PROJECT SEMINAR

ON

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

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CERTIFICATION

This is to certify that this project work on **"Effect of salbutamol, montelucast and prednisolone on lung tissue oxidant and antioxidant enzyme activities on ovalbumin induced female Sprague-Dawley rats"** was carried out by **ORE ALICE OMODUNNI**, with the matriculation number, **BMS2101665,** in partial fulfillment 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.

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EXTERNAL EXAMINER

DEDICATION

This project work is dedicated to God almighty who has been my source of strength and my parents, Mr and Mrs. O. Idowu, for financial, moral and support.

ACKNOWLEDGEMENTS

My sincere appreciation goes to God Almighty for His sufficient grace and mercy throughout the course of my undergraduate degree programme in the University of Benin, Benin City.

To my supervisor, Dr. MRS. M. I. Ebomoyi for all your unwavering support towards this work, I would also like to acknowledge Dr. E. O. Aloamaka, for his patience, enthusiasm, insightful comments, practical advice and unceasing ideas which have helped me tremendously at all times in my research and writing of this project. I would also like to acknowledge, with much appreciation, the crucial role of the teaching and non-teaching staff in the Department of Physiology, University of Benin.

Also, I wish to express my deepest thanks to my parents, Mr. & Mrs. O. Idowu and my siblings thanks for your unwavering support and encouragement; it is my source of strength.

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Thanks for your encouragements.

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ABSTRACT

Salbutamol, montelukast, and prednisolone are commonly prescribed for respiratory disorders. While their therapeutic effects on the airways are well documented, their impact on pulmonary oxidative stress and antioxidant defense mechanisms remains less understood. This study investigated the effects of these drugs and their combinations on oxidative stress biomarkers and total protein concentration in lung tissue. Experimental animals were assigned to eight groups (n = 4): 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-including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)-and oxidative markers, specifically hydrogen peroxide (H2O2) and nitric oxide (NO). Data were expressed as mean ±SEM and analyzed by one-way ANOVA with significance set at $p < 0.05$. Total protein concentration and catalase activity were not significantly altered in any treatment group compared with the negative control ($p > 0.05$). Prednisolone treatment significantly increased SOD activity relative to the negative control ($p < 0.05$), whereas GPx activity was elevated only in the positive control group. Montelukast significantly increased H202 levels compared with the negative control, but H202 levels in the salbutamol, montelukast, and prednisolone groups were significantly lower than the positive control. Nitric oxide concentration decreased in the positive control and prednisolone groups relative to the negative control, while montelukast treatment caused a significant increase in NO compared with the positive control ($p < 0.05$).

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Asthma is a chronic inflammatory disorder of the airways involving various cells and cellular elements, this inflammation leads to recurrent airflow limitation, dyspnea, thoracic constriction and productive or non-productive bronchial hyperresponsiveness, particularly during nocturnal or early morning hours (Kaufman, 2011). The major pathophysiological features of asthma include airway inflammation, bronchoconstriction and airway remodeling (Venkatesan *et al*., 2023). Asthma can be triggered by several factors such as genetic predisposition for example family history of asthma or atopic diseases, environmental exposures, and occupational hazards like exposure to chemicals or dust (Gary, 2007). Globally, over 235 million individuals suffer from asthma, with approximately 250,000 annual deaths due to asthma 80 percent of which occur in low- and middle-income countries (Wikipedia, 2025). Childhood asthma prevalence is higher than in adulthood, and while boys are more affected than girls, this pattern reverses post-puberty while adults tend to have greater asthma-related morbidity and mortality (Dharmage *et al*., 2019). The pathophysiology of asthma is characterized by chronic inflammation of the airways, primarily driven by immune system dysregulation, when allergens or irritants are inhaled, T helper 2 (Th2) cells, which release cytokines that stimulates B lymphocytes to produce Immunoglobulin E (IgE). The IgE binds to the mast cells, leading to the release of inflammatory mediators like histamine that cause bronchoconstriction and mucus secretion. Eosinophils, activated and recruited by interleukin-5(IL-5), infiltrate the airways and release cytotoxic granules, contributing to epithelial damage, airway inflammation, and hyperresponsiveness (Barnes, 2008).

In the treatment of asthma different drugs are administered in which each has their mechanism of action, salbutamol is a short-acting β2-adrenergic agonist (SABA) that stimulates beta 2 receptors on airway smooth muscle cells, leading to bronchodilation. It activates adenylate cyclase, increasing cyclic AMP (cAMP) levels, which in turn reduces intracellular calcium, causing muscle relaxation, Provides rapid relief from bronchospasm during acute asthma exacerbations (Marques and Vale, 2022). The drug prednisolone is a systemic glucocorticoid that suppresses inflammation by inhibiting the synthesis and release of inflammatory mediators. It

reduces the activity of immune cells involved in the inflammatory response, in acute asthma, prednisolone is administered orally or intravenously to decrease airway inflammation in the lungs (Connett *et al*., 1994). The drug montelukast is a leukotriene receptor antagonist that blocks cysteinyl leukotriene receptors, preventing leukotriene-mediated effects used for long-term control of asthma, particularly in patients with allergic asthma and exercise-induced bronchoconstriction (Connett *et al*., 1994). The combination of Salbutamol, Prednisolone, and Montelukast offers a well-rounded approach to managing asthma and other respiratory conditions. When combined, Salbutamol, Prednisolone, and Montelukast work synergistically to manage asthma in a Sprague-Dawley rat Salbutamol provides rapid bronchodilation, easing immediate breathing difficulties. Prednisolone reduces lung inflammation and prevents long-term airway. Montelukast blocks leukotriene activity, reducing inflammation, mucus production and bronchoconstriction (Dahlbäck *et al*., 1990).

Ovalbumin (OVA) is a protein found in egg white and is commonly used in experimental models to mimic allergic asthma in animals, especially Sprague-Dawley rats. When combined with an adjuvant like aluminum hydroxide and introduced into the body, OVA triggers an immune response similar to human asthma. This includes airway inflammation, eosinophil infiltration, mucus overproduction, and airway hyperresponsiveness (Thakur *et al*., 2019).

Oxidants also known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are highly reactive molecules produced as by products of cellular metabolism or during immune responses. Common examples include superoxide anion, hydrogen peroxide and nitric oxide (Rahman *et al*., 2006). Antioxidants are substances that help protect the lungs and other organs from the harmful effects of oxidants. The body produces its own antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione, which work to neutralize excess oxidants and maintain a healthy balance (Bowler and Crapo, 2002). The ovalbumin-Induced Asthma model in Sprague-Dawley Rats is a widely used experimental method to study asthma, characterized by airway inflammation, hyperresponsiveness and remodeling. induced by repeated ovalbumin exposure, this model mimics aspects of human asthma and exhibits increased sensitivity to airway constricting stimuli (Thakur *et al*., 2019).

1.2 AIM OF STUDY

The aim of this research is to evaluate the effect of salbutamol, prednisolone and montelucast and the combination of their impact on Lung Oxidant and Antioxidant Enzyme Activation and Histology in Ovalbumin-Induced Asthma in Sprague-Dawley Rats.

1.3 JUSTIFICATION OF THE STUDY

Study focuses the effects of commonly used anti-asthmatic drugs on oxidant/antioxidant enzyme balance.

1.4 RESEARCH QUESTIONS

1. What is the effect of salbutamol, montelukast and prednisolone on lung oxidant and antioxidant enzyme activities in ovalbumin-induced female Sprague-Dawley rats?
2. How do these drugs compare in modulating oxidative stress in lung tissues?
3. Does the combination of these drugs provide enhanced protection against oxidative damage?

1.5 SPECIFIC OBJECTIVES

1. To evaluate the levels of oxidant enzymes (e.g., MDA, NO) in lung tissues after treatment with salbutamol, montelukast, and prednisolone.
2. To assess the activity of antioxidant enzymes (Superoxide dismutase, catalase and glutathione peroxidase) in lung tissues of treated rats.
3. To compare the individual and combined effects of these drugs on oxidative stress markers in the lungs.

CHAPTER TWO

EPIDEMIOLOGY

2.1 ASTHMA

Asthma is a chronic inflammatory airway disorder marked by variable and reversible airflow limitation, airway hyperresponsiveness, and mucus hypersecretion (Dharmage *et al*., 2019). Globally, over 235 million individuals suffer from asthma, with approximately 250,000 annual deaths due to asthma 80 percent of which occur in low- and middle-income countries (Wikipedia, 2025). Despite a decrease in age-standardized rates over the past decades, the total number of cases continues to rise, with 262.4 million prevalent cases reported in 2019 and approximately 455,000 deaths annually (Wang *et al.*, 2023). This burden is disproportionately higher in low-and middle-income countries where access to healthcare and medications is limited (WHO, 2024). Key risk factors include genetic predisposition, exposure to allergens, smoking, and occupational irritants (Wang *et al*., 2023). Among global regions, only Central Europe showed a significant increase in asthma prevalence from 1990 to 2021 (Silverwood *et al*., 2025). High-income North America had the highest asthma prevalence in the 5 to 9 years age group in 2021 (Rutter *et al*., 2022).

2.2 TYPES OF ASTHMA AND CLASSIFICATION

Asthma is now understood to be a heterogeneous disease with multiple phenotypes and endotypes (Kuruvilla *et al*, 2018). The most recognized is Type 2-high asthma, which is characterized by elevated eosinophils, increased serum immunoglobulin E, and a strong T helper 2 cytokine response involving interleukin-4, interleukin-5, and interleukin-13 (Busse, 2020). Non-T2 asthma, which includes neutrophilic and pauci-granulocytic phenotypes, tends to occur in adult-onset or severe cases and often responds poorly to corticosteroids (Kim, 2024).

Intermittent asthma symptoms occurs less than twice a week, with occasional nocturnal disturbances and normal lung function. Mild persistent asthma, on the other hand, presents with symptoms occurring more than twice a week but not daily, resulting in minor activity restrictions. Moderate persistent asthma is defined by daily symptoms, more frequent nocturnal disturbances

and reduced lung function. Severe persistent asthma marked by continuous symptoms, frequent nighttime issues, and significant limitations in daily activities. This classification allows physicians to adjust treatment intensity based on the level of impairment and exacerbation risk (Koterba and Saltoun, 2012). Asthma can also be classified by underlying pathophysiology and immune involvement. One of the earliest models, proposed by Rackemann (1947), described two types: Extrinsic asthma, associated with allergens and occurring mostly in children and young adults. Intrinsic asthma, which occurs later in life, often without identifiable allergic triggers. In a more current view, Padem and Saltoun (2019) expanded this to include: Allergic asthma similar to extrinsic, often linked to IgE sensitization. Non-allergic asthma, which may involve neutrophilic or paucigranulocytic inflammation. Late-onset asthma, more common in adults with more persistent symptoms. Obesity-related asthma, often more severe and poorly responsive to corticosteroids. Asthma with fixed airflow limitation, due to chronic airway remodeling.

The classification of asthma is essential for tailoring treatments, especially in patients with poor response to standard therapies and they' are trigger-based classification which identifies asthma subtypes according to the specific stimuli that provoke symptoms it includes Exercise-induced asthma, where bronchospasm follows physical exertion. Aspirin-exacerbated respiratory disease (AERD), triggered by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Cough-variant asthma, presenting primarily as persistent coughing. Nocturnal asthma, where symptoms worsen at night (Padem and Saltoun, 2019). Additionally, occupational asthma is a recognized classification caused by exposure to allergens or irritants in the workplace. (Bernstein *et al*., 2006) emphasized two key types: Immunologic Ovalbumin, resulting from sensitization to a specific agent (e.g., flour, latex). Non-immunologic Ovalbumin, often caused by high-level irritant exposure (e.g., ammonia, chlorine) without prior sensitization.

2.3 PATHOPHYSIOLOGY OF ASTHMA

In asthma, chronic airway inflammation leads to airway remodeling, which includes thickening of the airway wall, subepithelial fibrosis, increased smooth muscle mass, and goblet cell hyperplasia (Huang and Qiu, 2022). According to (Frigas and Gleich, 1986) talked about the role of eosinophils as central effector cells in its pathophysiology. They showed that eosinophils release toxic proteins, particularly major basic protein (MBP), which damages respiratory epithelium, impairs mucociliary clearance, and worsens airway obstruction. Recurrent

inflammatory episodes generate reactive oxygen species (ROS), inducing oxidative stress that causes tissue damage and reduces lung function (Lewis *et al*., 2021). This oxidative stress also plays a role in the development of corticosteroid insensitivity, thereby complicating disease management (Lewis *et al*., 2021).

One of the main pathways linking asthma to cardiovascular disease is chronic systemic inflammation. Inflammatory mediators including interleukins, tumour necrosis factor-α and C-reactive protein, which are elevated in asthma contribute to endothelial dysfunction, arterial stiffness and an increased risk of atherosclerosis. This places patients with asthma at greater risk for hypertension, ischemic heart disease, and cerebrovascular events (Cazzola *et al*., 2023). Another mechanism lies in the effect of hypoxaemia and airway obstruction during acute asthma exacerbations, which can trigger myocardial stress and arrhythmias. Furthermore, β_2-agonists, a cornerstone of asthma therapy, may predispose some patients to tachycardia, palpitations, and, in rare cases, ischemic episodes, particularly in those with underlying heart disease. Corticosteroids, though effective in reducing airway inflammation, have also been associated with adverse cardiovascular outcomes when used long-term, including hypertension, dyslipidaemia and impaired glucose metabolism, which further increase CVD risk (Rajput *et al.*, 2023).

2.4 EFFECTS OF ASTHMA ON THE LUNG TISSUE OXIDANT AND ANTI OXIDANT ENZYME ACTIVITY

During asthmatic inflammation, excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs, mainly through the activation of nicotinamide adenine dinucleotide phosphate, reduced form oxidizes in inflammatory and structural lung cells. This leads to oxidative stress, damaging lipids, proteins, and DNA in airway tissues (Liang *et al*., 2020).

Clinical studies have demonstrated that in patients with bronchial asthma, the activities of key antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are significantly reduced compared to healthy individuals. Meanwhile, oxidant activity increases, as indicated by elevated levels of lipid peroxidation and oxidative biomarkers. Prolidase activity, which is important for collagen turnover and tissue repair, is also altered, suggesting impaired tissue remodeling processes in asthma (Kaleli *et al*., 2006).

Additionally, it has been proposed that the oxidative stress burden extends beyond the lungs, potentially disseminating into systemic circulation and resulting in oxidative imbalance within the bloodstream. This systemic oxidative stress further reduces antioxidant reserves and contributes to disease severity, exacerbations, and long-term airway damage (Nadeem *et al*., 2014).

2.5 ANIMAL MODEL OF ASTHMA

Salbutamol is a short-acting β2-adrenergic receptor agonist that relaxes the smooth muscles of the bronchioles.. These models have highlighted the importance of T-helper type 2 driven allergic responses in the progression of asthma and have been useful in the identification of potential drug targets for interventions involving allergic pathways (Zosky snd Sly, 2007). Animal models particularly mice, rats, guinea pigs, and non-human primates are widely used because they can reproduce key features of asthma such as airway hyperresponsiveness, inflammation, and structural remodeling. Sensitization is commonly achieved through allergens like ovalbumin or house dust mite, delivered intraperitoneally or intranasally, followed by repeated challenges to mimic chronic disease. mice are the most widely used because of their low cost, ease of handling, availability of genetically modified strains, and reproducibility of results (Aun *et al*., 2017).

The ovalbumin (OVA)-induced asthma model is a widely accepted experimental setup that mimics human allergic asthma in rodents. Sensitization and repeated airway challenges with OVA lead to airway eosinophilia, elevated IgE, increased Th2 cytokines, and airway hyperresponsiveness (Jedli *et al*., 2022). Inflammation in this model is also associated with oxidative damage, including elevated malondialdehyde (MDA) and decreased antioxidant enzymes like glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in lung tissues (Jedli *et al*., 2022). Histologically, lungs show epithelial desquamation, mucus plugging, peribronchial inflammation, and bronchial wall thickening (Jamal *et al*., 2022). These changes make the Ovalbumin model ideal for assessing the impact of drugs on oxidative stress and lung histology (Jedli *et al*., 2022).

2.6 MEDICATIONS OF ASTHMA

Quick-Relief (Rescue) Medications: these provide rapid relief from acute bronchospasm, the Short-Acting Beta-2 Agonists (SABAs) is a first choice for quick relief and examples are the albuterol (salbutamol) and levalbuterol while, Short-Acting Muscarinic Antagonists (SAMAs) Ipratropium is used, especially in acute severe asthma and in combination with SABAs. Systemic Corticosteroids can be administered to rapidly reduce airay inflammation during severe exacerbations. However, long-term use of systemic corticosteroids is avoided due to significant adverse effects such as osteoporosis, hypertension, diabetes, and adrenal suppression (Hashmi *et al.,* 2023).

Long term control medications: Inhaled Corticosteroids (ICS) is a first-line therapy for persistent asthma. They reduce airway inflammation, mucus secretion, and hyperresponsiveness. Examples are budesonide, fluticasone, beclomethasone. Long-Acting Beta-2 Agonists (LABAs) it is used in combination with Inhaled Corticosteroids, not as monotherapy. While they are effective in controlling symptoms, they must never be used alone in chronic asthma as this may increase the risk of asthma related mortality. they relax bronchial smooth muscle and improve lung function. Examples: salmeterol, formoterol. Leukotriene Receptor Antagonists are oral agents that block leukotriene-mediated inflammation and bronchoconstriction. These drugs are particularly useful in patients with aspirin-sensitive asthma and allergic rhinitis and example is montelukast.

Methylxanthines (Theophylline) is less commonly used due to narrow therapeutic index. They exert bronchodilation and mild anti-inflammatory effects (Hashmi and Chakraborty, 2023). Additional pharmacological options include theophylline, a bronchodilator with mild anti-inflammatory properties that works by inhibiting phosphodiesterase and increasing intracellular cyclic AMP. Although effective, its use has declined due to a narrow therapeutic window and significant side effects such as arrhythmias and seizures. Anticholinergic agents such as ipratropium bromide and tiotropium are also used, particularly in patients with severe asthma or overlapping features with chronic obstructive pulmonary disease. These drugs act by blocking muscarinic receptors in the airways, leading to reduced bronchoconstriction and mucus secretion (Falk *et al*., 2016).

2.7 SALBUTAMOL

Salbutamol is a short-acting β2-adrenergic receptor agonist that relaxes bronchial smooth muscles and provides symptomatic relief. Though primarily a bronchodilator, it has shown modest antioxidant effects in animal models. In a sepsis-induced lung injury study, salbutamol reduced lung inflammation without significantly increasing oxidative stress markers such as thiobarbituric acid-reactive substances (TBARS) (Cardoso-Sousa *et al*., 2019).

Earlier studies also reported that salbutamol reduces MDA and myeloperoxidase (MPO) levels while increasing SOD and GSH in lung tissues, suggesting some indirect antioxidant effect (Kushiya *et al*., 2006). Although not a classical antioxidant, salbutamol may contribute to redox balance via bronchodilation and reduced leukocyte infiltration.

Salbutamol reduces lung oxidative damage and improves antioxidant enzyme status in rat models of acute lung injury. When administered intranasally, salbutamol decreased lung lipid peroxidation and restored the activity of antioxidant enzymes, including superoxide dismutasek, catalase, and glucose-6-phosphate dehydrogenase (Cardoso-Sousa *et al*., 2019). Similarly, Özogul *et al* (2015) reported that both nebulized and oral salbutamol reduced cecal ligation and puncture-induced increases in oxidative stress markers, such as 8-iso-PGF2α, and restored lung antioxidants, including SOD and reduced glutathione, along with improvements in lung pathology.

2.8 MONTELUCAST

Montelukast is a leukotriene receptor antagonist that blocks the action of cysteinyl leukotrienes involved in bronchoconstriction, inflammation, and mucus production. It has been found to significantly reduce oxidative stress in asthma models (Zhu *et al*., 2023).

By blocking leukotriene-mediated immune activation, montelukast reduces ROS production from eosinophils and neutrophils. It also improves lung histology by reducing subepithelial fibrosis and goblet cell hyperplasia in chronic asthma models (Shin *et al*., 2013).

Montelukast reduces lung oxidative damage and supports antioxidant defenses. The comprehensive review reports increased activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase and reduced systemic oxidative stress after montelukast

treatment (Goel *et al*., 2025). Montelukast lowered lung lipid peroxidation markers (for example, malondialdehyde and isoprostane), preserved or restored lung antioxidant defenses, and improved lung histopathology in sepsis-induced injury (Alnfakh *et al*., 2022).

2.9 PREDNISOLONE

Prednisolone is a systemic corticosteroid that exerts potent anti-inflammatory effects. It suppresses cytokine release, immune cell recruitment, and reduces airway remodeling. While not a direct antioxidant, prednisolone reduces oxidative stress by controlling the underlying inflammation. Studies show that corticosteroids restore the redox environment, increasing responsiveness to therapy (Lewis *et al*., 2021). Dexamethasone, a related steroid, has been shown to lower MDA and raise SOD in lung tissues, indicating that glucocorticoids also support antioxidant defense indirectly (Dikmen *et al*., 2021). Histological changes in asthma include epithelial damage, mucus gland hypertrophy, basement membrane thickening, and dense inflammatory infiltrates (Jamal et al., 2022). OVA-induced models show clear features of asthma pathology, such as immune cell infiltration, bronchiolar wall thickening, and airway edema (Jedli *et al*., 2022).

prednisolone treatment significantly lowered lung tissue levels of malondialdehyde, a marker of lipid peroxidation, indicating reduced oxidative stress. At the same time, prednisolone increased the activity of key antioxidant enzymes in the lung, including superoxide dismutase, glutathione peroxidase, and glutathione reductase. These effects were observed both when prednisolone was administered alone and in combination with donepezil, and they were associated with improved lung histopathology. This demonstrates that prednisolone exerts a protective effect on lung tissue by both reducing oxidant damage and enhancing endogenous antioxidant enzyme activity (Mina *et al*., 2023).

CHAPTER THREE

METHODOLOGY

3.1 MATERIALS

Sprague-Dawley rat, Cotton wool, Syringes, Lancet, Pipettes, Measuring scale, Nebulizer exposure chamber, Analytical balance, Centrifuge, Spectrophotometer, Animal cages.

3.2 EXPERIMENTAL ANALYSIS

The study involves the use of female Sprague-Dawley rats. These rats were provided with appropriate animal care, in line with international guidelines for experimental animal handling and ethical approval obtained from the College of Medical Sciences ethics board. The Sprague-Dawley rats were housed in a clean, cool and sterile environment at 22°C room temperature, they were kept in cages, where they had access to food and water throughout the period of the experiment.

3.3 STUDY DESIGN

Female Sprague-Dawley rats weighing between 150-250g were divided into two main groups; the control group and Test group. All the groups consist of Eight (8) rats each. The control group received normal rat chow and water throughout the experimental period while the test groups were exposed to concentrations of Ovalbumin (OVA, egg albumin), and aluminum hydroxide to induce asthma after which they were treated with montelukast, prednisolone and salbutamol Experiment was carried out in phases.

PHASE 1

The female Sprague-Dawley rats were acclimatized for two weeks meaning they were allowed to adjust to their new environment and were not given any treatments during this time.

CONTROL GROUPS

- **GROUP 1:** control
- **GROUP 2:** asthmatic not treated

TEST GROUP

- **GROUP 3:** Asthma induced and treated with salbutamol
- **GROUP 4:** Asthma induced and treated with montelucast
- **GROUP 5:** Asthma induced and treated with prednisolone

- At the end of the 14-day acclimatization period, all rats were weighed to determine their initial body weights. All groups, except the negative control, received an intraperitoneal injection of ovalbumin (OVA, 1 mg) emulsified in aluminium hydroxide [$Al(OH)_3$, 100 mg]. Drug treatments began on day 14, the first day of OVA challenge, and continued daily.

- All experimental groups (groups 3, 4, 5, 6, 7, and 8) were sensitized with OVA (1 mg) and aluminium hydroxide (1.020 g) dissolved in 1.0 mL of saline on days 0 and 7. From day 7 of treatment until the last day, the rats were challenged twice weekly with OVA (1.0% w/v, adsorbed in 1.0 mL saline). For each challenge, the rats were placed in a plastic chamber measuring 70 cm in diameter and 40 cm in length, connected to a Medel Family Nebulizer (REF 90543 MEDEL FAMILY SILVER AEROSOL), and exposed to OVA aerosol for 15 minutes per group.

PHASE 2

After confirmation of asthma in all test groups, treatment began each rat received their respective drugs. Group 3 was administered Salbutamol (1 mg/kg, orally), Group 4 was administered Montelukast (10 mg/kg, orally) and Group 5 was administered Prednisolone (3 mg/kg, orally).

3.4 RESEARCH PROCEDURES

Groups	Treatment	Description
1	Negative control	No induction, no drug treatment. Kept under the same conditions as other groups.
2	Positive control	Ovalbumin induced no treatment.
3	Salbutamol	1 mL of 2 mg/mL solution daily.
4	Montelucast	1 mL of 10 mg/mL solution daily.
5	prednisolone	1 mL of 3 mg/mL solution daily.

3.5 WEIGHT MEASUREMENTS The weight was measured using a Measuring scale, A plastic bowl was placed on the measuring scale and tared back to 0. The rats were gently placed in the bowl and placed back on the scale. The weight of the rats was recorded in gram (g).

3.6 SAMPLE COLLECTION AND BIOCHEMICAL ANALYSIS

After the treatment period, rats were anesthetized with chloroform. lung tissues were excised, washed with ice-cold phosphate-buffered saline (PBS), and homogenized. The homogenates were centrifuged, and the supernatants were used to assay the following parameters using standardized commercial kits:

●Antioxidant enzymes: Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).

●Oxidative stress markers: Malondialdehyde, hydrogen peroxide (H_2O_2), nitric oxide (NO).

●Total Protein Concentration: Determined by the Bradford method to normalize enzymatic activity data.

3.7 STATISTICAL ANALYSIS

All data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to compare differences among groups, followed by Tukey's post hoc test for multiple comparisons.

A p-value of < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism software (version 10.2.2; GraphPad Inc., San Diego, CA, USA).

CHAPTER 4

RESULTS

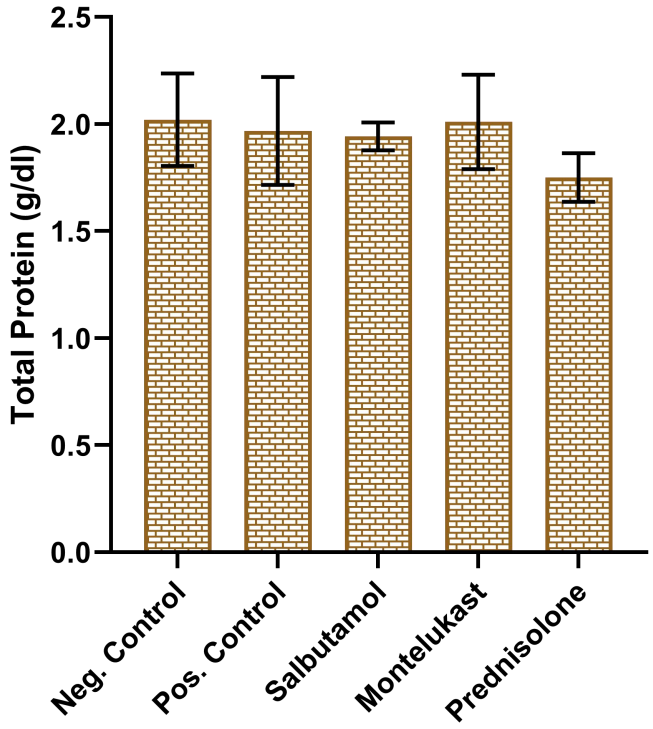

Fig. 4.1: Chart showing the effect of salbutamol, montelukast, prednisolone, and their combinations on lung tissue total protein concentration.

Results show no statistically significant difference in lung tissue total protein concentration in all 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.

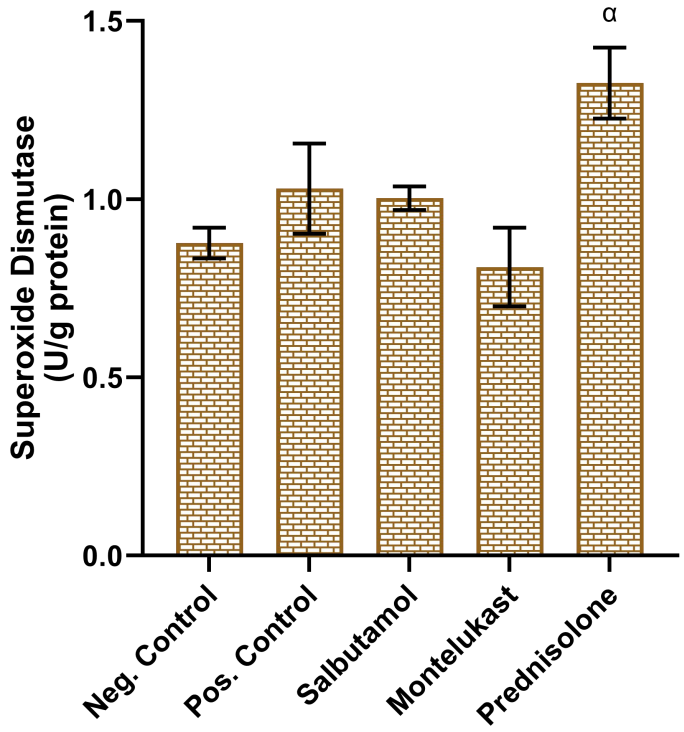

Fig. 4.2: Chart showing the effect of salbutamol, montelukast, prednisolone, and their combinations on lung tissue superoxide dismutase enzyme activity.

Results show a statistically significant increase in lung tissue superoxide dismutase enzyme activity in the prednisolone-treated group compared with the negative controls ($p<0.05$), but no statistically significant difference among the positive control, salbutamol, and 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.

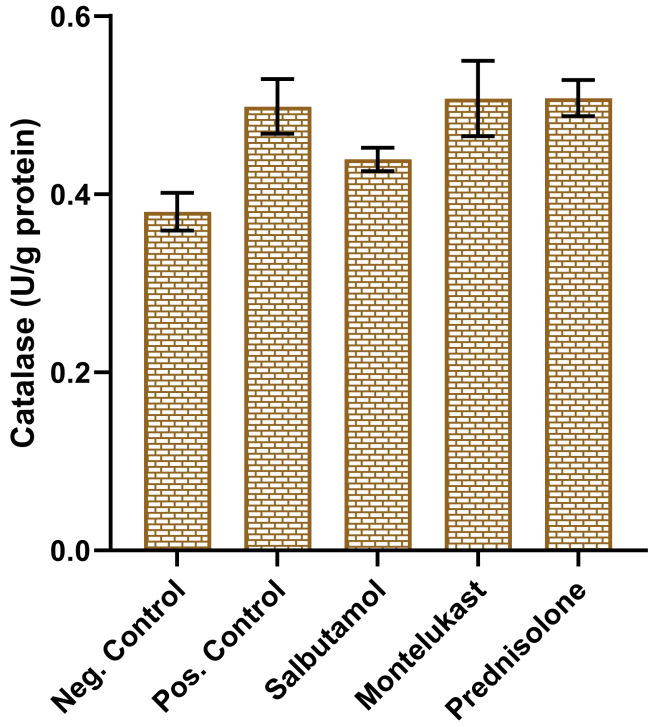

Fig. 4.3: chart showing the effect of salbutamol, montelukast, prednisolone, and their combinations on lung tissue catalase enzyme activity.

Results show no statistically significant difference in lung tissue catalase enzyme activity in all 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.

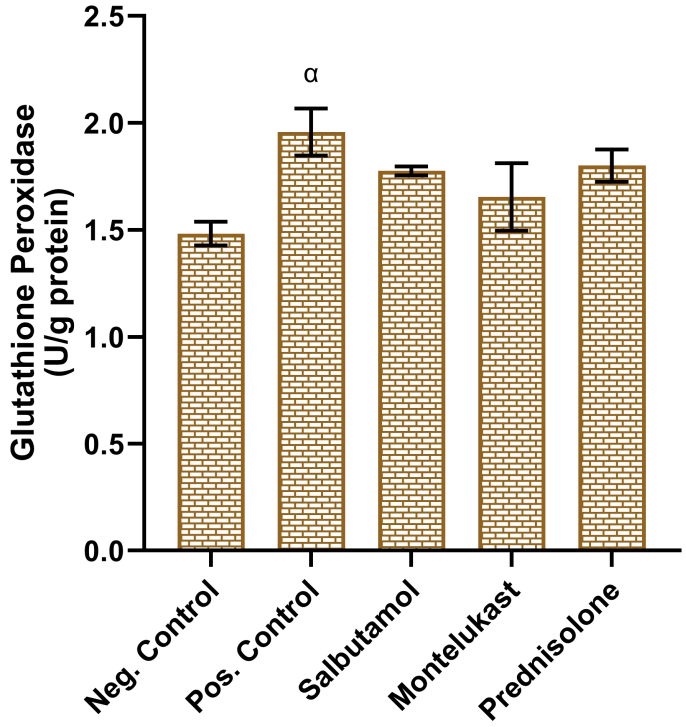

Fig. 4.4: Chart showing the effect of salbutamol, montelukast, prednisolone, and their combinations on lung tissue glutathione peroxidase enzyme activity.

Results show a statistically significant increase in lung tissue glutathione peroxidase enzyme activity in the positive control group compared with the negative controls ($p<0.05$), but no statistically significant difference among the salbutamol, montelukast, and prednisolone groups compared with the negative control ($p>0.05$).

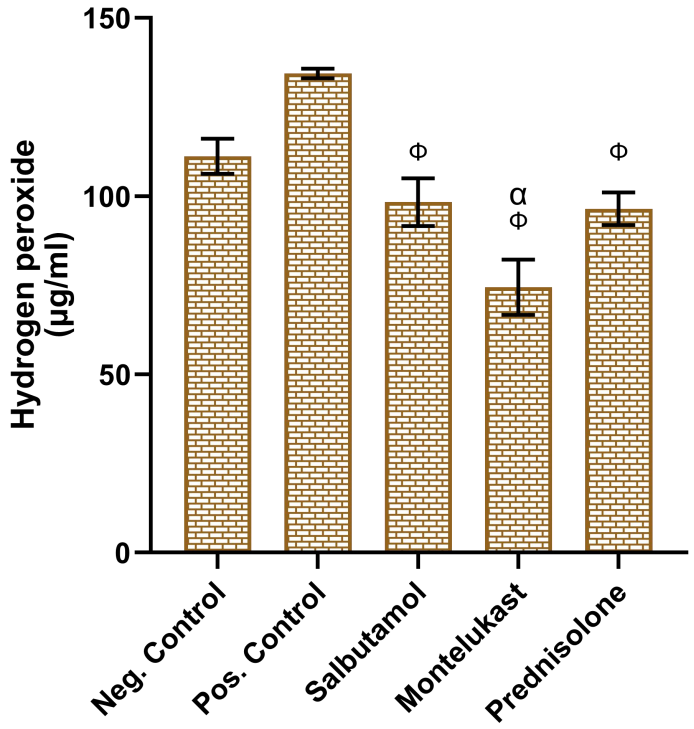

Fig. 4.5: Chart showing the effect of salbutamol, montelukast, prednisolone, and their combinations on lung tissue hydrogen peroxide concentration.

Results show a statistically significant increase in lung tissue hydrogen peroxide concentration in the montelukast-treated group compared with the negative control ($p<0.05$), but no statistically significant difference among the positive control, salbutamol, and prednisolone-treated groups compared with the negative control ($p>0.05$). Also, there was a statistically significant decrease in the salbutamol, montelukast, and prednisolone-treated groups compared with the positive control group ($p<0.05$).

n=4 ± SEM.

$^{\alpha}p<0.05$ compared to negative control;

$^{\Phi}p<0.05$ compared to positive control.

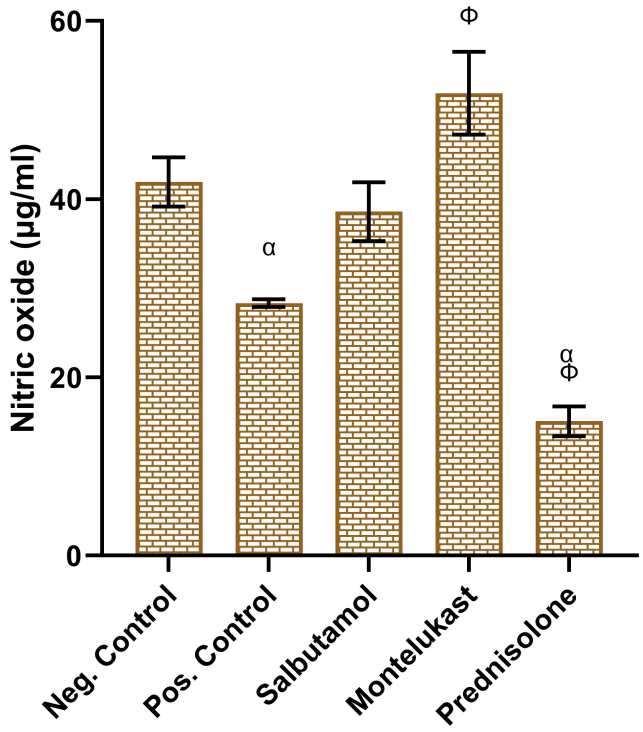

Fig. 4.6: Chart showing the effects of salbutamol, montelukast, prednisolone, and their combinations on lung tissue nitric oxide concentration and activity.

Results show a statistically significant decrease in lung tissue nitric oxide concentration in the positive control and prednisolone-treated groups compared with the negative control ($p>0.05$), but no statistically significant difference in the salbutamol- or montelukast-treated groups compared with the negative control ($p>0.05$). Also, there was a statistically significant increase in the montelukast and a decrease in the prednisolone-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 FIVE

DISCUSSIONS AND CONCLUSION

5.1 DISCUSSION

In this study, **Figure 1**, there was no statistically significant difference in **lung tissue total protein concentration** among all the treatment groups when compared with the negative control. This indicates that administration of the drugs did not alter protein synthesis or degradation in lung tissue. Thus, the structural integrity of the lungs remained unaffected by the treatments under the experimental conditions.

In **Figure 2**, **superoxide dismutase (SOD) enzyme activity** showed a statistically significant increase in the prednisolone-treated group compared with the negative control ($p<0.05$). This suggests that prednisolone enhanced antioxidant defense by increasing the conversion of superoxide radicals to hydrogen peroxide. This agrees with reports that corticosteroids can modulate oxidative stress by stimulating antioxidant enzymes. However, salbutamol and montelukast did not significantly affect SOD activity, implying a weaker antioxidant response through this pathway.

The data in **Figure 3** show no statistically significant difference in **catalase (CAT) enzyme activity** across all treatment groups. This suggests that none of the drugs significantly influenced the breakdown of hydrogen peroxide to water and oxygen. It also indicates that catalase may not be the principal enzyme modulated by these agents in maintaining oxidative balance in lung tissue.

In **Figure 4**, **glutathione peroxidase (GPx) enzyme activity** was significantly increased in the positive control compared with the negative control ($p<0.05$), but no significant difference was observed in the drug-treated groups. This suggests that the induced oxidative condition in the positive control enhanced the body's antioxidant defense system, while administration of the drugs did not further elevate GPx activity beyond that response.

Figure 5 shows that **hydrogen peroxide (H_2O_2) concentration** was significantly increased in the montelukast-treated group compared with the negative control ($p<0.05$). However, hydrogen peroxide levels were significantly decreased in all drug-treated groups compared with the

positive control ($p<0.05$). This pattern suggests that while montelukast may cause a mild rise in ROS generation compared with normal baseline levels, all three drugs contributed to reducing oxidative stress relative to the disease or stress state represented by the positive control.

Finally, as illustrated in **Figure 6**, **nitric oxide (NO2) concentration** significantly decreased in both the positive control and prednisolone-treated groups when compared with the negative control, while a significant increase was observed in the montelukast-treated group relative to the positive control. The reduction in NO2 observed with prednisolone might result from its anti-inflammatory properties, particularly the suppression of inducible nitric oxide synthase (iNOS) activity. Conversely, the increase in NO2 levels with montelukast could reflect a compensatory mechanism aimed at restoring endothelial function or countering oxidative imbalance.

Overall, the findings states that **prednisolone** has a more pronounced antioxidant effect, primarily by enhancing SOD activity and reducing NO2 concentration, while **montelukast** displays a dual effect slightly increasing ROS but improving NO2 availability. **Salbutamol** produced relatively mild effects across most parameters. The combination treatments maintained oxidative balance without significantly altering protein levels, indicating a potential stabilizing or protective effect on lung tissue exposed to oxidative or inflammatory stress.

5.2 CONCLUSION

In, conclusion, this study shows that standard asthma treatments have tissue-specific effects on oxidative balance in OVA-induced asthmatic rats. While monotherapies with salbutamol, montelukast, or prednisolone caused minimal changes, the combination of salbutamol and prednisolone (SAL/PRED) uniquely altered the pulmonary antioxidant system. By increasing SOD and CAT levels while decreasing GPx, this regimen created a targeted imbalance in oxidative defenses that did not lead to measurable tissue damage, likely due to compensatory mechanisms and the anti-inflammatory effects of prednisolone.

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