 5–15 minutes), and its duration of effect lasts approximately 4–6 hours, making it ideal for on-demand symptom relief. Clinically, salbutamol provides immediate relief from symptoms such as wheezing, coughing, chest tightness, and shortness of breath. It is a cornerstone treatment in both mild intermittent asthma and as a supplemental therapy in persistent asthma, used alongside controller medications like corticosteroids (Taşar *et al.,* 2005; continuous albuterol in children, 2007).

2.7.1.3 Effect of Salbutamol on Lung Tissue Oxidant Activities

Salbutamol (also known as albuterol) is a short-acting β_2-adrenergic receptor agonist widely used in the management of asthma and other obstructive airway diseases due to its potent bronchodilatory properties. While its primary mechanism involves relaxing airway smooth muscles by increasing cyclic adenosine monophosphate (cAMP), emerging evidence suggests that salbutamol may also influence oxidative stress pathways in the lung tissue (Ricciardolo *et al*., 2004; Barnes, 2011). In the context of asthma, oxidative stress arises from the overproduction of reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), primarily released by activated immune cells like eosinophils, neutrophils, and macrophages in response to allergens or inflammatory stimuli (Comhair and Erzurum, 2010).

Several studies have shown that salbutamol can indirectly modulate oxidant activities in lung tissue. For instance, its anti-inflammatory effect has been associated with reduced recruitment and activation of inflammatory cells, thereby potentially limiting ROS generation (Mak *et al.,* 2002). Additionally, β_2 agonists may attenuate oxidative stress by downregulating pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins, and leukotrienes, which otherwise promote oxidative burst in immune cells (Kunzli *et al*., 2005). However, high doses or prolonged use of salbutamol have been reported in some experimental models to paradoxically increase oxidative stress due to overstimulation of β_2 receptors, which may lead to mitochondrial dysfunction and excessive ROS production (Zeng *et al*., 2013). This dual effect underscores the need to consider dosage and treatment duration when evaluating its impact on oxidant activity.

In ovalbumin-induced asthma models, oxidative stress markers such as malondialdehyde (MDA), a byproduct of lipid peroxidation, are typically elevated due to heightened ROS activity. Salbutamol treatment has been shown in some studies to reduce MDA levels and other oxidative markers, suggesting a possible protective effect against allergen-induced oxidative injury in the lungs (Gurgueira *et al*., 2002). However, these effects may vary depending on the severity of inflammation, duration of exposure, and whether salbutamol is used alone or in combination with other anti-inflammatory agents. Overall, while salbutamol primarily acts as a bronchodilator, it may exert secondary effects on lung tissue oxidant activities by modulating inflammatory pathways and ROS production.

2.7.1.4 Effect of Salbutamol on Lung Tissue Antioxidant Enzyme Activities

Salbutamol's effect on lung tissue antioxidant activities is closely tied to its anti-inflammatory properties and its influence on redox homeostasis. Antioxidants, both enzymatic (e.g., superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx]) and non-enzymatic (e.g., glutathione, vitamins C and E), are crucial for neutralizing reactive oxygen species (ROS) and preventing oxidative damage in lung tissues (Rahman, 2002). In asthmatic lungs, a decrease in antioxidant enzyme activity is frequently observed, contributing to an imbalance between oxidants and antioxidants, a condition that exacerbates tissue injury and airway remodeling (Comhair and Erzurum, 2010).

Salbutamol may help restore this balance by indirectly enhancing the antioxidant defense system. Through its ability to reduce inflammatory cell infiltration and ROS production, salbutamol minimizes the burden placed on endogenous antioxidant enzymes, thereby preserving or even improving their activity levels (Mak *et al.*, 2002). Some experimental models have shown increased activity of SOD, CAT, and GPx in salbutamol-treated groups compared to untreated asthmatic controls, suggesting that salbutamol contributes to maintaining antioxidant capacity in the lungs (Ogunlana *et al.,* 2006). This is particularly important in chronic asthma, where persistent inflammation leads to sustained oxidative stress and antioxidant depletion.

Moreover, by increasing intracellular cAMP levels, salbutamol may exert antioxidant effects at the molecular level, as cAMP has been shown to regulate redox-sensitive transcription factors like nuclear factor erythroid 2-related factor 2 (Nrf2), which enhances the expression of antioxidant enzymes (Lee and Yang, 2012). This cAMP-Nrf2 pathway may be a key mechanism through which salbutamol contributes to antioxidant activity, although more studies are needed to fully elucidate this effect.

Nevertheless, the antioxidant benefits of salbutamol may not be universally consistent across all conditions. In some models, especially those involving high oxidative loads or chronic β_2-agonist use, antioxidant enzyme levels remain suppressed despite salbutamol treatment, possibly due to receptor desensitization or insufficient control of underlying inflammation (Barnes, 2006). Thus, while salbutamol shows promise in enhancing antioxidant defense, its efficacy may depend on factors such as disease severity, treatment regimen, and combination with other therapeutic agents like corticosteroids or leukotriene antagonists.

2.7.2 MONTELUKAST

Montelukast is a leukotriene receptor antagonist with the molecular formula $C_{35}H_{36}ClNO_3S$. The molecule features a quinoline ring, a cyclopropane acetic acid group, and specific substituents designed to enhance its effectiveness and selectivity for the cysteneinyl leukotriene receptor type 1. Montelukast is an orally administered leukotriene receptor antagonist (LTRA) commonly used as a controller medication in the long-term management of bronchial asthma and allergic rhinitis (Koyama *et al.*, 2002). Unlike bronchodilators that act rapidly to relieve acute symptoms, montelukast works by targeting the underlying inflammatory processes that contribute to chronic asthma. It is approved for use in both adults and children and is generally well tolerated, making it a convenient and effective option for maintenance therapy (Koyama *et al.*, 2002).

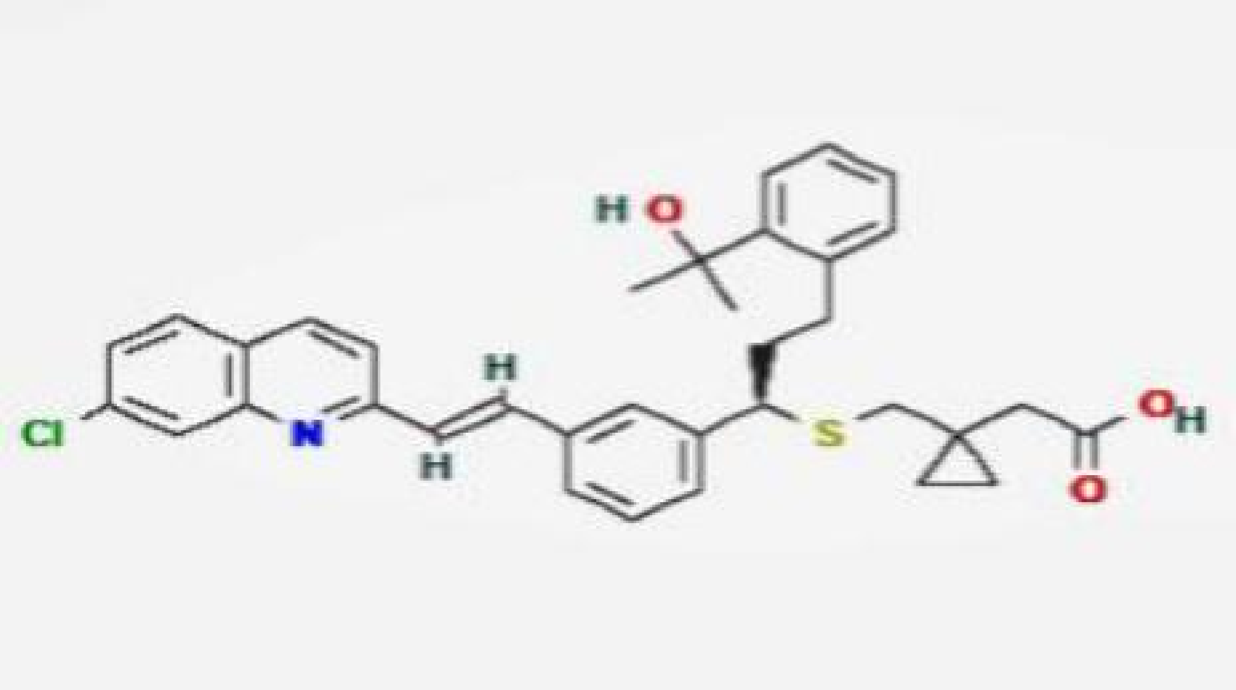

Figure 3: chemical structure of Montelukast (Okumu *et al.,* 2008).

2.7.2.1 Mechanism of Action of Montelukast

The mechanism of action of montelukast involves selective and competitive inhibition of the cysteinyl leukotriene receptor 1 (CysLT1), which is located on airway smooth muscle cells and various inflammatory cells. Leukotrienes such as LTC_4, LTD_4, and LTE_4 are potent inflammatory mediators derived from arachidonic acid through the 5-lipoxygenase pathway. These molecules play a critical role in the pathophysiology of asthma by promoting bronchoconstriction, increasing vascular permeability, stimulating mucus secretion, and attracting eosinophils to the airways. By blocking the interaction between leukotrienes and the CysLT1 receptor, montelukast effectively reduces airway inflammation, improves airflow, and decreases the severity and frequency of asthma symptoms and exacerbations (Koyama *et al*., 2002).

2.7.2.2 Role of Montelukast in Asthma

In clinical practice, montelukast serves as a controller medication used for the prevention of asthma symptoms rather than immediate relief. It is particularly useful in patients with aspirin-sensitive asthma, exercise-induced bronchospasm, and asthma associated with allergic rhinitis. Although it does not directly induce bronchodilation like short-acting β_2-agonists, montelukast plays a significant role in reducing asthma flare-ups by modulating the leukotriene pathway. Its oral route of administration and favorable safety profile enhance patient compliance, and it is often used as an add-on therapy in individuals who do not achieve full symptom control with inhaled corticosteroids alone (Koyama *et al*., 2002).

2.7.2.3 Effect of Montelukast on Lung Tissue Oxidant Activities

Montelukast is a leukotriene receptor antagonist (LTRA) that selectively blocks cysteinyl leukotriene receptor 1 ($CysLT_1$), thereby preventing the pro-inflammatory actions of leukotrienes such as LTC_4, LTD_4, and LTE_4, which are elevated in allergic asthma and contribute to bronchoconstriction, mucus secretion, eosinophilic infiltration, and oxidative stress (Capra *et al*., 2012). In the context of asthma, increased production of reactive oxygen species (ROS) and oxidative stress plays a significant role in airway inflammation and tissue damage. Immune cells such as eosinophils and macrophages generate high levels of ROS during allergic responses, contributing to oxidative injury and airway remodeling (MacNee, 2001).

Montelukast has demonstrated promising effects in reducing oxidant activity in various experimental asthma models. By antagonizing leukotriene signaling, montelukast not only attenuates bronchial inflammation but also limits the recruitment and activation of ROS-producing cells in the lung tissue. For instance, studies in ovalbumin-induced asthmatic rats have shown that montelukast significantly reduces levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, suggesting its ability to suppress oxidative damage in pulmonary tissues (Nakamura *et al.*, 2003). Furthermore, montelukast decreases levels of inducible nitric oxide synthase (iNOS) and nitric oxide (NO), which are also involved in oxidative stress-mediated lung injury (Hoshino *et al.*, 2009).

The reduction in oxidant activity following montelukast administration may be linked to both direct and indirect mechanisms. Directly, montelukast inhibits leukotriene-mediated oxidative bursts. Indirectly, it downregulates pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (e.g., IL-4, IL-5), thereby reducing immune activation and ROS generation (Di Gangi *et al.,* 2009). By curbing oxidative stress, montelukast helps to preserve lung tissue integrity and reduces airway hyperresponsiveness.

However, the extent of its oxidant-reducing effects may vary depending on the severity and chronicity of the disease, dosage, and timing of administration. Some studies have reported partial reductions in oxidative markers, indicating that while montelukast is effective, it may be more beneficial when used in combination with other anti-inflammatory agents such as corticosteroids (Antczak *et al.,* 2005). Overall, montelukast contributes significantly to reducing oxidative burden in asthmatic lungs, which is critical for improving respiratory function and preventing long-term structural damage.

2.7.2.4 Effect of Montelukast on Lung Tissue Antioxidant Enzyme Activities

In addition to reducing oxidant activity, montelukast has been shown to enhance or preserve antioxidant enzyme activities in lung tissue, thereby contributing to the restoration of redox balance in asthmatic conditions. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play critical roles in neutralizing harmful ROS and protecting against oxidative damage. In asthmatic lungs, especially in ovalbumin-induced models, these enzymes are often depleted due to excessive oxidative stress and ongoing inflammation (Rahman, 2002).

Montelukast appears to mitigate this depletion by reducing oxidative burden and indirectly upregulating antioxidant defenses. Experimental studies have demonstrated that montelukast treatment increases the activity of SOD and CAT in lung tissues, which helps convert superoxide radicals to less harmful molecules like hydrogen peroxide and subsequently to water and oxygen (Nakamura *et al.*, 2003; Tug Tunc *et al.*, 2007). This enzymatic action is crucial in preventing lipid peroxidation and maintaining cellular integrity in the lungs.

Moreover, montelukast has been found to increase glutathione (GSH) levels in lung tissue. Glutathione is a vital non-enzymatic antioxidant that works in conjunction with GPx to detoxify peroxides and regenerate other antioxidants. By elevating GSH and GPx activity, montelukast further contributes to reducing oxidative injury (Yildiz *et al.,* 2010). These effects may be partly mediated through the suppression of inflammatory signaling pathways, particularly NF-κB, which is known to regulate the expression of both oxidative and antioxidant genes (Rahman and Adcock, 2006).

The antioxidant-enhancing effects of montelukast are particularly beneficial in chronic asthma models where prolonged oxidative stress leads to sustained damage and remodeling of the airways. Some studies also suggest that montelukast can modulate redox-sensitive transcription factors like Nrf2, which upregulates antioxidant enzyme expression, although this mechanism requires further investigation (Lee and Yang, 2012).

2.7.3 PREDNISOLONE

Prednisolone is a synthetic glucocorticoid derived from cortisol and widely used as an anti-inflammatory and immunosuppressive agent. It is commonly prescribed in the treatment of various inflammatory and autoimmune disorders, including bronchial asthma, due to its potent ability to modulate immune responses and reduce inflammation (Rhen and Cidlowski, 2005; Barnes, 2010).

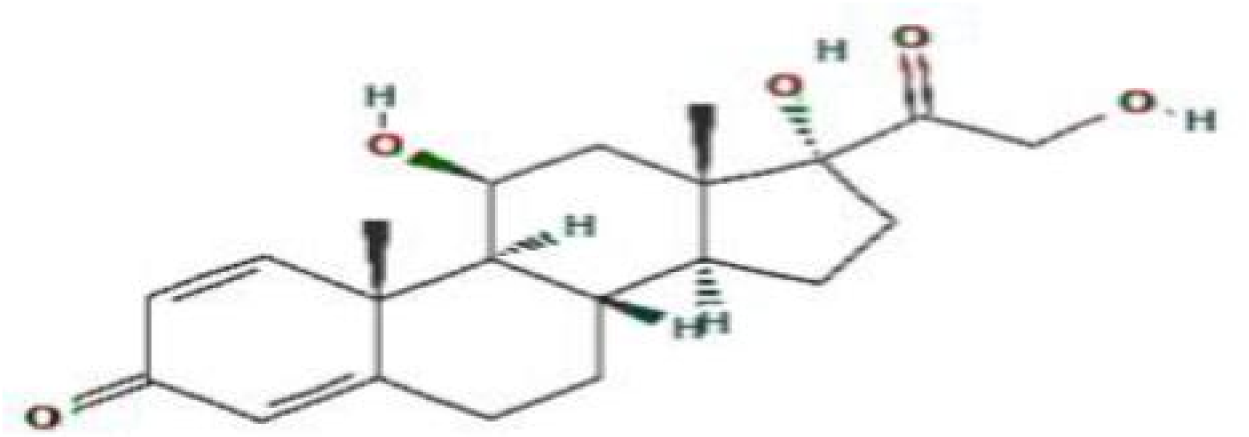

Figure 4: Chemical structure of Prednisolone (Burns and Davenport, 2020).

2.7.3.1 Mechanism of Action of Prednisolone

Prednisolone exerts its effects by binding to intracellular glucocorticoid receptors in the cytoplasm. This receptor-drug complex then translocates to the nucleus, where it interacts with glucocorticoid response elements on DNA to modulate gene expression. Through this mechanism, prednisolone suppresses the transcription of pro-inflammatory cytokines such as interleukins, tumor necrosis factor-alpha (TNF-α), and interferon-gamma. Simultaneously, it enhances the expression of anti-inflammatory proteins like lipocortin-1 (annexin A1), which inhibits phospholipase A2 and subsequently the arachidonic acid cascade, a key pathway in the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes (Newton and Holden, 2007; Barnes, 2011). This multifaceted regulation accounts for its broad-spectrum anti-inflammatory and immunosuppressive effects.

2.7.3.2 Role of Prednisolone in Asthma

In the context of bronchial asthma, prednisolone plays a critical role in the management of acute exacerbations and in cases of severe, persistent asthma that do not respond adequately to inhaled corticosteroids alone. It helps to rapidly reduce airway inflammation, decrease mucosal edema, and limit immune cell infiltration within the bronchial mucosa (GINA, 2024). Prednisolone accomplishes this by inhibiting the activation and recruitment of key inflammatory cells such as eosinophils, mast cells, and T lymphocytes (Adcock and Caramori, 2001). Furthermore, it enhances the sensitivity and responsiveness of β2-adrenergic receptors, thereby improving the bronchodilatory efficacy of medications like salbutamol (Barnes, 2006).

2.7.3.3 Effect of Prednisolone on Lung Tissue Oxidant Activities

Prednisolone, a synthetic glucocorticoid, is widely recognized for its potent anti-inflammatory and immunosuppressive effects in the treatment of asthma and other inflammatory lung diseases. In the context of oxidant activity, prednisolone plays a pivotal role in mitigating oxidative stress by modulating the production and activity of reactive oxygen species (ROS) in lung tissues. Asthma is characterized by chronic inflammation that stimulates the activation of immune cells such as eosinophils, neutrophils, and macrophages, which in turn release large amounts of ROS, contributing to oxidative lung injury and airway remodeling (Rahman and MacNee, 1996).

Prednisolone reduces oxidant activity by suppressing the activation of nuclear factor kappa B (NF-κB), a redox-sensitive transcription factor responsible for upregulating pro-inflammatory cytokines and enzymes like inducible nitric oxide synthase (iNOS) and NADPH oxidase, both of which are major sources of ROS in inflamed tissues (Barnes, 2011). By inhibiting these pathways, prednisolone significantly decreases ROS production and dampens oxidative stress. Studies involving ovalbumin-induced asthma models in rats have shown that prednisolone treatment reduces levels of oxidative stress markers such as malondialdehyde (MDA) and nitric oxide (NO), indicating its effectiveness in controlling oxidant activity in the lungs (Mishra *et al*., 2012; Al-Rikabi *et al*., 2018).

Moreover, prednisolone limits the recruitment and activation of inflammatory cells into the airway mucosa, which indirectly lowers ROS production. This includes the downregulation of adhesion molecules and chemokines involved in leukocyte migration to the lungs (Barnes, 2006). It also stabilizes lysosomal membranes and inhibits phospholipase A2, thereby reducing the release of arachidonic acid, a precursor of several inflammatory and oxidative mediators such as prostaglandins and leukotrienes (Rhen and Cidlowski, 2005).

Although prednisolone does not act as a direct antioxidant, its ability to suppress oxidative stress lies in its capacity to halt the inflammatory cascade that underpins ROS generation. The extent of oxidant reduction may vary depending on the dose and duration of therapy, but overall, prednisolone is effective in restoring redox homeostasis and preventing ROS-induced pulmonary damage in asthma and other respiratory disorders.

2.7.3.4 Effect of Prednisolone on Lung Tissue Antioxidant Enzyme Activities

Beyond reducing oxidant production, prednisolone has been shown to exert protective effects on the antioxidant defense system in lung tissues. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play vital roles in neutralizing ROS and preventing oxidative damage in asthmatic lungs. In inflammatory conditions like asthma, these enzyme systems are often compromised due to persistent oxidative stress and inflammation, leading to impaired redox balance and tissue injury (Comhair and Erzurum, 2010).

Prednisolone helps restore antioxidant enzyme activity by attenuating inflammation-induced enzyme depletion and enhancing cellular antioxidant capacity. Research on ovalbumin-induced asthma models has revealed that treatment with prednisolone significantly increases the activities of SOD, CAT, and GPx in lung tissue, suggesting a reversal of oxidative damage and restoration of enzymatic defense (Kirkham and Rahman, 2006; Mishra *et al.*, 2012). This is likely mediated through the suppression of ROS-generating pathways and reduced burden on endogenous antioxidants, allowing enzymes to regain functionality.

Furthermore, prednisolone may indirectly promote antioxidant activity by modulating transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates the expression of antioxidant enzymes (Rahman and Adcock, 2006). While this mechanism is more established for natural antioxidants and certain synthetic compounds, some evidence suggests that corticosteroids may influence Nrf2 signaling, especially under oxidative stress conditions (Biswas and Rahman, 2008).

Additionally, prednisolone has been observed to restore levels of glutathione (GSH), a critical non-enzymatic antioxidant, thereby enhancing glutathione-dependent antioxidant pathways such as those involving GPx and glutathione reductase (GR). This replenishment of GSH also contributes to reducing lipid peroxidation and maintaining membrane integrity in lung tissues (Haddad and Harb, 2005).

Overall, prednisolone's effect on antioxidant enzymes reflects its broader role in preserving redox equilibrium in the lungs. By mitigating inflammatory stimuli and oxidative insults, it supports the recovery and activity of essential antioxidant defenses. This dual action of reducing oxidant production while enhancing antioxidant enzyme activities makes prednisolone a valuable therapeutic agent in managing asthma-associated oxidative stress.

2.8 ANIMAL MODELS OF ASTHMA

Animal models of asthma provide a valuable tool for studying asthma within a fully functioning immune and respiratory system. These models have highlighted the role of T-helper type 2 mediated allergic responses in asthma development and have been instrumental in identifying potential drug targets related to allergic pathways. However, several drugs that show effectiveness in animal models of asthma have had limited clinical success 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.

Mice Models: Mice are the most commonly used species in asthma research, largely due to their genetic manipulability and the ability to induce allergic responses through sensitization with allergens like ovalbumin (Woodrow *et al.*, 2023).

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 (Woodrow *et al.,* 2023).

Rat Models: Asthma symptoms, such as airway hyper responsiveness and inflammation, are generally easier to replicate in rats than in mice. Rats are also larger and more manageable, allowing for the collection of larger sample volumes, making rat models of asthma increasingly valuable (Camps-Bossacoma *et al.,* 2015). However, strains like Wistar, Sprague-Dawley, Fisher, and Lewis rats do not always develop an allergic response with IgE production. Despite this, asthma models have been successfully established in Wister and Sprague-Dawley rats (Kucharewicz *et al.,* 2008). On the other hand, the Brown Norway rat is an atopic strain that readily produces IgE responses after allergen sensitization, making it a more suitable model for studying allergic asthma. Rats, such as the Brown-Norway strain, are used in asthma research, though less frequently than mice and guinea pigs. They can develop allergic responses and exhibit airway hyperresponsiveness, but typically require higher doses of antigens compared to other animal models (Zosky and Sly, 2007).

Larger Animals: Larger animals like sheep, dogs, and non-human primates are also employed in asthma studies. Non-human primates, in particular, share similar allergen responses with humans, making them valuable for understanding more complex immune responses. However, their use is more costly and logistically challenging compared to smaller animal models (Zosky and Sly, 2007).

CHAPTER 3

METHODOLOGY

3.0 MATERIALS

Materials used for this study include:

Feed

Clean water

Plastic cages

Chloroform

Dissection materials

Aluminium Hydroxide

Ovalbumin (OVA)

Compressor Nebulizer

Oral gavage

Syringes

Gloves

EDTA Bottles

Formaldehyde

Weighing machine

Cotton wool

Beaker

Plastic cages

Challenge box

Salbutamol

Montelukast

Prednisolone

Saline water

Refrigerator

Mentholated spirit

Saw dust

3.1 EXPERIMENTAL ANIMALS

This study was carried out using female Sprague-Dawley rats. They all 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, cool and sterile environment at room temperature and they were kept in cages where they had access to food and water and libitum throughout the experimental period.

3.2 EXPERIMENTAL DESIGN

Forty (40) female Sprague-Dawley rats weighing between 180-250g were sourced from the animal house in Lagos Nigeria and transported to the university of Benin. The rats were acclimatized for two weeks after which they were randomly assigned into five (5) groups of eight (8) rats per group.

3.3 DURATION OF STUDY

This study was conducted for a period of seven (7) weeks, during which there were two(2) weeks of acclimatization, one(1) week of sensitization and four (4) weeks of administration and challenge.

3.4 EXPERIMENTAL PROTOCOL/DESIGN

Experiment was carried out in phases

3.4.1 PHASE 1

Rats were acclimatized for two weeks into their new environment after which they were assigned into groups of five (5) with eight (8) rats per group.

Test groups

Group 1: Negative control

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

Group 3:Asthma induced and treated with prednisolone and salbutamol

Group 4:Asthma induced and treated with montelukast and prednisolone

Group 5:Asthma induced and treated with salbutamol and montelukast

: The positive control group and all test groups were induced with asthma following the modified guideline outlined by (Bai *et al*., 2019; Wu *et al*., 2019). All experimental groups (2,3,4 and 5) were sensitized to intraperitoneal injection of 1mg OVA and 20mg aluminium hydroxide dissolved in 0.9 saline on days 1 and 7. After which they were challenged by placing them in a transparent box measuring 50cm by 40cm by 30cm pumped full with an aerosolised solvent consisting of 1% w/v OVA dissolved in 0.9 saline using a compressor Nebulizer (CNB 69009) with aerosol delivery rate of > 0.25 ml/min for 15minutes bi-weekly for 28 days.

The negative control group was sensitized and challenged with an intraperitoneal injection of saline and aerosolised saline, respectively. Asthma induction was verified the first week after the challenge with evidence of neutrophilia and eosinophilia in randomly selected rat groups induced with asthma and compared against the negative control (Bai *et al*., 2020, wu e*t al*., 2019).

3.4.2 PHASE 2

After confirming asthma in all test groups, Treatment began with 2mg/ml salbutamol (oral)(Nair and prabhavalkar, 2021), 10mg/ml montelukast, 3mg/ml prednisolone (oral) for 28 days (4 weeks).

At the end of the treatment period, the blood pressure of all groups was measured using IITC noninvasive mouse rat blood pressure apparatus , using a noninvasive tail cuff method.

3.4.3 PHASE 3

All animals were euthanized after the drug administration. Blood and tissue samples were collected for antioxidants assay and histology.

3.5 BLOOD, TISSUE SAMPLE COLLECTION AND ANALYSES

3.5.1 BLOOD SAMPLING AND SERUM ISOLATION

Blood samples were immediately collected from euthanised rats via cardiac puncture and the portal vein. They were kept at room temperature for 30min, followed by centrifugation at 5000rpm (rounds per minute) for 15 minutes, and serum isolated by aspiration .The separated serum was frozen for the later quantitative determination of some biomarkers (Thakur *et al*., 2019).

3.5.2 HISTOLOGICAL ANALYSIS

Dissected lung tissues were washed with normal saline , immediately in 10% (V/V) formaldehyde solution and embedded in paraffin. Tissue specimens were sectioned and stained with haematoxylin and cosin (H & E) dye. Images of selected sections were captured at 10X magnifications using a 200 m digital camera (Thakur *et al*., 2019).

3.5.3 DETERMINATION OF TOTAL AND DIFFERENTIAL CELL COUNTS

Total and differential leukocytes (Neutrophils, Eosinophils, lymphocytes, and Monocytes) were measured in the blood using a fully automated cell counter.

3.6 STATISTICAL ANALYSIS

All the data obtained from the experiments was expressed as mean + Standard Error of Mean(SEM): Statistical analysis was performed by one- way analysis of variance (ANOVA) to assess differences amongst multiple groups, followed by Tukey's post-hoc test using Graph pad prism 10.0.3 statistical analysis software (Graphpad, San Diego, CA). $P < 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

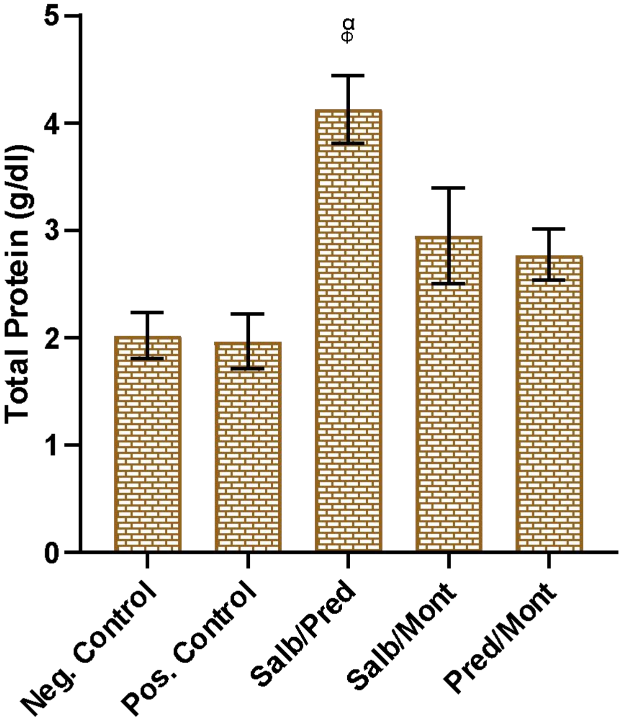

Figure 4.1: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone on lung tissue total protein activities in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show a statistically significant increase in lung tissue total protein concentration in the salbutamol/prednisolone-treated group compared with the negative controls ($p<0.05$), but no statistically significant difference among the positive control, salbutamol/montelukast, and prednisolone/montelukast groups compared with the negative control ($p>0.05$). $n=4$ ± SEM. $\alpha p<0.05$ compared to negative control; $\Phi p<0.05$ compared to positive control.

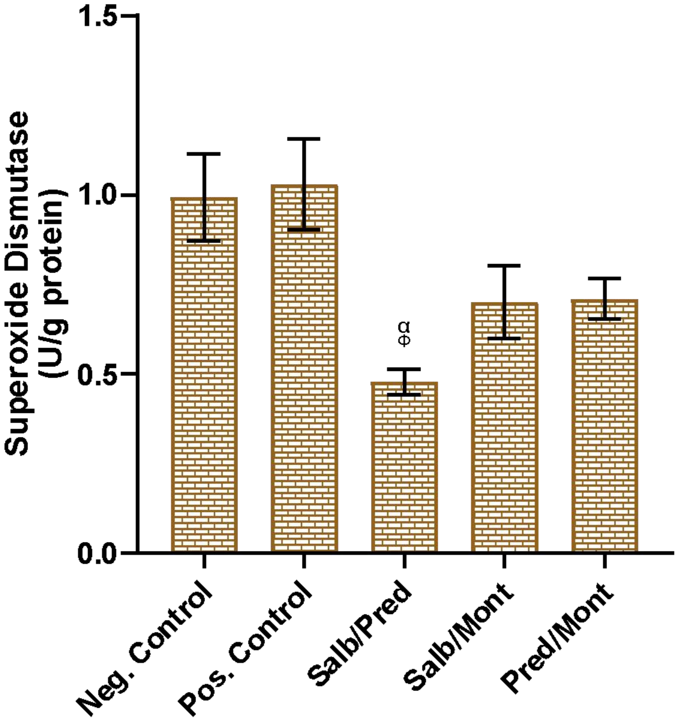

Figure 4.2: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone on lung tissue Superoxide dismutase activities in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show a statistically significant decrease in lung tissue superoxide dismutase enzyme activity in the salbutamol/prednisolone-treated group compared with the negative controls ($p<0.05$), but no statistically significant difference among the positive control, salbutamol/montelukast, and prednisolone/montelukast groups compared with the negative control ($p>0.05$). n=4 ± SEM. α$p<0.05$ compared to negative control; Φ$p<0.05$ compared to positive control.

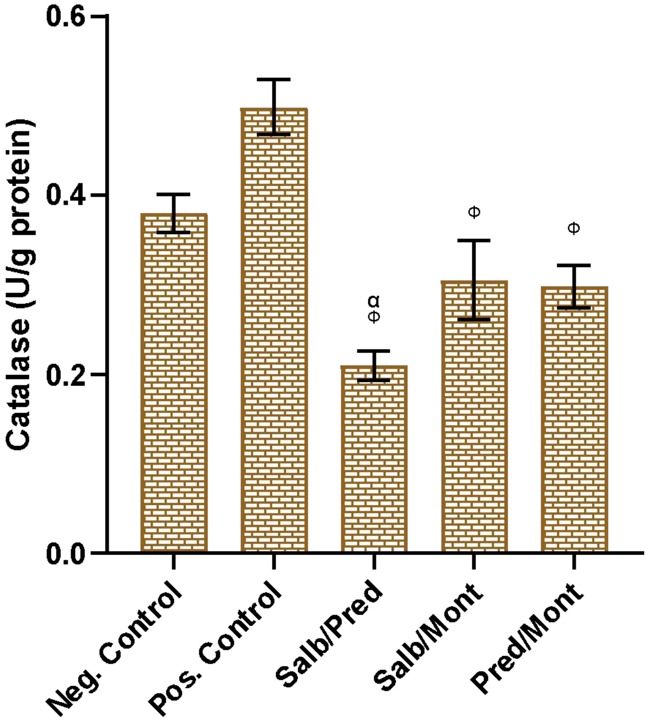

Figure 4.3: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone on lung tissue catalase activities in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show a statistically significant decrease in lung tissue catalase enzyme activity in the salbutamol/prednisolone-treated group compared with the negative and positive controls ($p<0.05$), but no statistically significant difference among the positive control, salbutamol/montelukast, and prednisolone/montelukast groups compared with the negative control ($p>0.05$). Also, there was a statistically significant decrease in the salbutamol/montelukast and prednisolone/montelukast-treated groups compared to the positive control ($p<0.05$). n=4 ± SEM. $\alpha p<0.05$ compared to negative control; $\Phi p<0.05$ compared to positive control.

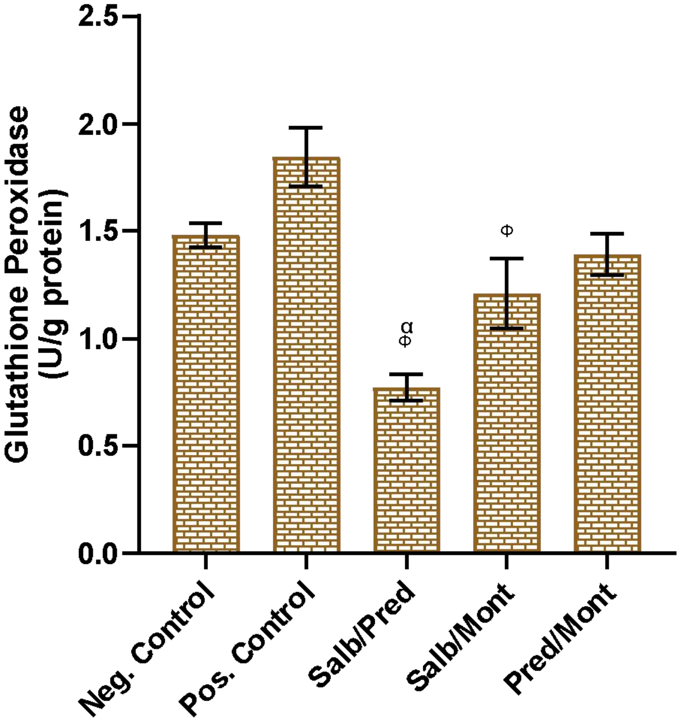

Figure 4.4: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone on lung tissue glutathione peroxidase activities in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show a statistically significant decrease in lung tissue glutathione peroxidase enzyme activity in the salbutamol/prednisolone-treated group compared with the negative and positive controls ($p<0.05$), but no statistically significant difference among the positive control, salbutamol/montelukast, and prednisolone/montelukast groups compared with the negative control ($p>0.05$). Also, there was a statistically significant decrease in the salbutamol/montelukast-treated groups compared to the positive control ($p<0.05$). n=4 ± SEM. $\alpha p<0.05$ compared to negative control; $\Phi p<0.05$ compared to positive control.

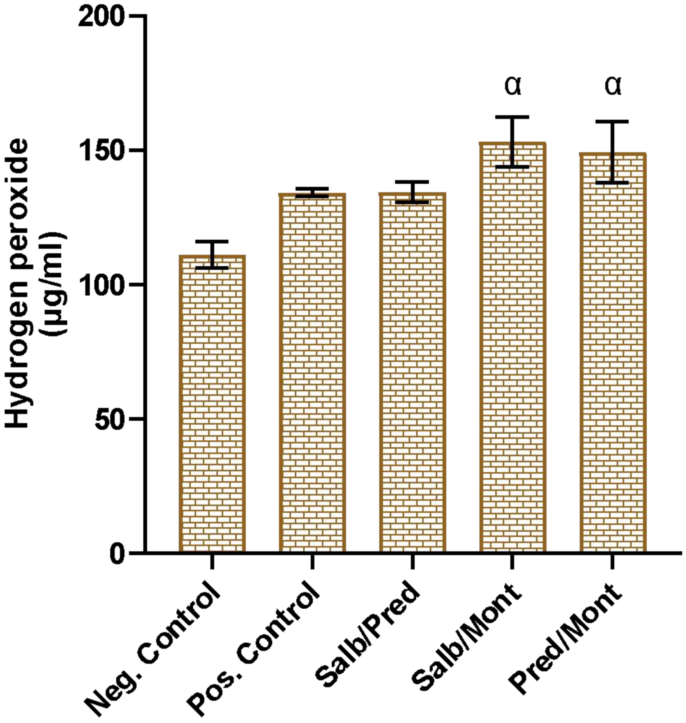

Figure 4.5: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone combination on lung tissue hydrogen peroxide concentration in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show a statistically significant increase in lung tissue hydrogen peroxide concentration in the salbutamol/montelukast and prednisolone montelukast-treated groups compared with the negative control ($p<0.05$), but no statistically significant difference among the positive control, and salbutamol/prednisolone groups compared with the negative control ($p>0.05$). n=4 ± SEM. $\alpha p<0.05$ compared to negative control; $\Phi p<0.05$ compared to positive control.

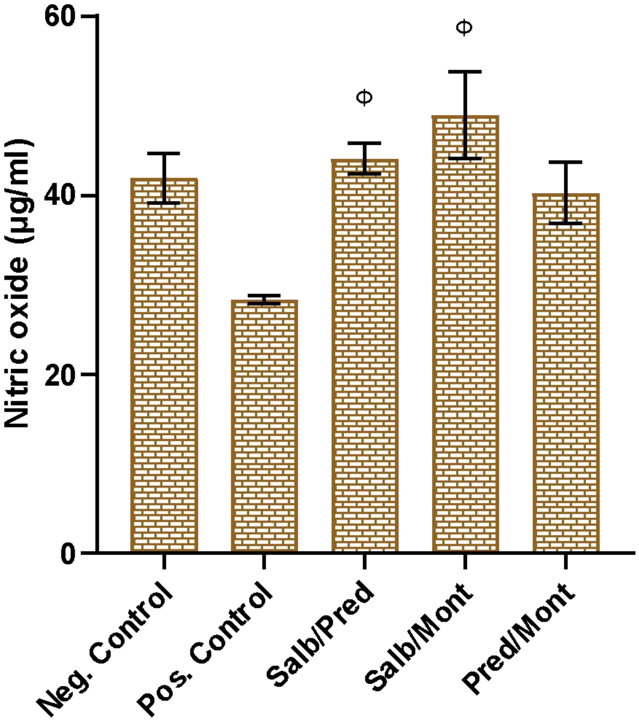

Figure 4.6: Chart showing the effect of the combinations of salbutamol, montelukast and prednisolone combination on lung tissue nitric oxide concentration in Ovalbumin-induced asthma in female Sprague-Dawley rats.

Results show no statistically significant difference in lung tissue nitric oxide concentration in all the groups compared with the negative control ($p > 0.05$). Also, there was a statistically significant increase in the salbutamol/prednisolone and salbutamol/montelukast-treated group compared with the positive control ($p < 0.05$). n=4 ± SEM. $\alpha p < 0.05$ compared to negative control; $\Phi p < 0.05$ compared to positive control.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study examined the effects of salbutamol, montelukast, prednisolone, and their combinations on lung tissue oxidative stress and antioxidant enzyme activities by assessing total protein, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), hydrogen peroxide (H_2O_2), and nitric oxide (NO). The findings show that while these asthma medications are mainly used for airway management, they can also influence lung tissue biochemistry and antioxidant defense mechanisms in different ways.

The combination of salbutamol and prednisolone produced the most notable effects across several parameters. There was a significant increase in total protein concentration in the lungs of animals treated with this combination compared with the negative control group. This may indicate enhanced protein synthesis or an accumulation of inflammatory or structural proteins in the lung tissue as a response to drug interaction or mild oxidative stress. However, this same group showed a decrease in the activities of SOD, CAT, and GPx, suggesting a reduction in antioxidant enzyme defense. This decline could mean that while tissue protein content increased, the antioxidant protection was weakened, making the lung more vulnerable to oxidative damage.

The salbutamol/montelukast and prednisolone/montelukast combinations also showed significant decreases in catalase activity compared to the positive control. Catalase plays a key role in converting hydrogen peroxide to water and oxygen, so its reduction implies reduced capacity to neutralize reactive oxygen species. This was further supported by the increased hydrogen peroxide concentration observed in these same groups. The rise in hydrogen peroxide suggests the presence of oxidative stress in the lungs, possibly due to excessive production of reactive oxygen species or reduced antioxidant enzyme activity.

For superoxide dismutase and glutathione peroxidase, both essential for neutralizing harmful radicals, there was a significant decrease in the salbutamol/prednisolone group compared with both controls. This confirms that the combination may impair the body's ability to counter oxidative stress in the lungs. Such suppression of enzymatic antioxidants could lead to an imbalance between oxidants and antioxidants, favoring oxidative damage.

Interestingly, nitric oxide levels did not show significant changes across all groups when compared to the negative control. However, the salbutamol/prednisolone and salbutamol/montelukast groups showed an increase compared with the positive control. Nitric oxide is a signaling molecule that helps regulate airway tone and inflammation, but when elevated under oxidative conditions, it may react with other radicals to form reactive nitrogen species that further contribute to tissue injury. The mild increase in NO could therefore represent an adaptive response or early sign of oxidative disturbance.

Taken together, the results suggest that combining asthma medications, especially salbutamol with prednisolone or montelukast, can alter the balance between oxidative and antioxidant processes in lung tissue. The observed decrease in antioxidant enzyme activities along with increased hydrogen peroxide levels indicates a shift towards oxidative stress. This highlights that while these drugs are beneficial for managing asthma symptoms, their combinations could potentially reduce antioxidant defense in lung tissue, leading to mild oxidative injury if used over a long period.

5.2 CONCLUSION

This study shows that salbutamol, montelukast, and prednisolone, either alone or in combination, affect lung tissue oxidative balance differently. The combination of salbutamol and prednisolone increased total protein concentration but decreased antioxidant enzyme activities, indicating reduced antioxidant protection. Similarly, the combinations involving montelukast showed increased hydrogen peroxide levels and reduced catalase activity, suggesting higher oxidative stress in the lungs. Overall, these findings indicate that while individual drugs have mild effects, combining them—especially salbutamol with prednisolone or montelukast—can lead to reduced antioxidant capacity and increased oxidative stress. Continuous monitoring and careful use of these drug combinations are therefore recommended to prevent possible oxidative damage to lung tissue.

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