EFFECTS OF SALBUTAMOL , MONTELUKAST AND PREDNISOLONE AND THEIR COMBINATION ON SERUM OXIDANT AND ANTIOXIDANTS ENZYMES ACTIVITIES IN OVALBUMIN INDUCED SPRAGUE-DAWLEY RATS.

BY

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NOVEMBER, 2025.

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

NOVEMBER, 2025.

CERTIFICATION

We the undersigned hereby certify that MOHAMMED WOSIRATU (BMS2101647) carried out this research in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Physiology.

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External Examiner **(Date)**

DEDICATION

This project is dedicated, first and foremost, to Allah (SWT), the Almighty, the source of all knowledge, wisdom, and infinite guidance.

In loving and eternal memory, I dedicate this work also to the **Late Mr. Great Onorame Yamai**. Your profound influence, enduring spirit, and quiet commitment to excellence serve as a continuous source of inspiration that guided me through this challenging journey. May your soul rest in perfect peace.

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First and foremost, I am thankful to **ALLAH (SWT),** the Almighty, the source of all knowledge, wisdom, and infinite guidance.

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CHAPTER ONE

1.0INTRODUCTION

Asthma is characterized by chronic inflammation of the lower respiratory tract, a state frequently seen alongside inflammatory disorders of the upper airway (Mims, 2015). The 1995 GINA guidelines classified a patient's off-treatment asthma status (intermittent to severe persistent) to guide initial therapy. However, modern guidelines have abandoned this severity-based classification in favor of a focus on achieving and maintaining disease control. Current clinical practice defines asthma severity by the level of pharmacological treatment required to keep the disease controlled; in the most difficult cases, severity is determined by the condition's refractoriness to therapy(Song *et al.,* 2019). Asthma is typically suspected based on a patient's recurring symptoms and their positive response to a bronchodilator medication, which helps relax the airway muscles(Sockrider and Fussner, 2020). For individuals over the age of five, the diagnosis is usually confirmed with a breathing test called spirometry (a type of pulmonary function test or PFT), which detects airway narrowing or obstruction. However, a normal spirometry result does not rule out asthma(Sockrider and Fussner, 2020). First-line asthma medications include the short-acting β2-adrenergic agonist salbutamol for rapid relief of bronchoconstriction, the leukotriene receptor antagonist montelukast for its anti-inflammatory and bronchodilatory effects, and the corticosteroid prednisolone for potent anti-inflammatory action(Whirledge and Cidlowski, 2010), these drugs exert their effects through different mechanisms, including bronchodilation, anti-inflammatory actions, and inhibition of leukotriene-mediated inflammation (Barnes, 2008). The induction of airway inflammation using Ovalbumin (OVA) in Sprague-Dawley rats serves as a widely recognized model for studying asthma (Kumar and Herbert, 2013). This model triggers a Th2-mediated inflammatory response, which subsequently elevates the production of reactive oxygen species (ROS), resulting in oxidative stress (Rahman and MacNee, 2000).

1.1 JUSTIFICATION OF STUDY

This study Evaluates the combined effect of these drugs on serum oxidants and antioxidant enzymes, which may differ from individual drug effects and influence overall treatment efficacy or systemic side effects.

1.2 AIM OF STUDY

The study aims to investigate the effects of single and combined asthma medications on the serum oxidant and antioxidant enzyme activities in an ovalbumin-induced asthma model using Sprague-Dawley rats.

1.3 RESEARCH QUESTIONS

What are the effects of Salbutamol, Montelukast, and Prednisolone monotherapies on antioxidants enzymes activities levels in an ovalbumin-induced asthmatic rats?

1.4 SPECIFIC OBJECTIVES

The specific objective is to evaluate and compare the effects of Salbutamol, Montelukast, and Prednisolone monotherapies on the activities of antioxidant enzymes in the serum of OVA-induced Sprague Dawley rats.

CHAPTER 2

2.0 ASTHMA

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction, bronchial hyperresponsiveness and underlying inflammation (Barnes, 2008). This respiratory disease is characterised by airway inflammation, causing intermittent airflow obstruction and bronchial hyperresponsiveness (Hashmi and Cataletto, 2024). The inflammatory process in asthma involves a variety of immune cells, including mast cells, eosinophils, and T lymphocytes, which release a cascade of inflammatory mediators such as histamine, leukotrienes, and cytokines (Holgate, 2008).

The hallmark asthma symptoms include cough, wheezing, and shortness of breath, which are often exacerbated by triggers ranging from allergens to viral infections (Lee and McDonald, 2018).

Asthma has been increasingly recognized as a heterogeneous disease comprised of both allergic and non-allergic phenotypes, a feature not captured in prior surveys(Enilari and Sinha, 2019).

Clinical diagnosis is usually established based on the presence of symptoms and documented variability in expiratory airflow limitation as measured by pulmonary function testing(Enilari and Sinha, 2019).

Presently, asthma is a major chronic disease affecting approximately 334 million people worldwide. The epidemic spares no age group, race or ethnicity, however ethnicity and socioeconomic status do influence the prevalence, morbidity and mortality of asthma in the United States and various countries throughout the world(Enilari and Sinha, 2019). Prior

studies have shown that the prevalence of asthma ranges from 15% to 20% in many countries, especially in the developed nations (Enilari and Sinha, 2019).

In the United States (US), the current prevalence of asthma among adults is approximately 7.6%, but rates vary dramatically among different ethnic groups. Prevalence is 9.1% among Black non-Hispanics and 13.6% among Puerto Ricans but only approximately 5% for Mexican and Asians(Enilari and Sinha, 2019).

Several studies have reported increasing rates of asthma in less developed countries in Asia and Africa(Enilari and Sinha, 2019). There are several studies also highlighting the burden of asthma in children in Nigeria, with a prevalence ranging from 5.1% to 14.3%.6,8 Nevertheless, there remains a dearth of literature on the burden of asthma in adults in Nigeria. There have been attempts at championing the global study of the burden of asthma by the International Study of Asthma and Allergies in Childhood (ISAAC) group, which included Nigerian participants(Musa and Aliyu, 2014).

Mortality from asthma is low compared to other chronic diseases and accounts for less than 1% of deaths globally. However, given the high prevalence worldwide, asthma is still responsible for 250,000 potentially preventable deaths annually(Enilari and Sinha, 2019).

Asthma also accounts for the loss of over 15 million disability adjusted life years (DALY) annually and ranks among the highest causes of DALY for children. Children aged 10–14 years, followed by elderly patients have the highest DALYs Conversely, adults aged 30–34 years have the least disability from the disease(Enilari and Sinha, 2019). Morbidity due to asthma is underappreciated in the elderly. Asthma affects 7–9% of the US population older than 65 years, and causes substantial morbidity, as older adults are four times more likely to die from asthma(Enilari and Sinha, 2019).

2.0.1: PATHOPHYSIOLOGY OF ASTHMA

Asthma is a chronic, heterogenous disease of the airways characterized by airway hyperresponsiveness (AHR), airway inflammation, and airway structural changes termed remodeling(Peter *et al.,* 2024). These features lead to airflow obstruction due to bronchoconstriction, mucus plugging, airway edema, and airway wall thickening. This airflow obstruction is potentially reversible, occurring either spontaneously or in response to bronchodilator (β-agonists) and anti-inflammatory (inhaled corticosteroid [ICS]) therapy(Peter *et al.,* 2024).

Airway hyperresponsiveness: Airway hyperresponsiveness Is defined as the predisposition of airways to narrow (bronchoconstriction) in response to stimuli that produce little to no effect in healthy individuals. AHR is responsible for recurrent symptoms such as wheezing and breathlessness(Peter *et al.,* 2024). AHR results from an intricate and complex interplay between airway smooth muscle (ASM) hypercontractility, airway inflammation, and airway remodeling(Peter *et al.,* 2024).

Diagnosis of asthma is confirmed objectively by demonstrating variable airflow obstruction or Airway hyperresponsiveness using a bronchoprovocation test. The type of Airway hyperresponsiveness in response to indirect versus direct bronchoprovocation testing may be informative regarding underlying drivers of bronchoconstriction in an individual patient and assessment may impact disease management(Peter *et al.,* 2024).

The airway epithelium plays a critical role as both a structural element and an immune barrier to the external environment(Peter *et al.,* 2024). Airborne pathogens, pollutants, and allergens can damage airway epithelial cells and trigger the release of epithelial-derived cytokines and growth factors that drive characteristic downstream inflammatory pathways(Peter *et al.,* 2024). Airway narrowing and closure is influenced by a multitude of processes, including

inflammatory infiltrates consisting of mononuclear cells and eosinophils, surfactant disruption leading to increased propensity for airway closure, and reduced lung elastic recoil, which reduces driving pressure(Peter *et al.,* 2024).

Airway Inflammation: Chronic inflammation of the airways is a central component of asthma. This inflammatory process involves the infiltration of various immune cells into the airway walls, including mast cells, eosinophils, T lymphocytes (particularly Th2 cells), and neutrophils in some phenotypes (Holgate, 2008). These cells release a multitude of inflammatory mediators, such as histamine, leukotrienes, prostaglandins, cytokines (e.g., IL-4, IL-5, IL-13), and chemokines (Barnes, 2008). This event triggers the airway smooth muscle contraction within minutes and may stimulate reflex neural pathways (Riccio and Proud, 1996). Subsequently, an influx of inflammatory cells, including monocytes, dendritic cells, neutrophils, T lymphocytes, eosinophils, and basophils may lead to postponed bronchoconstriction several hours later (Riccio and Proud, 1996).

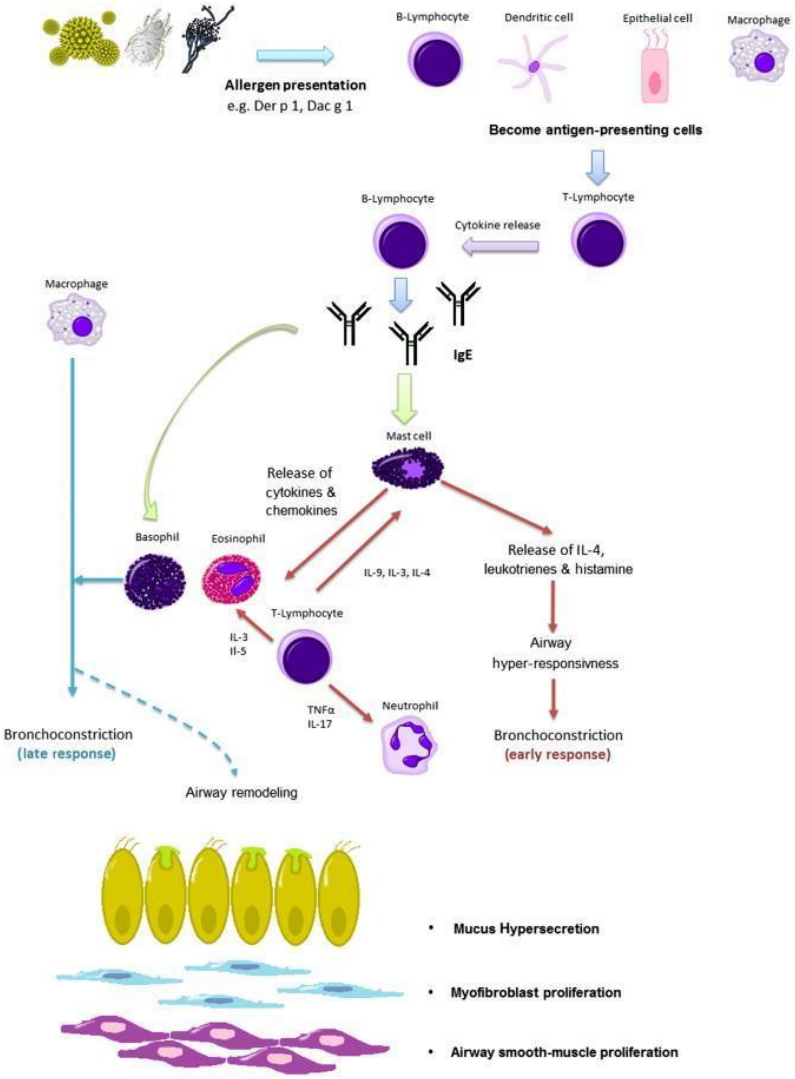

Figure 2.1**:** Pathogenesis of Allergic Asthma

Airflow Obstruction

The narrowing of the airway lumen all through the tracheobronchial tree is caused by the contraction of airway smooth muscle, thickening of the airway wall due to oedema, mucus stopping within the airways, and airway remodelling, which collectively contributes to changing levels of airflow obstruction (Limb *et al*., 2005). Mediators such as histamine and leukotrienes, discharged from inflammatory cells or through reflex neural pathways, trigger the contraction and relaxation of airway smooth muscle (Riccio and Proud, 1996). Airway remodelling, which includes thickening of the basement membrane, deposition of collagen,

and shedding of epithelial cells, can lead to irreversible changes within the airways (Hashmi and Cataletto, 2024). This process quickens the decrease in lung function, especially in people with severe and early-onset asthma (Limb *et al*., 2005).

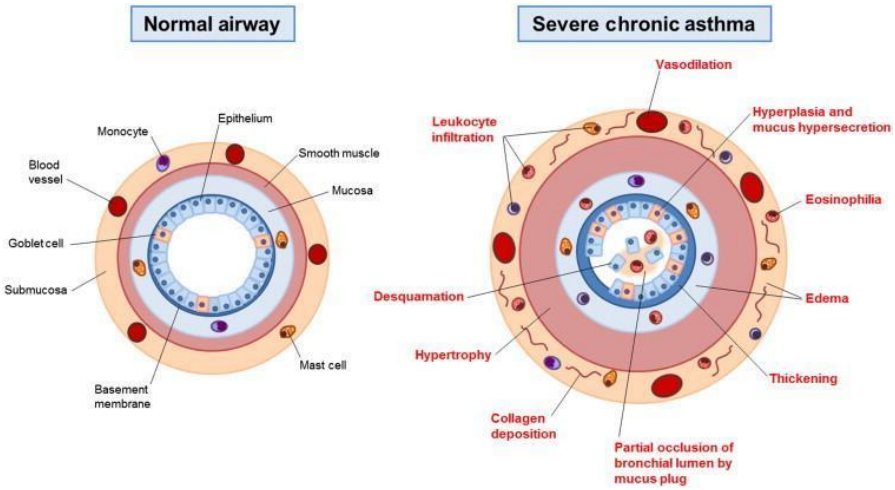

Figure 2.2: Airway Remodeling in Asthma

2.0.2: CLASSIFICATION OF ASTHMA

Asthma can be broadly categorized based on triggers, age of onset, and severity (Bacharier *et al.,* 2012). One common classification is based on the **trigger** of asthma symptoms:

Allergic Asthma (Extrinsic): This is the most common type, often beginning in childhood. Symptoms are triggered by exposure to allergens such as pollen, dust mites, pet dander, and mold (Holgate, 2008).

Non-Allergic Asthma (Intrinsic): This type is less common and often develops in adulthood. Triggers are typically non-allergen related, including irritants like smoke, cold air, exercise, stress, and viral infections (Wenzel, 2005).

Exercise-Induced Bronchoconstriction (EIB): Formerly known as exercise-induced asthma, this involves the transient narrowing of airways that occurs during or immediately after physical exertion (Anderson and Kippelen, 2008). After intense physical activity, individuals who experience asthma symptoms, such as wheezing and shortness of breath, are affected (McFadden & Gilbert, 1994). This reaction is triggered by excessive airway sensitivity to cold or dry air, which occurs during the increased breathing rate seen in exercise (McFadden and Gilbert, 1994). Symptoms usually appear after exercise and can last up to an hour.

Asthma can also be categorized by the **age of onset**:

Childhood-Onset Asthma: Asthma that develops in children, which may persist into adulthood or resolve in some cases (Martinez, 1995).

Adult-Onset Asthma: Asthma that first appears in adulthood. This type is often non-allergic and can be more severe or difficult to manage (Goodman *et al.,* 2003).

2.1: REACTIVE OXIDATIVE STRESS

Oxidative stress is the condition that occurs when the body's antioxidant defenses are overcome by a buildup of reactive oxygen species (ROS) such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radical ($OH^\cdot$) leading to cellular damage(Yadav and saini, 2016). The oxidative stress is an important component as well as consequence of asthma pathogenesis and various

factors like age, duration and lifestyle determine the overall impact of disease(Yadav and saini, 2016).

Reactive oxygen species (ROS), which cause oxidative stress, are primarily generated by mitochondria during normal cellular respiration. However, they are also produced by enzymes like lipoxygenases (LOX) and cyclooxygenases (COX) as part of arachidonic acid metabolism, and by various endothelial and inflammatory cells under both healthy and diseased conditions(Pizzino *et al.,* 2017). The overproduction of the reactive oxygen species (ROS) is as a result of inflammation of the airways that causes oxidative stress by interfering with the tissues in our body like proteins, lipids and DNA and causing dysfunction of these molecules(Yadav and saini, 2016). They exaggerate airways inflammation by inducing many proinflammatory mediators including macrophages, neutrophils and eosinophils(Yadav and saini, 2016).

In asthmatic patients, there is an increased production of reactive oxygen species (ROS) and reactive nitrogen species(Milos *et al.,* 2017). Asthmatic patients consistently show elevated markers of oxidative stress in their blood. A common way to measure this damage is by assessing the level of malondialdehyde (MDA). MDA is a byproduct of lipid peroxidation, which is the oxidative damage to cell membranes(Morales and Munné-Bosch, 2019).

Studies frequently find higher serum MDA levels in individuals with asthma, particularly in those whose condition is poorly managed or severe, thereby indicating a clear link between increased oxidative damage and the severity of the disease(Tofiq *et al.,* 2015). Research has shown that asthmatic patients exhibit substantially higher serum MDA levels (e.g., 13.23 ± 3.86 µmol/L) compared to controls (e.g., 7.75 ± 2.99 µmol/L), with statistically significant

differences ($p < 0.001$). This increase in MDA has been linked to an exacerbation of bronchoconstriction (Tofiq *et al.,* 2015).

Research using real-time monitoring of protein hydroperoxide (HP) formation in the peripheral blood of asthmatic individuals has demonstrated that this process is significantly faster and results in higher overall accumulation compared to healthy subjects, this finding points to enhanced systemic oxidative stress in asthma(Stanislawa *et al.,*2022). Furthermore, children with poorly controlled asthma show increased serum concentrations of Nitric Oxide Metabolites (NOx)(including nitrate), which suggests a greater presence of reactive nitrogen species(Behnaz *et al.,* 2021).

2.2 THE ENZYMATIC ANTIOXIDANTS

The Enzymatic antioxidants include catalase, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD)(Miguel *et al.,*2025). These antioxidant defense systems form a tightly regulated antioxidant network to resist any change in the redox environment of intra- as well as extracellular space(Miguel *et al.,*2025). The enzymatic antioxidants such as catalase present in the pulmonary fluid and interstitial spaces of the lungs and also in the blood vessels and airways help convert the potent oxidant hydrogen peroxide (H_2O_2) to H_2O thus helping to reduce systemic oxidant level. These enzymatic antioxidants have been reported to be decreased in asthmatic patients, which further aids in systemic oxidative stress(Yadav and saini, 2016).

Superoxide Dismutase (SOD) is a crucial enzyme that converts superoxide radicals into hydrogen peroxide. While it is a primary defense, studies often report *decreased* SOD activity in the serum and erythrocytes of asthmatic patients, suggesting an impaired ability to neutralize superoxide (Shokry and El-Tarahony, 2013). The reduction in SOD activity

appears to be more pronounced with increasing severity of asthma. For instance, studies have shown that lower SOD levels are observed in moderate and severe asthma compared to mild asthma (Shokry and El-Tarahony, 2013).

Catalase (CAT) breaks down hydrogen peroxide into water and oxygen. Similar to SOD, reduced CAT activity has been observed in asthmatic patients, indicating a diminished capacity to handle hydrogen peroxide accumulation (Ali *et al* 2024).

Glutathione Peroxidase (GPx) plays a vital role in reducing hydrogen peroxide and organic hydroperoxides. Lower GPx activity has also been noted in the serum and erythrocytes of asthmatic children, further highlighting compromised antioxidant defenses (Shokry and Tarahony, 2013) while Glutathione (GSH) is a key non-enzymatic antioxidant. Decreased serum GSH levels and a lower GSH/GSSG (oxidized glutathione) ratio are indicative of increased oxidative stress and depleted antioxidant reserves in asthmatic patients, especially in those with poorly controlled asthma (Miguel *et al.*, 2025).

The total antioxidant status or capacity in the serum of asthmatic patients is often significantly reduced compared to healthy individuals, particularly during acute exacerbations (Katsoulis *et al.,* 2003). This suggests a systemic depletion of antioxidant reserves (Katsoulis *et al.,* 2003).

2.3 DRUGS IN TREATMENT OF ASTHMA

2.3.1 SABUTAMOL

Salbutamol is a short-acting β2 -adrenergic receptor agonist (β2 -agonist) widely used as a bronchodilator for the relief of acute asthma symptoms (Lipworth, 1999). The drug is a racemic mixture containing two enantiomers: (R)-salbutamol and (S)-salbutamol. The (R)-salbutamol enantiomer is responsible for its therapeutic effects, including bronchodilation

(Ehrhardt *et al*., 2005), while (S)-salbutamol is associated with airway hypersensitivity and bronchoconstriction(Nakpheng *et al*., 2017). Despite its enantioselective properties, salbutamol is typically administered as a 1:1 racemic mixture, although this may influence its overall efficacy and side-effect profile (Nakpheng *et al*., 2017). By stimulating β2 -receptors on airway smooth muscle, salbutamol induces relaxation and bronchodilation, thereby improving airflow. While its primary action is bronchodilation, some studies suggest that β2 -agonists may also possess modest anti-inflammatory and antioxidant properties (Kobayashi *et al.,* 2005).

Oral formulations, including tablets, syrups, and extended-release preparations, are used when inhalation therapy is not feasible (Florea *et al.,* 2003). However, the oral route is less favoured due to lower bioavailability and systemic side effects resulting from extensive hepatic metabolism. Inhalation formulations, such as dry powder inhalers (DPIs) and pressurised metered dose inhalers (pMDIs), are the most commonly used forms (Domínguez-Romero *et al.,* 2013). These are designed to deliver salbutamol directly to the airways, enhancing its therapeutic effect while minimising systemic exposure. Additionally, injectable formulations of salbutamol are used in emergency situations, such as severe asthma exacerbations, where rapid and systemic bronchodilation is required (Nakpheng *et al.,* 2017).

2.3.1.1 MECHANISM OF ACTION OF SABUTAMOL

Salbutamol exerts its effects by stimulating β2 adrenergic receptors on bronchial smooth muscle cells(Gumbhir-Shah *et al.,* 1999). Activation stimulates adenylate cyclase, an enzyme that increases the production of cyclic AMP (cAMP) (Gumbhir-Shah *et al.,* 1999). Elevated cAMP levels relax bronchial smooth muscle, thereby relieving bronchoconstriction (Gumbhir-Shah *et al.,* 1999). In addition to its bronchodilator effects, salbutamol has anti-inflammatory properties. It inhibits the release of inflammatory mediators from mast cells

and eosinophils while suppressing the production of cytokines reducing airway inflammation (Hamilton *et al.,* 2001).

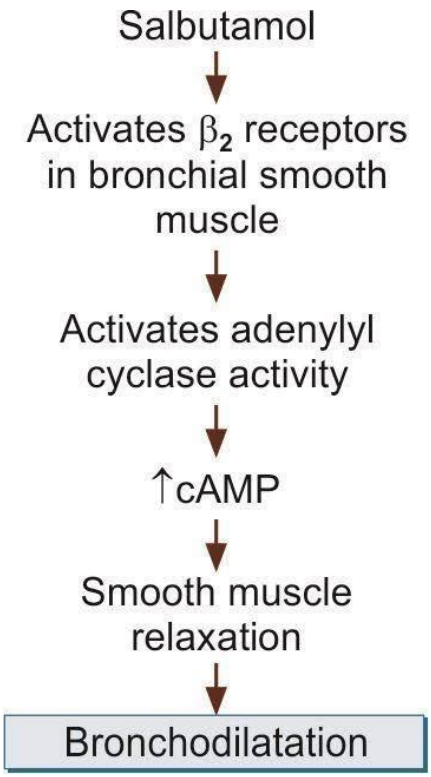

Figure 2.3: Schematic diagram of the mechanism of action of salbutamol.

2.3.1.2: EFFECTS OF SABUTAMOL ON OXIDANTS AND ANTIOXIDANTS

Specifically, salbutamol increases the activity of superoxide dismutase (SOD), a key enzyme that neutralises harmful superoxide radicals and boosts levels of glutathione. This vital intracellular antioxidant protects cells from damage (Eteraf-Oskouei *et al.,* 2017). Salbutamol demonstrates notable antioxidant effects, which significantly contribute to its anti-inflammatory properties. In various models of inflammation, salbutamol has been observed to reduce myeloperoxidase (MPO) activity, an enzyme that produces reactive oxygen species that exacerbate oxidative stress (Kumar *et al.,* 2023). Additionally, it decreases lipid peroxidation (LPO) levels, a marker of cellular damage caused by oxidative stress (Uzkeser *et al.,* 2012). Concurrently, salbutamol enhances the activity of superoxide dismutase (SOD).

This critical antioxidant enzyme neutralises harmful superoxide radicals and boosts glutathione levels, a key molecule in cellular antioxidant defence mechanisms (Eteraf-Oskouei *et al.,* 2017).

These effects collectively suggest that salbutamol mitigates oxidative stress, likely by activating β2-adrenergic receptors. By reducing oxidative damage and promoting antioxidant defences, salbutamol provides therapeutic benefits in managing inflammatory conditions such as asthma(Eteraf-Oskouei *et al.,* 2017).

2.3.2: MONTELUKAST

Montelukast is a selective leukotriene receptor antagonist (LTRA) that blocks the action of cysteinyl leukotrienes (LTC 4 , LTD 4 , and LTE 4) at the CysLT1 receptor (Drazen *et al.,* 1999). Leukotrienes are potent inflammatory mediators that contribute to bronchoconstriction, mucus secretion, and airway inflammation in asthma. By inhibiting leukotriene signaling, montelukast helps to reduce these inflammatory processes. Emerging evidence suggests that leukotrienes can also contribute to oxidative stress in the airways (Dworski *et al.,* 1991).

It is commonly prescribed for managing chronic asthma and allergic rhinitis. The medication disrupts the signalling pathways of leukotrienes in various cells and tissues, which are responsible for airway muscle constriction, abnormal pulmonary fluid buildup (airway oedema) and in some cases, inflammation in the lungs (McCarthy, 2023). It effectively alleviates rhinitis symptoms and offers asthma relief, as demonstrated in a study that significantly enhanced Daily Rhinitis Symptom scores compared to a placebo (Philip *et al.,* 2004).

Pharmaco-kinetically, montelukast is rapidly and efficiently absorbed orally, reaching peak serum concentrations approximately two hours after administration, with an average bioavailability of 73% (Li *et al.,* 2021).

2.3.2.1: MECHANISM OF ACTION OF MONTELUKAST

Montelukast binds strongly to cysteinyl leukotriene receptors, particularly those for leukotrienes D_4 and E_4. These leukotrienes, released by various cells, such as mast cells, play a role in the inflammatory processes associated with asthma and allergic rhinitis. Leukotriene receptors exist in airway cells, including smooth muscle cells and macrophages (Wermuth *et al.,* 2023). It blocks the physiological effects of leukotrienes such as swelling of the airway, contraction of smooth muscles, and cellular dysfunction without showing agonist activity. In asthmatic individuals, a low dose of montelukast (5 mg) significantly inhibits bronchoconstriction caused by leukotriene D_4 (Yan *et al.,* 2024).

2.3.2.2: EFFECTS OF MONTELUKAST ON SERUM OXIDANT AND ANTIOXIDANTS.

Montelukast has shown considerable antioxidant properties in several studies. It boosts glutathione (GSH) levels and improves the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in tissues impacted by oxidative stress, including the lungs, liver, and kidneys (Dengiz *et al.,* 2007). In children with asthma, montelukast decreased free radical production in whole blood and polymorphonuclear neutrophils (PMNs), suggesting its role in regulating oxidative stress (Al Saadi *et al.,* 2011). Furthermore, it reduces oxidative damage in sepsis and endotoxemia models by boosting the activity of antioxidant enzymes (Mohamadin *et al.,* 2011).

2.3.3: PREDNISOLONE

Prednisolone, a commonly used glucocorticoid in asthma management, is critical in reducing airway inflammation and enhancing lung function. Its anti-inflammatory properties help alleviate asthma symptoms, as evidenced by a study where oral prednisolone significantly reduced asthma severity scores, decreased the need for albuterol (a bronchodilator) and

improved forced expiratory volume in one second (FEV_1) in patients experiencing severe asthma exacerbations (Djukanović *et al.,* 1997). In pediatric cases of acute asthma, prednisolone has been shown to increase hospital discharge rates compared to placebo (Connett *et al.,* 1994).

Furthermore, prednisolone acts on inflammatory pathways by modulating airway inflammatory cells. It reduces eosinophil and T lymphocyte counts, which are critical contributors to asthma's underlying inflammation and hyper-responsiveness (Bentley *et al.,* 1996).

Prednisolone demonstrates intricate pharmacokinetics, marked by fast absorption and considerable individual variability. Following oral administration, peak plasma levels are usually attained within 1 to 3 hours, with a bioavailability ranging from approximately 70% to 90% (Bergmann *et al.,* 2012). The drug exhibits extensive protein binding, ranging from 65% to 91%, and is predominantly metabolised in the liver, resulting in a half-life of approximately 2 to 3 hours (Ryu *et al.,* 2018).

2.3.3.1: MECHANISM OF ACTION OF PREDNISOLONE

Prednisolone primarily exerts its effects through the glucocorticoid receptor (GR). Once prednisone is converted to prednisolone in the liver, it binds to the cytosolic GR, forming a steroid-receptor complex that moves to the nucleus. This complex influences gene expression by interacting with specific DNA regions, suppressing pro-inflammatory cytokines and stimulating anti-inflammatory proteins (Boudinot *et al.,* 1986). The mechanism also involves reducing vascular permeability and inhibiting leukocyte migration to inflammatory sites, which contributes to its anti-inflammatory and immunosuppressive effects. Furthermore, prednisolone can induce apoptosis in specific immune cells, enhancing its therapeutic action in conditions such as asthma and autoimmune disorders (Bergmann *et al.,* 2012).

2.3.3.2: EFFECTS OF PREDNISOLONE ON SERUM OXIDANTS AND ANTIOXIDANTS.

Prednisolone has been shown to affect antioxidant levels and oxidative stress significantly. In a rat study, prednisolone administration resulted in reduced activities of antioxidant enzymes like glutathione peroxidase and catalase, along with a decrease in glutathione (GSH) levels, which suggests an increase in oxidative stress. The study also found elevated lipid peroxidation, indicating that prednisolone may cause oxidative damage (Bardas *et al.,* 2020).

In contrast, another study showed that acute prednisolone treatment improved the total reactive antioxidant potential (TRAP) suggesting a potential protective effect against reactive oxygen species (ROS). However, chronic treatment increased lipid peroxidation without altering TRAP levels, implying that prolonged use could result in oxidative damage despite the initial antioxidant benefits (Torres *et al.,* 2014).

2.4: ANIMAL MODELS OF ASTHMA

Animal models of asthma provide a valuable tool for studying asthma within a fully functioning immune and respiratory system (Zosky and Sly, 2007). These models have highlighted the role of T-helper type 2 mediated allergic 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 due to their genetic manipulability and ability to induce allergic responses through sensitisation with

allergens like ovalbumin. They are essential for studying airway hyperresponsiveness and inflammation mechanisms, although they may not wholly mimic all aspects of human asthma (Woodrow *et al*., 2023). The BALB/c strain is particularly favoured because of its immune system characteristics and ease of genetic modification. Other strains, such as C57BL/6 and A/J, are used less frequently (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. Historically, guinea pigs have been preferred for asthma studies because of their strong natural airway hyperresponsiveness and sensitivity to various allergens, allowing for effective therapeutic intervention testing (Woodrow *et al*., 2023).

Rat Models: Asthma symptoms, such as airway hyperresponsiveness and inflammation, are generally easier to replicate in rats than in mice. Rats are also more significant and more manageable, allowing for the collection of larger sample volumes and making rat models of asthma increasingly valuable (Camps-Bossacoma *et al*., 2015). However, strains like Wistar, Sprague-Dawley, Fisher, and Lewis rats do not continually develop an allergic response to IgE production (Kucharewicz *et al*., 2008). Despite this, asthma models have been successfully established in Wistar 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 sensitisation, making it a more suitable model for studying allergic asthma. Rats like 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 than 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 than smaller animal models (Zosky and Sly, 2007).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials

The materials used in this study included:

- Standard rodent feed and clean water
- Plastic cages
- Dissection materials and syringes
- EDTA bottles and formaldehyde
- Aluminum Hydroxide and Ovalbumin (OVA)
- Compressor Nebulizer (CNB 69009)
- Oral gavage
- Anesthetic agents such as chloroform
- Protective gloves
- Saline solution
- Pharmacological agents: Salbutamol (1 mg), Montelukast (10 mg), and Prednisolone (3 mg).

3.2. Experimental Animals

64 female Sprague-Dawley rats were purchased for this study. The animals were housed in clean, well-equipped cages within a cool and sterile environment maintained at a room temperature. They were provided with standard rodent chow and water ad libitum throughout the experimental period, in accordance with international guidelines for the handling of experimental animals.

For individual identification, each rat was labeled on different body parts (e.g., head, belly, back, left hand, tail) using picric acid and Gentian Violet stains.

3.3 Study Design

The study spanned a seven-week duration, structured into three phases: a two-week acclimatization period, followed by one week for asthma induction, and a four-week treatment phase.

For the study, 64 female Sprague Dawley rats weighing between 180g and 250g were organized into eight groups, with each group having a maximum of eight rats. The eight experimental groups were as follows:

- GROUP 1: Negative Control
- GROUP 2: Positive control (Asthma induced and not treated)
- GROUP 3: Asthma induced and treated with Salbutamol
- GROUP 4: Asthma induced and treated with Montelukast
- GROUP 5: Asthma induced and treated with Prednisone
- GROUP 6: Asthma induced and treated with Salbutamol and Prednisolone
- GROUP 7: Asthma induced and treated with Salbutamol and Montelukast
- GROUP 8: Asthma induced and treated with Prednisolone and Montelukast

3.4 Experimental Procedure

The experiment was carried out in phases:

3.4.1: Phase 1

Asthma was induced in the positive control group (GROUP 2) and all test groups (GROUP 3-8) following the modified guideline outlined by Bai *et al.,* 2019) and Wu *et al.,* (2019). The

sensitization phase involved an intraperitoneal injection of a solution containing 56 mg of OVA and 1020 mg of aluminium hydroxide dissolved in 56 mL of saline. Each rat received 1 mL of this mixed solution on days 1 and 7.

Following sensitization, the rats were challenged by placing them in a transparent box (50 cm x 40 cm x 30 cm) filled with an aerosolized solution. The aerosol was generated from 0.6 g of OVA dissolved in 115 mL of saline using a Compressor Nebulizer (CNB 69009) with an aerosol delivery rate of ≥ 0.25 mL/min for 15 minutes weekly.

The negative control group (GROUP 1) was sensitized with an intraperitoneal injection of saline and challenged with aerosolized saline, respectively, to serve as a baseline for comparison.

3.4.2: Phase 2

Asthma induction was verified one week after the first challenge. This was confirmed by observing evidence of neutrophilia and eosinophilia in the rats from the induced groups, which were then compared against the negative control group (Bai *et al.,* 2019; Wu *et al.,* 2019).

A four-week treatment phase commenced. treatment began with 1 mg/kg salbutamol (oral) (Nair and Prabhavalkar, 2021), 3 mg/kg prednisolone (oral) (Pourmehdi *et al*., 2020) and 10 mg/kg montelukast (oral)

3.4.3: Phase 3

All animals were euthanised after drug administration. Blood and tissue samples were collected for biomarker 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 from the portal vein. They were kept at room temperature for 30 min, followed by centrifugation at 5000 rpm (rounds per minute) for 15 min, and serum isolated by aspiration. The separated serum was frozen for the later quantitative determination of some biomarkers (Thakur *et al*., 2019).

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 GraphPad Prism 10.2.2 statistical analysis software (GraphPad, San Diego, CA). $P < 0.05$ was considered statistically significant.

CHAPTER 4

4.0: RESULTS

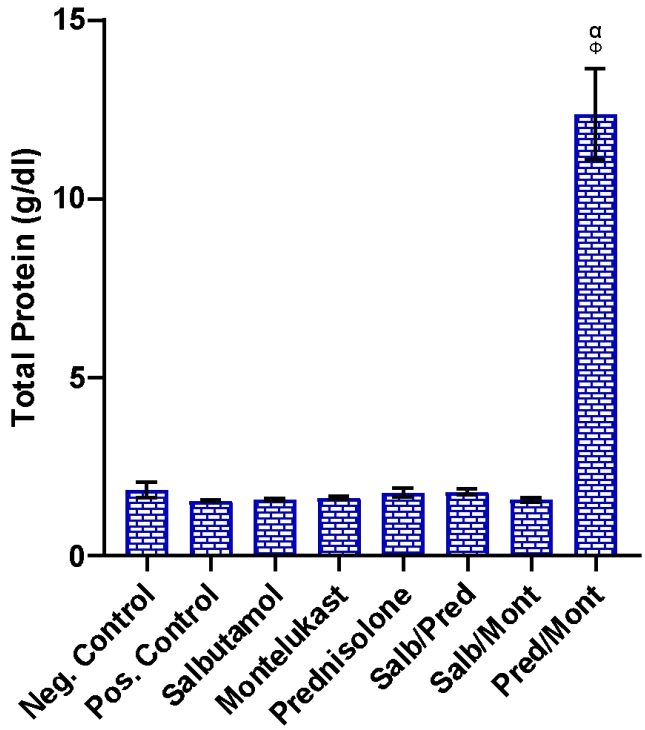

Figure 4.1: Charts showing the effects of salbutamol, montelukast, prednisolone and their combinations on serum total protein concentration.

The Results show a statistically significant increase in heart tissue total protein concentration in the prednisolone/montelukast-treated group compared with the negative and positive controls. ($p<0.05$), but no statistically significant difference among the positive control, salbutamol, montelukast, prednisolone, salbutamol/prednisolone, and salbutamol/montelukast-treated 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.

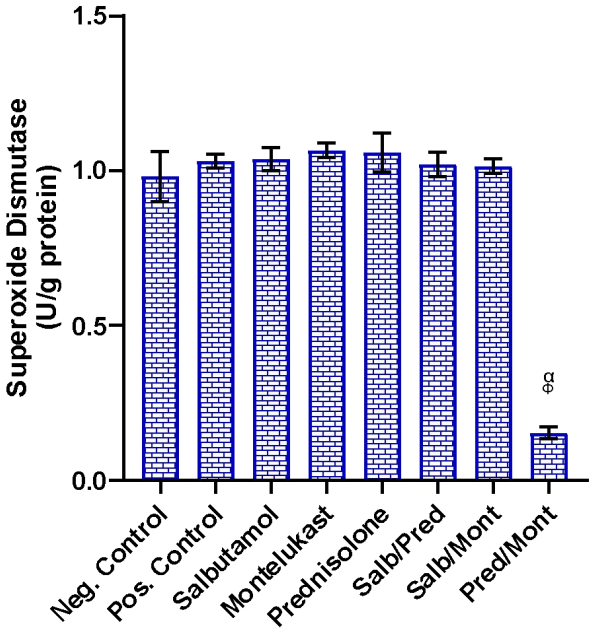

Figure 4.2: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum superoxide dismutase enzyme activity

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

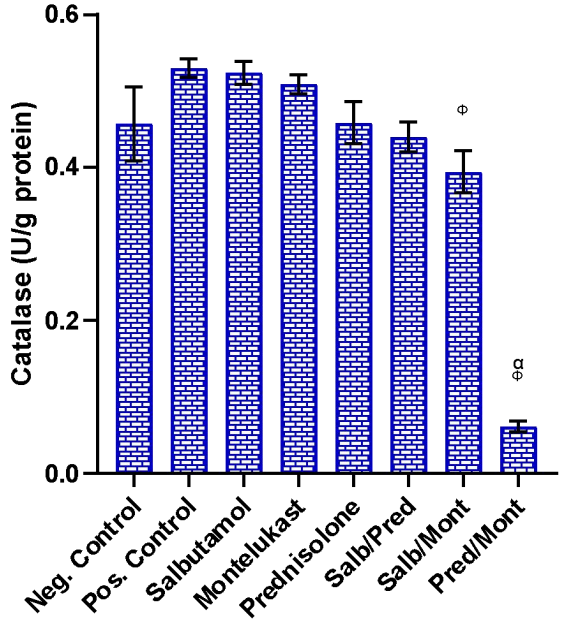

Figure 4.3: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum catalase enzyme activity

The result show a statistically significant decrease in catalase enzyme activity in the prednisolone/montelukast-treated group compared with the negative and positive controls. ($p<0.05$), but no statistically significant difference among the positive control, salbutamol, montelukast, prednisolone, salbutamol/prednisolone, and salbutamol/montelukast-treated groups compared with the negative control ($p>0.05$). Also, there was a statistically significant decrease in the salbutamol/montelukast-treated group 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.

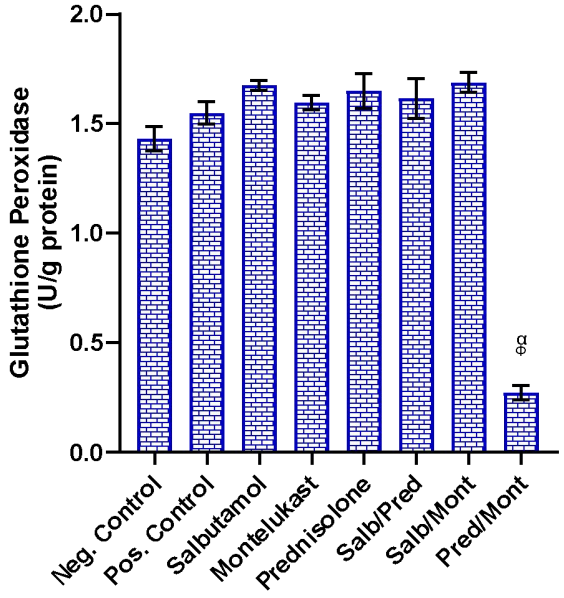

Figure 4.4: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum glutathione peroxidase enzyme activity

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

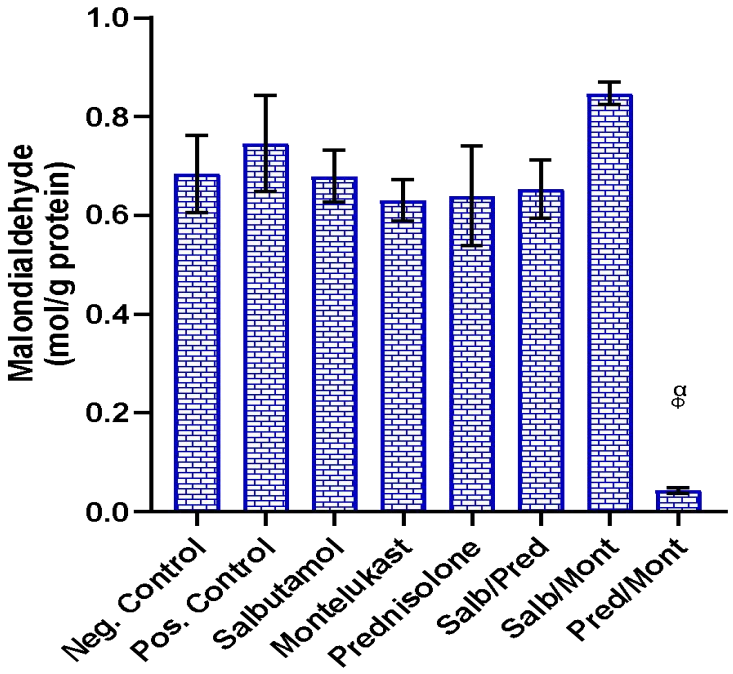

Figure 4.5: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum malondialdehyde concentration

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

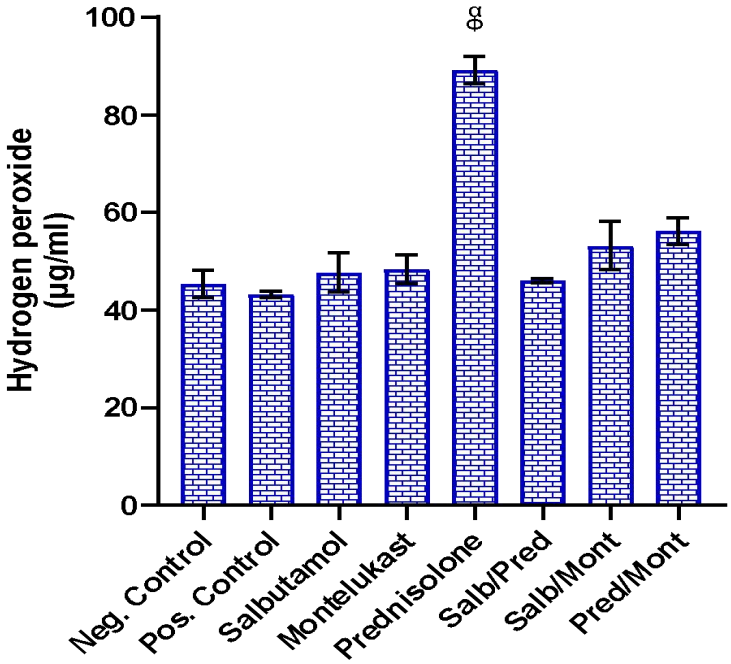

Figure 4.6: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum hydrogen peroxide concentration

The Results show a statistically significant increase in hydrogen peroxide concentration in the prednisolone-treated group compared with the negative and positive controls. ($p<0.05$), but no statistically significant difference among the positive control, salbutamol, montelukast, salbutamol/prednisolone, salbutamol/montelukast, and prednisolone/montelukast-treated 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.

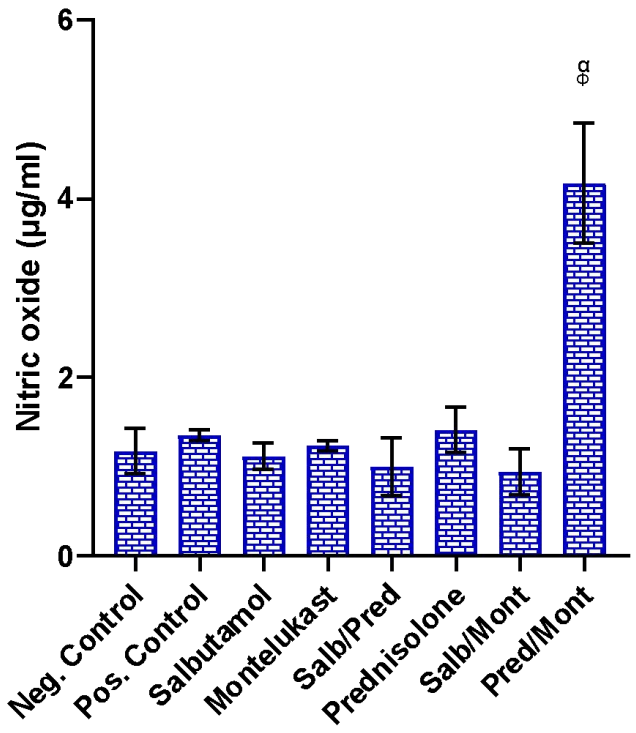

Figure 4.7: Charts showing the effects of salbutamol, montelukast, prednisolone, and their combinations on serum nitric oxide concentration

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

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Total Protein measures the amount of protein in the blood, specifically in the fluid portion called serum. It's a clinical chemistry test that can help diagnose several health conditions. In asthma, alterations in serum protein levels can reflect the systemic acute-phase response(Panteghini, 2016).

An increase could indicate a heightened state of systemic inflammation or a specific metabolic response to treatment. The result reveals a significant finding, the combination of prednisolone and montelukast induced a statistically significant increase in heart tissue total protein concentration, an effect not observed with any other monotherapy or combination therapy, including salbutamol with either drug.

This suggests that co-administration of prednisolone and montelukast may have synergistic effects on protein synthesis or hepatic protein metabolism, possibly due to glucocorticoid-induced enhancement of protein synthesis and montelukast's modulation of inflammatory mediators (Benson *et al.,* 2018).

The absence of significant changes in other groups implies that salbutamol and individual drug treatments do not markedly alter systemic protein levels.

Superoxide Dismutase is an Antioxidant enzyme that protects the heart from ischemia and the lungs from inflammation and fibrosis (Coksun *et al.,* 2011). It is a class of enzymes that restrict the body's biological oxidant cluster enzyme system, which can effectively respond to cellular oxidative stress as well as lipid metabolism, inflammation, and oxidation. In asthma, superoxide dismutase activity is significantly lower in epithelial lining fluid and airway epithelial cells in asthma.

A significant decrease in SOD activity was observed in the prednisolone/montelukast group compared to controls ($p<0.05$). Since SOD protects cells against oxidative stress by dismutating superoxide radicals, its reduction indicates possible oxidative imbalance. The finding may be attributed to prednisolone's oxidative side effects and montelukast's potential pro-oxidant influence when co-administered (Kang *et al.,* 2020).

Glutathione Peroxidase is a family of enzymes that protect cells from oxidative damage caused by Reactive Oxygen Species (ROS). It helps to scavenge free radicals, which in turn helps to prevent lipid peroxidation. In Asthma, lower levels of glutathione peroxidase activity contribute to oxidative stress in the airways of asthmatics, which can exacerbate inflammation and airway hyperresponsiveness.

This study shows a statistically significant decrease in GPx activity was recorded for the prednisolone/montelukast group ($p<0.05$). GPx is essential for detoxifying hydrogen peroxide and lipid peroxides; its reduction indicates heightened oxidative vulnerability. The observation may be linked to steroid-related oxidative damage and possible interference of montelukast in redox balance (Park *et al.,* 2019).

Catalase is an antioxidant enzyme present in all aerobic organisms. Which catalyses the decomposition of hydrogen peroxide into water and oxygen (Coksun *et al.,* 2011). It is an essential enzyme in protecting the cell from oxidative damage by Reactive Oxygen Species (ROS) (Coksun *et al.,* 2011). Asthma is associated with a decrease in catalase activity, meaning that people with asthma tend to have lower levels of this antioxidant enzyme in their bodies, particularly in the lung lining, which contributes to the oxidative stress experienced in the airways during asthma attacks; this reduction in catalase activity is often due to oxidative modifications like nitration, effectively impairing its function.

Catalase activity significantly decreased in both prednisolone/ montelukast and salbutamol/ montelukast groups ($p<0.05$). Catalase breaks down hydrogen peroxide, and its suppression may enhance oxidative stress. This aligns with reports that corticosteroids and β-agonists can reduce antioxidant enzyme activity through mitochondrial stress mechanisms (Ahmed and Oluwole, 2021). The results indicate that combined therapy may impair antioxidant defense systems more than monotherapy.

Malondialdehyde is a toxic molecule that is a by-product of lipid peroxidation (Uzkeser *et al.,* 2012). It's a known mutagen and carcinogen that can damage DNA and is used as a biomarker of oxidative stress in clinical research. In asthma, the inflammatory process within the airways can lead to increased production of reactive oxygen species (ROS), which causes lipid peroxidation and results in elevated MDA levels.

There was a significant decrease in serum MDA in the prednisolone/montelukast group compared to controls ($p<0.05$). Since MDA is a marker of lipid peroxidation, the reduction suggests lower lipid oxidative damage despite reduced antioxidant enzymes. This paradox could reflect anti-inflammatory suppression of free radical generation by the drug combination, as glucocorticoids often inhibit lipid peroxidation via NF-κB pathway inhibition (Ramos and de Souza, 2022).

Hydrogen Peroxide (H_2O_2) is produced endogenously primarily through the dismutation of superoxide radicals, a reaction catalyzed by the enzyme superoxide dismutase (SOD) (Sies,2017). It can directly cause hyperresponsiveness in airway smooth muscle, increase mucus secretion, induce damage to the airway epithelium, and act as a chemoattractant for more inflammatory cells, thereby perpetuating the inflammatory cycle (Nadeem *et al.,* 2008). The concentration of H_2O_2 is normally kept in check by antioxidant enzymes like catalase and glutathione peroxidase. Therefore, an increase in H_2O_2 reflects either an overproduction from its precursor (superoxide), an impairment in its decomposition, or both.

The result for hydrogen peroxide concentration presents a distinct and intriguing pattern compared to the previous findings. Here, the significant effect is driven by prednisolone monotherapy, which caused a statistically significant increase in H_2O_2 compared to both negative and positive controls. This indicates that prednisolone may induce oxidative stress through enhanced ROS production. Corticosteroids are known to increase mitochondrial ROS generation, contributing to oxidative tissue injury if unopposed by antioxidant enzymes (Sarkar *et al.,* 2017).

Nitric oxide is expressed in airway epithelial and inflammatory cells in response to inflammatory cytokines (Barnes, 2010). High concentrations of NO can react with superoxide ($O_2^{\bullet -}$) to form peroxynitrite ($ONOO^-$), a potent nitrosative stress molecule that damages proteins, lipids, and DNA, thereby exacerbating airway injury and hyperresponsiveness. The result demonstrating a statistically significant increase in serum nitric oxide concentration

specifically in the prednisolone/montelukast-treated group is a critical and paradoxical finding. Elevated NO suggests enhanced inflammatory signaling or endothelial activation. However, excessive NO can contribute to nitrosative stress, especially when antioxidant defenses are reduced. The dual modulation of inflammation and oxidative pathways by montelukast and prednisolone might explain this outcome (Okafor and Moses, 2020).

CONCLUSION

This study reveals that first-line asthma drugs have distinct extrapulmonary effects on oxidative homeostasis. The Prednisolone-Montelukast combination critically impairs the enzymatic antioxidant system, while Prednisolone alone can elevate oxidant levels. These findings highlight that the choice of asthma therapy, especially long-term combination regimens, requires consideration of systemic oxidative balance, moving beyond airway-specific effects to ensure comprehensive patient management and mitigate potential long-term metabolic consequences.

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