EFFECT OF MONTELUKAST AND HYDROCORTISONE ON ANTIOXIDANT LEVELS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS

BY

ABIODUN GOD'SGIFT OSHONAME (BMS1802572)

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

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CERTIFICATION

This is to certify that this project work on **EFFECT OF MONTELUKAST AND HYDROCORTISONE ON ANTIOXIDANT LEVELS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS** was carried out by **ABIODUN GOD'SGIFT OSHONAME (BMS1802572)** in partial fulfilment of 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.

_____ _____

ABIODUN GOD'SGIFT OSHONAME **DATE**
Student

_____ _____

MRS. UA PETERS **DATE**

Project Supervisor

_____ _____

PROF. O.K UCHE **DATE**

Head of Department

_____ _____

EXTERNAL EXAMINER **DATE**

DEDICATION

This project work is dedicated to God Almighty who is lavishing in knowledge and understanding. I also dedicate it to my family and friends for their care and support through this undergraduate journey of mine.

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CHAPTER ONE

1.0 INTRODUCTION

Asthma is a chronic inflammatory disorder with variable airway obstruction and bronchial hyper-responsiveness that results in recurrent episodes of wheezing, coughing, chest tightness, and shortness of breath in asthmatics. Asthma is caused

by heterogenic gene-environment interactions that are not fully understood. (Mims, 2015).

Cough variant asthma, exercise-induced asthma, aspirin-exacerbated respiratory disease (AERD), and vocal cord dysfunction (VCD) are all variations of asthma. However, vocal cord dysfunction (VCD) deserves special attention because it is a classic mimic of asthma and may also be a comorbid condition (Wu *et al*., 2019).

Health professionals who treat upper or lower airway inflammation should be aware of the diagnosis and pathophysiology of asthma since it is crucial that scientific understanding of the disease continue to advance (Mims, 2015).

The past two years have seen significant advancements in understanding the probable causes of asthma exacerbations and development, and these innovative insights have helped identify and establish promising new routes for potential therapeutic intervention (Miller *et al*., 2021).

While asthma was once thought to have a single diagnosis and standardized treatments for all patients, it is now recognized as a heterogeneous, multifactorial disorder with a variety of genetic and environmental factors, where targeted therapies improve asthma control (Melissa and Tatyana, 2020).

Despite a wide range of treatment options, nearly half of adults with asthma report having one or more attacks in the previous year, highlighting the significance of symptom management and disease control (Mazurek and Syamlal, 2018).

Target-directed agents are now readily available for patients with severe persistent asthma as a result of improved knowledge of the pathophysiology and biomarkers of asthma (Melissa and Tatyana, 2020). Anti-asthmatic medications like Montelukast and Hydrocortisone are two examples.

Montelukast is a leukotriene inhibitor that is frequently used to treat chronic asthma and allergic rhinitis by obstructing leukotriene-produced molecular signaling pathways in a variety of cells and tissues throughout the human body that result in airway muscle tightening, the production of abnormal pulmonary fluid (airway edema): and in some cases, pulmonary inflammation (McCarthy, 2023).

Using Montelukast can improve pulmonary function by lowering inflammatory markers (Khan *et al*., 2022). Montelukast, a leukotriene receptor antagonist, has a smaller effect size than inhaled corticosteroids for asthma exacerbations of varying severity (Zhang *et al*., 2014).

On the other hand, glucocorticoids like hydrocortisone are used to treat a variety of inflammatory and allergy conditions, including many pulmonary illnesses like asthma, chronic obstructive pulmonary disease, influenza, and bronchitis (Barnes, 2010): as well as bronchopulmonary dysplasia in infants (Rademaker *et al*., 2008; Morris *et al*., 2019; Doyle *et al*., 2010).

The glucocorticoid potency of hydrocortisone, which is structurally closest to endogenous cortisone, is weakest, but it has a higher mineralocorticoid effect; because of this, special attention must be paid to the risk of elevated blood pressure (Sule *et al*., 2021).

Both the anti-asthma medications montelukast and hydrocortisone are successful in reducing the severity of asthma symptoms and asthma-related deaths. Nevertheless, there is little research on the application and efficacy of hydrocortisone for asthma (Sule *et al*., 2021).

Asthma, characterized by airway inflammation and hyperresponsiveness, has a complex relationship with antioxidant enzyme levels, which play a crucial role in defending against oxidative stress. Some of these antioxidant enzymes include Superoxide Dismutase (SOD): Catalase (CAT), Glutathione Peroxidase (GPx), and Glutathione Reductase, among others. Individuals with asthma often exhibit altered antioxidant enzyme levels, influenced by genetic, environmental, and disease-related factors (Sackesen *et al*., 2008). This imbalance can contribute to increased susceptibility to oxidative stress, airway inflammation, and worsened disease severity (Cazzoletti *et al*., 2006). Additionally, antioxidant enzyme levels have been associated with asthma severity, control, and lung function (Cazzoletti *et al*., 2006). Supplementation with antioxidants, such as vitamins C and E, and dietary modifications emphasizing antioxidant-rich foods, may offer potential therapeutic benefits by restoring antioxidant balance and mitigating oxidative stress (García-Larsen *et al*., 2017; Riccioni *et al*., 2005).

1.1 JUSTIFICATION OF STUDY

Asthma is a common respiratory condition that affects people of all ages worldwide. According to the Global Burden of Disease Study 2017, an estimated

339 million people were living with asthma globally. This study also reported that asthma was responsible for approximately 417,918 deaths in 2017, hence there is a pressing need in the modern world for asthma to be treated more successfully. As molecules that fight free radicals in the body, antioxidant levels are impacted by asthma, making them a diagnostic and therapeutic marker for asthma. Anti-asthma medications like hydrocortisone and montelukast may have an impact on antioxidant levels in people with asthma.

1.2 AIM

The purpose of this study is to ascertain how Montelukast and Hydrocortisone affect antioxidant levels in asthma-induced Sprague Dawley rats.

1.3 SPECIFIC OBJECTIVES

To determine how the asthma-induced Sprague Dawley rats' levels of antioxidants, such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase, etc., may be impacted by the use of the asthma medications Montelukast and Hydrocortisone.

1.4 RESEARCH QUESTIONS

1. Does Montelukast affect antioxidant levels in asthma-induced Sprague Dawley rats?

2. Does Hydrocortisone affect antioxidant levels in asthma-induced Sprague Dawley rats?

3. If yes, by what physiological mechanism does this occur?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ASTHMA

About 350 million individuals worldwide suffer from asthma, a chronic inflammatory disease of the airways (Vos *et al*., 2015). Now that this heterogeneous disease is being investigated at the cellular and molecular levels, there are new options for its prevention and control (Wenzel, 2012; Fajt and Wenzel, 2015).

The underlying pathophysiology of severe asthma is chronic damage and modification of airway epithelial cells (AECs) and airway smooth muscle cells (Jiang *et al*., 2021). In other words, asthma pathophysiology involves chronic inflammation of both large and small airways, as well as bronchial remodeling (Bara *et al*., 2010).

The immune pathophysiology of asthma involves the activation of both the innate and adaptive immune systems to stimulate chronic airway inflammation, which can result in airway obstruction and hyperresponsiveness. Some known factors that cause chronic airway inflammation include allergens, infections, obesity, hormones, tobacco smoke, exercise, cold air, genetic mutations, and systemic eosinophilia (Melissa and Tatyana, 2020). Various cells, including eosinophils, cytokines, and mediators, are also involved in asthma (Niimi, 2013). A few asthma phenotypes have been identified from recognizable clusters of demographic, clinical, and/or pathophysiological traits, including allergic/non-allergic, late-onset, fixed airflow obstructed, obesity-induced asthma, and electronic nose-derived inflammatory phenotype (Wenzel, 2012; Brinkman *et al*., 2019; Moore *et al*., 2010). Studies have revealed metabolic problems and various metabolic phenotypes in asthma patients (Reinke *et al*., 2017; Kelly *et al*., 2017; Claudia *et al*., 2014). The coordination of a vast number of metabolites is necessary for normal human body function. A new therapeutic approach for asthma may be individualized care depending on the phenotype. Additionally, there is a growing body of study on asthma nowadays, and its pathophysiology is being thoroughly elaborated, albeit it is still unclear how molecular mediators of asthma, such as metabolites like lipids, act in vivo (Wang, 2021).

2.1.1 TREATMENT OF ASTHMA

Asthma was previously thought to be a single diagnosis with standardized treatments for all patients; however, asthma is now recognized as a heterogeneous, multifactorial disorder with a variety of genetic and environmental factors, where targeted therapies result in improved asthma control, and thus there is an urgent need to treat asthma more effectively (Melissa and Tatyana, 2020). Chronic airway inflammation is a hallmark of asthma and, as such, an important target for treatment (Tashkin *et al*., 2019). The use of glucocorticosteroids (corticosteroids) has been studied in both illnesses (Tashkin *et al*., 2019): with the first successful treatment of asthma with an oral corticosteroid (OCS) reported in 1950 (Crompton, 2006). Target-directed medicines are now readily available for patients with severe, persistent asthma thanks to advances in our understanding of underlying pathophysiology and biomarkers (Melissa and Tatyana, 2020). Treatments of asthma range from the use of corticosteroids down to Long-acting Beta Agonists, Leukotriene Modifier, Theophyllines, Long-acting Muscarinic Agents, Macrolide Antibiotics, Vitamin D, Allergen Immunotherapy, Aspirin Desensitization, Dihydrofolate Reductase Inhibitor, hormones, Anti-IgE, CRTh2 Antagonist, Bronchial Thermoplasty etc (Melissa and Tatyana, 2020). The first-line medications for acute severe asthma include oxygen, corticosteroids, salbutamol (albuterol), and anticholinergics, while the second-line medications include heliox, magnesium sulfate, ketamine, and inhalational anesthetics, with future therapies including furosemide, leukotriene modifiers, antihistamines, and phosphodiesterase inhibitors (Lucian *et al*., 2001). Two drugs of focus in this review are Montelukast (a leukotriene modifier or antagonist) and Hydrocortisone (a corticosteroid).

2.2 MONTELUKAST

Montelukast, the most widely used leukotriene-modifying agent (LTMA), is a selective leukotriene receptor antagonist and is currently indicated for prophylactic and chronic treatment of asthma, relief of symptoms of allergic rhinitis, and acute prevention of exercise-induced bronchoconstriction (Paljarvi *et al*., 2022).

Leukotriene receptor antagonists (LTRAs) are useful for treating chronic asthma, exercise-induced asthma, and aspirin-induced asthma; some patients respond to LTRA better than ICS, hence a customized approach to asthma pharmacotherapy is

advised (Sabin *et al*., 2012). Montelukast significantly reduces mild, moderate, and part of severe exacerbations in chronic mild to moderate asthma, but it has inferior efficacy to inhaled corticosteroids (ICs) (Zhang, 2014). Montelukast interferes with molecular signaling pathways produced by leukotrienes in a variety of cells and tissues throughout the human body leading to the tightening of airway muscles, production of aberrant pulmonary fluid (airway edema), and in some cases, pulmonary inflammation (McCarthy, 2023).

2.3 HYDROCORTISONE

Hydrocortisone is the name given to cortisol when it is administered as medicine (Becker, 2001). Hydrocortisone is a glucocorticoid that is used to treat a variety of inflammatory and allergy conditions, including many pulmonary illnesses like asthma, chronic obstructive pulmonary disease, influenza, and bronchitis (Barnes, 2010), as well as bronchopulmonary dysplasia in infants (Rademaker *et al*., 2008; Morris *et al*., 2019; Doyle *et al*., 2010). The adrenocortical steroid called hydrocortisone prevents or suppresses cell-mediated immune responses as well as tissue responses to inflammatory processes. It also inhibits the accumulation of inflammatory cells at inflammatory sites, phagocytosis, the release and synthesis of lysosomal enzymes, and the release of inflammatory mediators ('H', 2007). It is a corticosteroid with a potency of 1, a relative sodium retention potency of 1, and a half-life of 8-12 hours (Alangari *et al*., 2014). Hydrocortisone, the most structurally similar to endogenous cortisone, is the weakest agent in terms of glucocorticoid potency but possesses a higher mineralocorticoid effect, and due to its higher mineralocorticoid effects, special attention needs to be given to the risk for higher blood pressure (Sule *et al*., 2021). Side effects associated with the use of hydrocortisone just like other corticosteroids could include, hypothalamic-pituitary-adrenal axis suppression, physical appearance changes: moon facies, buffalo hump, central trunk, obesity, growth suppression, hirsutism, acne, insomnia, increased appetite, hyperglycemia, muscle wasting, reduced bone mineral density and osteoporosis, increased disability, immunosuppression, etc (Williams, 2018).

2.4 EFFECT OF MONTELUKAST ON ASTHMA

In patients with cough variant asthma, montelukast was beneficial in treating cough symptoms, reducing cough reflex sensitivity, and soothing eosinophilic airway inflammation; the antitussive effect and anti-eosinophilic airway inflammation were comparable (Yi *et al*., 2022). Montelukast is a recommended alternative for the treatment of asthma even after treatment with inhaled

corticosteroids (ICS) alone or 11 with ICS plus a long-acting β2-agonist (LABA) (Hoshino *et al*., 2019). In this study by Hoshino *et al*. (2019), eighty-seven patients with asthma were treated with budesonide and formoterol (640/18 μg); then, the patients were randomly allocated to three groups to receive oral montelukast (10 mg/day), inhaled tiotropium (5 μg/day), or no add-on to the maintenance therapy for 48 weeks of which fractional exhaled nitric oxide (FeNO) and pulmonary function were measured, and quantitative computed tomography was performed with the interpretation of results showing that montelukast may provide additive benefits concerning the pulmonary function and airway inflammation or remodeling in patients with asthma (Hoshino *et al*., 2019).

In 2013, Niimi published an initial finding showing that 4 weeks of treatment with a leukotriene receptor antagonist (LTRA) montelukast had an anti-inflammatory impact as demonstrated by a decrease in sputum eosinophils, in addition to attenuating cough VAS and capsaicin cough sensitivity. The results indicate that the antitussive effect of montelukast in asthma may be due to its anti-inflammatory properties rather than bronchodilation (Niimi, 2013). Spirometry, airway responsiveness, and impulse oscillation indices (respiratory resistance and reactance) were unaffected by the medication.

Kawai *et al* (2008) administered montelukast (10 mg/day) orally to 36 CVA patients (25 women and 11 men; median age, 37.5 years) after allowing the patients' 12 bronchial mucosae to undergo a biopsy with a fiberoptic bronchoscope with biopsy specimens being double stained with anti-CD63 antibody and anti-human tryptase antibody reported that cough symptoms improved in 22 patients (the effective group) but did not improve in 14 patients (the ineffective group). The bronchial mucosa biopsy specimens showed that the proportion of CD63-positive cells in tryptase-positive mast cells was significantly higher in the effective group than in the ineffective group; although the total numbers of mast cells were not different between the two groups.

Once more, a close look at the tissue of the airways demonstrates that montelukast reduces the amount of mast cells and eosinophils in asthma (Ramsay *et al*., 2009; Tenero *et al*., 2016). According to Seiko *et al*. (2008), eosinophils and mast cells are crucial components in asthmatic patients.

According to Calapai *et al*. (2014), side effects of using Montelukast included agitation, anxiety, sadness, sleep disturbance, hallucinations, suicidal ideation and behavior, tremors, dizziness, sleepiness, neuropathies, and seizures.

2.5 EFFECT OF HYDROCORTISONE ON ASTHMA

As a corticosteroid, hydrocortisone is a crucial medication for managing chronic asthma. It has anti-inflammatory effects on the airway and is an effective therapy for maintaining asthma control while also lowering morbidity and mortality from asthma (Raissy *et al*., 2013). The use and efficacy of hydrocortisone for treating asthma are subjects of scant research (Sule *et al*., 2021). In a 1974 trial, Pierson *et al*. employed injectable hydrocortisone for treating asthma in kids and discovered that it was more effective than a placebo in improving arterial hypoxemia. According to Pierson *et al*. (1974), hydrocortisone was well tolerated and had no major side effects. To treat 40 adult asthma patients every six hours for five days, Raimondi *et al*. compared high (80 mg/kg/day) and moderate (6 mg/kg/day) doses of IV hydrocortisone. Spirometric analysis of their findings with the two research groups revealed no appreciable differences (Raimondi *et al*., 1986).

In a study to determine the effect of intravenous hydrocortisone on nocturnal airflow limitation in childhood asthma, hydrocortisone was given over some time in a double-blind randomized crossover design to a selected number of subjects and FEV1, blood eosinophils and airway responsiveness to methacholine and adenosine 5′- monophosphate (AMP) were measured with results showing that substitution of lower endogenous values of cortisol with hydrocortisone, specifically improves lung function at the nadir time points of circadian cortisol levels. Furthermore, a short period of hydrocortisone infusion reduced the number of circulating eosinophils, which can be considered a marker of inflammation, whereas it does not change 14 the severity of airway hyperresponsiveness obstruction in asthma (Landstra *et al*., 2003).

Asthma in children frequently causes nocturnal airway obstruction, which is brought on by an increase in airway inflammation (Landstra *et al*., 2005). Hydrocortisone improved FEV in asthmatic children, but this wasn't because it reduced the activity of circulating peripheral blood mononuclear cells; rather, it was because it had an impact on the epithelial and/or fibroblasts in the local lung tissue, which reduced airway inflammation and vascular leakage (Landstra *et al*., 2005).

2.6 ANTIOXIDANT AND ANTIOXIDANT ENZYMES

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, plays a pivotal role in the pathogenesis of various diseases, including cardiovascular diseases, cancer, neurodegenerative disorders, and metabolic syndromes. Antioxidants and antioxidant enzymes are critical components of the body's defense system against oxidative damage (Adapted from multiple sources).

2.6.2 SOURCES OF OXIDATIVE STRESS

Oxidative stress has been implicated in the pathogenesis of numerous diseases, including cardiovascular diseases, cancer, neurodegenerative disorders, and metabolic syndromes (Sies, 1997). Oxidative stress can arise from various sources, including endogenous processes (e.g., mitochondrial respiration and inflammation), exogenous factors (e.g., environmental pollutants and radiation), and lifestyle choices (e.g., smoking and high-fat diets) (Cadenas and Davies, 2000). These sources contribute to the generation of ROS, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2).

2.6.3 ENDOGENOUS ANTIOXIDANT

The human body possesses a robust defense system of endogenous antioxidants, including enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play pivotal roles in detoxifying ROS within cells (Sies, 1997). For catalase (CAT) or glutathione peroxidase (GPx) to effectively neutralize hydrogen peroxide (H_2O_2): superoxide dismutase (SOD) must first convert superoxide anions ($O_2^{\bullet}$) into hydrogen peroxide (H_2O_2) (Sies, 1997).

Antioxidant enzymes take on the role of the main sentinels within the cellular fortress, directing a symphony of protective responses against the constant barrage of reactive oxygen species (ROS). The body's antioxidant defense system is built on these extraordinary enzymatic protectors, each of which plays a specific role in cellular health maintenance and ROS neutralization.

2.6.3.1 SUPEROXIDE DISMUTASE (SOD)

The first line of defense against ROS is sentineled by superoxide dismutase (SOD). Superoxide anions ($O_2^{\bullet}$), one of the most prevalent and harmful ROS, are catalyzed into less reactive hydrogen peroxide (H_2O_2) (McCord and Fridovich,

1969). Organisms lacking this enzyme frequently experience severe oxidative damage, underscoring the crucial necessity of SOD. The three separate types of SOD, which are found in various cellular compartments, including the cytoplasm (CuZn-SOD), mitochondria (Mn-SOD), and extracellular spaces (EC-SOD), ensure precise control over the levels of superoxide within particular cellular domains.

2.6.3.2 CATALASE (CAT)

A crucial part in controlling the dangerous hydrogen peroxide (H_2O_2) is played by catalase (CAT): which is largely found in peroxisomes. The quick conversion of H_2O_2 into water (H_2O) and molecule oxygen (O_2) is what gives it its catalytic power (Chance *et al*., 1979). Because too much H_2O_2 can produce extremely reactive hydroxyl radicals (•OH), which can cause damage to biological components, this enzymatic function is crucial. An essential defense mechanism against the effects of oxidative stress is the strong enzyme CAT, which can handle enormous amounts of H_2O_2.

2.6.3.3 GLUTATHIONE PEROXIDASE (GPX)

A family of enzymes called glutathione peroxidase (GPx) is at the forefront of preventing oxidative damage to cellular membranes and constituents. To reduce and neutralize lipid peroxides and hydrogen peroxide (H_2O_2): these enzymes use the cofactor selenium in a precise mechanism. Glutathione is used by GPx as a reducing agent to maintain the redox state of cells and the integrity of cell membranes (Arthur, 2000). Each distinct isoform of the numerous and varied GPx enzymes, which are dispersed across multiple cellular compartments, is specifically designed to meet the demands of its particular microenvironment.

2.6.4 ANTIOXIDANTS: NON-ENZYMATIC DEFENDERS

The term "antioxidant" refers to a broad class of substances, both endogenous and exogenous, that work to prevent oxidative stress. These non-enzymatic defenses combat ROS, stop oxidative cellular component destruction, and preserve cellular redox equilibrium in a variety of ways. Vitamins (such as vitamin C and vitamin E): minerals (such as selenium): and polyphenols (such as flavonoids and resveratrol) are important non-enzymatic antioxidants.

2.6.4.2 VITAMIN C (ASCORBIC ACID)

Vitamin C is a water-soluble antioxidant known for its ability to donate electrons and neutralize free radicals, effectively protecting against oxidative damage (Padayatty and Levine, 2001).

2.6.4.3 VITAMIN E (TOCOPHEROLS AND TOCOTRIENOLS)

Vitamin E, a lipid-soluble antioxidant, plays a crucial role in protecting cell membranes from oxidative stress by interrupting lipid peroxidation (Traber and Stevens, 2011).

2.6.4.4 SELENIUM

Selenium is an essential trace element that forms the active site of antioxidant enzymes like glutathione peroxidase (Rayman, 2012). It is integral to the body's antioxidant defense system.

2.6.4.5 POLYPHENOLS

Polyphenols have powerful antioxidant capabilities and have been researched for their possible health advantages (Scalbert *et al*., 2005). They are widely present in fruits, vegetables, and beverages like tea and red wine.

2.7 EFFECTS OF ASTHMA ON ANTIOXIDANT LEVELS

Asthma, a chronic respiratory condition characterized by airway inflammation and hyperresponsiveness, represents a significant global health burden. It affects millions of individuals, with a diverse spectrum of severity and control (Global Initiative for Asthma, 2021). While the pathophysiology of asthma is multifaceted, one emerging facet of interest is the intricate interplay between oxidative stress and antioxidant enzyme levels within the asthmatic airways. Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, has garnered attention for its role in asthma pathogenesis (Holguin *et al*., 2013).

A distinctive oxidative environment, including elevated ROS generation, compromised antioxidant defenses, and altered antioxidant enzyme levels, distinguishes the airways of asthmatics. Asthma is associated with increased oxidative stress, which is a result of these alterations (Cazzoletti *et al*., 2006). Importantly, the following crucial antioxidant enzymes are impacted:

Superoxide Dismutase (SOD): Reduced SOD activity has been observed in the airways of individuals with asthma (Comhair and Erzurum, 2010). SOD plays a central role in neutralizing superoxide anions ($O_2^{\bullet-}$), a potent ROS, by converting them into hydrogen peroxide (H_2O_2). Decreased SOD activity in asthma may contribute to the accumulation of $O_2^{\bullet-}$ and oxidative stress.

Catalase (CAT): Impaired CAT activity within asthmatic airways has also been reported (Comhair and Erzurum, 2010). CAT is responsible for decomposing hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Reduced CAT activity may result in the accumulation of H_2O_2, which can lead to the formation of highly reactive hydroxyl radicals ($^{\bullet}OH$).

Glutathione Peroxidase (GPx): The activity of GPx, an enzyme crucial for neutralizing hydrogen peroxide (H_2O_2) and lipid peroxides, may be diminished in asthmatic airways (Kharitonov *et al*., 1994). Reduced GPx activity may render cells more susceptible to oxidative damage, particularly to lipid peroxidation.

Changes in antioxidant enzyme levels in asthma have a substantial impact on the etiology of the condition. According to Sugiura *et al*. (2002): increasing oxidative stress inside the airways can cause inflammation, airway remodeling, and enhanced bronchial hyperresponsiveness. According to Zhang *et al*. (2014): lipid peroxidation brought on by oxidative stress can harm cell membranes and impair biological processes. Furthermore, exacerbations, increased asthma severity, and decreased lung function have all been connected to oxidative stress (Holguin *et al*., 2013).

Understanding the effect of asthma on antioxidant enzyme levels opens avenues for therapeutic interventions. Strategies aimed at restoring antioxidant balance within asthmatic airways, such as antioxidant supplementation or dietary modifications emphasizing antioxidant-rich foods, may hold promise for improving asthma control and mitigating oxidative stress-induced damage (García-Larsen *et al*., 2017; Riccioni *et al*., 2005).

2.8 EFFECT OF ANTIOXIDANT LEVELS ON ASTHMA

Asthma is associated with a dysregulated antioxidant defense system, marked by changes in key antioxidant enzymes:

Superoxide Dismutase (SOD): Superoxide dismutase, responsible for converting superoxide anions ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2): is altered in individuals with asthma. Variations in SOD activity have been linked to asthma severity and susceptibility (Comhair and Erzurum, 2010).

Catalase (CAT): Catalase, which decomposes hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2): may also exhibit changes in asthma. Reduced CAT activity within asthmatic airways can contribute to increased oxidative stress and inflammation (Comhair and Erzurum, 2010).

Glutathione Peroxidase (GPx): The activity of glutathione peroxidase, a critical enzyme for neutralizing hydrogen peroxide (H_2O_2) and lipid peroxides, may be compromised in asthma. Diminished GPx activity can lead to increased susceptibility to oxidative damage and exacerbation of airway inflammation (Kharitonov *et al*., 1994).

Altered antioxidant enzyme levels in asthma have far-reaching consequences for disease outcomes. Reduced antioxidant enzyme activity is associated with increased oxidative stress, which can exacerbate airway inflammation, enhance bronchial hyperresponsiveness, and contribute to asthma symptom severity (Cazzoletti *et al*., 2006).

Furthermore, the dysregulation of antioxidant enzyme levels can impact the effectiveness of conventional asthma therapies, potentially influencing the response to corticosteroids and other anti-inflammatory medications (Holguin *et al*., 2013). Understanding the relationship between antioxidant enzyme levels and asthma opens the door to potential therapeutic interventions. Strategies aimed at restoring optimal antioxidant enzyme activity, such as targeted enzyme supplementation or lifestyle modifications to enhance endogenous antioxidant defenses, hold promise for improving asthma control and reducing oxidative stress-induced damage (García-Larsen *et al*., 2017; Riccioni *et al*., 2005).

2.9 EFFECT OF MONTELUKAST AND HYDROCORTISONE ON ANTIOXIDANT LEVELS

Montelukast and hydrocortisone are two pharmacological agents used in the management of various respiratory conditions, including asthma. Understanding their impact on antioxidant enzyme levels and overall antioxidant status is crucial for comprehending their mechanisms of action and potential implications for patient care.

Montelukast, a leukotriene receptor antagonist primarily used to alleviate airway constriction and inflammation in asthma, has its main mechanism of action as anti-inflammatory. However, recent studies have suggested that Montelukast may possess antioxidant properties as well. Specifically, Montelukast has been associated with increased activity of antioxidant enzymes, including Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) (Ragab *et al*., 2018). This dual action is thought to contribute to its ability to mitigate oxidative stress in the airways of individuals with asthma.

While Montelukast's primary mechanism of action is anti-inflammatory, targeting the inflammatory pathways associated with asthma (Hakonarson and McFadden, 2002): there is limited evidence to suggest that it may also indirectly influence antioxidant enzyme levels by reducing airway inflammation (Ahmed *et al*., 2011). This reduction in airway inflammation can alleviate oxidative stress and, consequently, may lead to a modulation of antioxidant enzyme levels.

Hydrocortisone, a corticosteroid, is a potent anti-inflammatory medication commonly employed in asthma management. While its primary focus is on reducing inflammation, emerging research suggests that corticosteroids like Hydrocortisone may also influence antioxidant enzyme levels. Studies have indicated that corticosteroids are associated with increased expression and activity of antioxidant enzymes, including Superoxide Dismutase (SOD) and Catalase (CAT), within airway cells (Yao *et al*., 2017). This dual action is believed to contribute to the reduction of oxidative stress and inflammation in asthma.

As a corticosteroid, Hydrocortisone exerts robust anti-inflammatory effects by suppressing various aspects of the immune response, including inflammation in the airways (Adcock and Caramori, 2001). Corticosteroids, including Hydrocortisone, are recognized for their capacity to upregulate the expression of antioxidant enzymes, notably Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), as part of their anti-inflammatory mechanisms (Rhen and Cidlowski, 2005). This

increase in antioxidant enzyme levels due to Hydrocortisone treatment can significantly enhance the cellular defense against oxidative stress (Pizzino *et al*., 2017).

Montelukast and hydrocortisone have distinct effects on overall antioxidant levels in individuals with asthma. Beyond their influence on antioxidant enzyme levels, these medications may affect the availability of crucial antioxidants, such as vitamin C and glutathione, which play a vital role in neutralizing reactive oxygen species (ROS) and maintaining cellular redox balance. Several studies suggest that both montelukast and hydrocortisone may enhance the presence of antioxidants within the airways (Sugiura *et al*., 2002; Hosoki *et al*., 2013).

Montelukast's primary anti-inflammatory action indirectly reduces oxidative stress, potentially preserving or increasing overall antioxidant levels. In contrast, hydrocortisone, by elevating antioxidant enzyme levels, enhances the cellular capacity to neutralize reactive oxygen species (ROS): leading to the preservation of overall antioxidant levels.

In conclusion, while montelukast and hydrocortisone are primarily recognized as anti-inflammatory agents, they have secondary effects on antioxidant enzyme levels and overall antioxidant status. Montelukast's impact on antioxidant enzyme levels is linked to its anti-inflammatory properties, while hydrocortisone's influence includes the upregulation of antioxidant enzymes. These pharmacological actions may collectively contribute to the modulation of oxidative stress and have implications for the management of respiratory conditions, including asthma.

CHAPTER THREE

3.0 RESEARCH DESIGN AND METHODOLOGY

3.1 MATERIALS

Sprague Dawley rats

Cages

Chloroform

Nebulizer

Dissection materials

Electronic scale

Picric acid

Universal bottles

Syringes

Cotton wool

Aluminium hydroxide

Ovalbumin

Hydrocortisone

Montelukast

Saline solution.

3.2 EXPERIMENTAL ANIMALS

This study involved the use of female Sprague-Dawley rats. They all received proper animal care in line with the international guidelines for experimental animal handling. Ethical approval was obtained from the College of Medical Sciences ethics board. The Sprague-Dawley rats were housed in a clean, cool and sterile environment at 22^0C room temperature, they were kept in cages, where they had access to food and water *ad libitum* throughout the period of the experimental process.

3.3 STUDY DESIGN

Sprague-Dawley rats weighing between 180-250 g were divided into two (2) main groups; the Control group and Test group. The test group was further divided into three (3) subgroups in which one group consisted of asthmatic rats which were not treated with anti-asthmatic drugs and the other two which was treated with anti-asthmatic drugs. All the groups consisted of twenty (20) rats each (n=5). 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 grade II) and aluminum hydroxide to induce asthma after which they were treated with Montelukast and Hydrocortisone.

Experimental protocol/design

Experiment was carried out in phases

Phase 1

Rats were allowed to acclimatize into their new environment for two (2) weeks after which they were divided into four (4) groups of twenty (20) rats per group.

Test groups

GROUP 1: Control

GROUP 2: Asthmatic not treated

GROUP 3: Asthmatic and treated with Montelukast

GROUP 4: Asthmatic and treated with Hydrocortisone

All test groups were induced with asthma following the modified guideline outlined by (Bai *et al*., 2019; Wu *et al*., 2019). All experimental groups (2, 3 and 4) were sensitized 1 mg OVA and 200 mg aluminum hydroxide dissolved in 0.9 saline on day 0 and 7, challenged with OVA (1 % w/v, adsorbed in 0.9 saline) twice weekly from day 7 of treatment until the last day.

For the challenge 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) with aerosol delivery of 0.28 ml/min.

Normal control group were sensitized and challenged with intraperitoneal injection and aerosolized saline respectively. Asthma induction was verified first week after challenge with evidence of neutrophilia and eosinophilia in all test groups compared to control (Bai *et al*., 2019; Wu *et al*., 2019).

Phase 2

After confirmation of asthma in all test groups, treatment began with 5 mg/kg Hydrocortisone (i.p) (Ekpo and Pretorius, 2008) and 10 mg/kg Montelukast.

During this period of treatment, the blood pressure of all groups were monitored via non-invasive tail-cuff method.

Phase 3

At the end of drug administration, all animals were euthanized. Blood and tissue samples were collected for Antioxidant levels determination and histology

Phase 4

Antioxidant enzymes such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), Glutathione and total antioxidant capacity were assayed in blood plasma

Blood Pressure Measurement

i. Day 7 of treatment

ii. Day 28 (last day) of treatment

3.4 BLOOD SAMPLING AND SERUM ISOLATION

Blood was collected from retro-orbital plexus of rats under light diethyl ether anaesthesia in a non-heparinized tube. 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 were stored at frozen for the later quantitative determination of Antioxidant levels (Thakur *et al*., 2019).

3.5 DETERMINATION OF ANTIOXIDANT LEVELS

Antioxidant enzymes such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), Glutathione were measured by enzyme-linked immunosorbent assay (ELISA) in the serum using spectrophotometry-based kits. All the plates were analysed on an automated plate reader.

3.6 STATISTICAL ANALYSIS

All the data obtained from the experiments were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) for assessing differences amongst multiple groups, followed by Tukey's test using Graphpad Prism 8.1 software (Graphpad, San Diego, CA). $P < 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 RESULTS OF STATISTICAL ANALYSIS

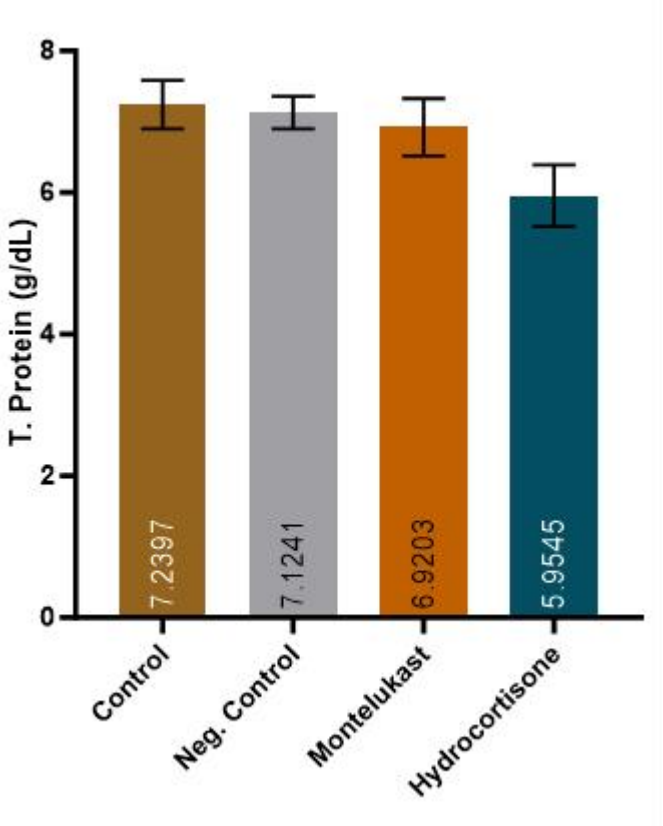

Fig. 4.1: chart show effect of montelukast and hydrocortisone on total protein in asthma induced Sprague Dawley rats

Result shows no statistically significant difference in total protein $p>0.05$

*$p<0.05$ compared to control

α$p<0.05$ compared to negative control

ϕ$p<0.05$ compared to montelukast

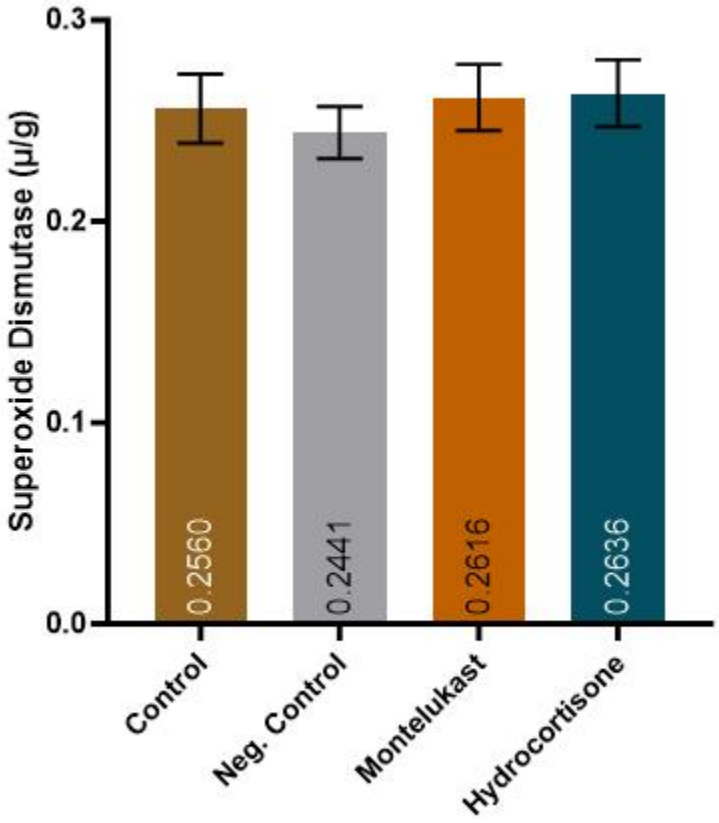

Fig. 4.2: chart show effect of montelukast and hydrocortisone on superoxide dismutase in asthma induced Sprague Dawley rats

Result shows no statistically significant difference in superoxide dismutase $p>0.05$
*$p<0.05$ compared to control
α$p<0.05$ compared to negative control
ϕ$p<0.05$ compared to montelukast

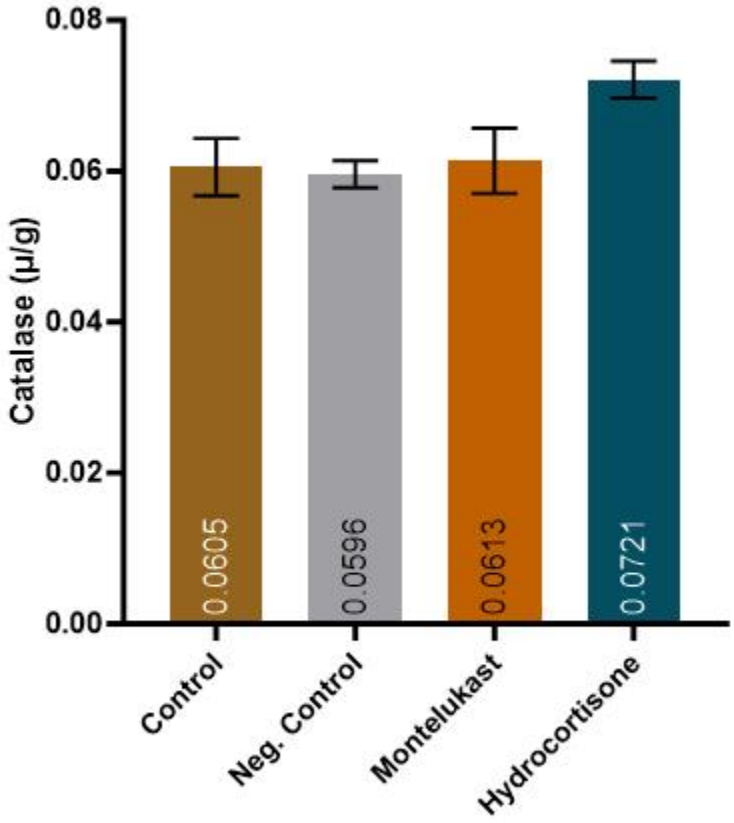

Fig. 4.3: chart show effect of montelukast and hydrocortisone on catalase in asthma induced Sprague Dawley rats

Result shows no statistically significant difference in catalase $p>0.05$
*$p<0.05$ compared to control
α$p<0.05$ compared to negative control
ϕ$p<0.05$ compared to montelukast

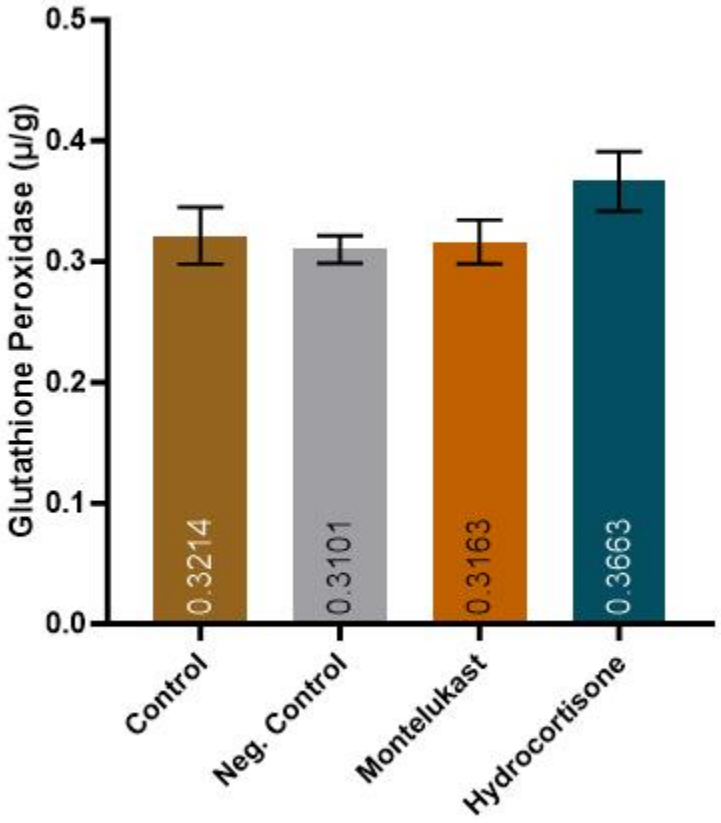

Fig. 4.4: chart show effect of montelukast and hydrocortisone on glutathione peroxidase in asthma induced Sprague Dawley rats

Result shows no statistically significant difference in glutathione peroxidase
$p>0.05$
$*p<0.05$ compared to control
$\alpha p<0.05$ compared to negative control
$\phi p<0.05$ compared to montelukast

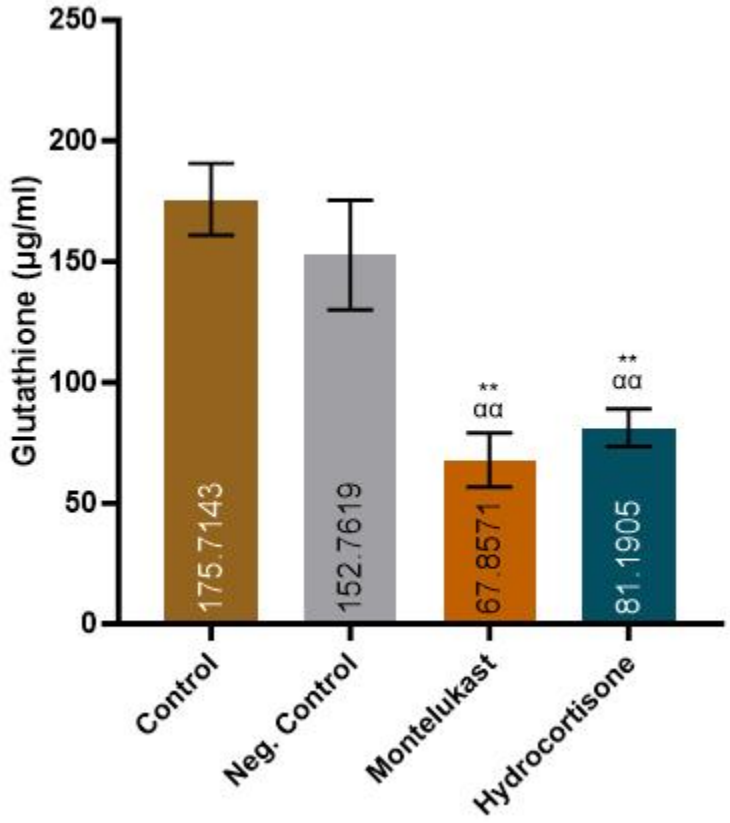

Fig. 4.5: chart show effect of montelukast and hydrocortisone on glutathione in asthma induced Sprague Dawley rats

Result shows a statistically significant difference in glutathione $p<0.05$

*$p<0.05$ compared to control

α$p<0.05$ compared to negative control

ϕ$p<0.05$ compared to montelukast

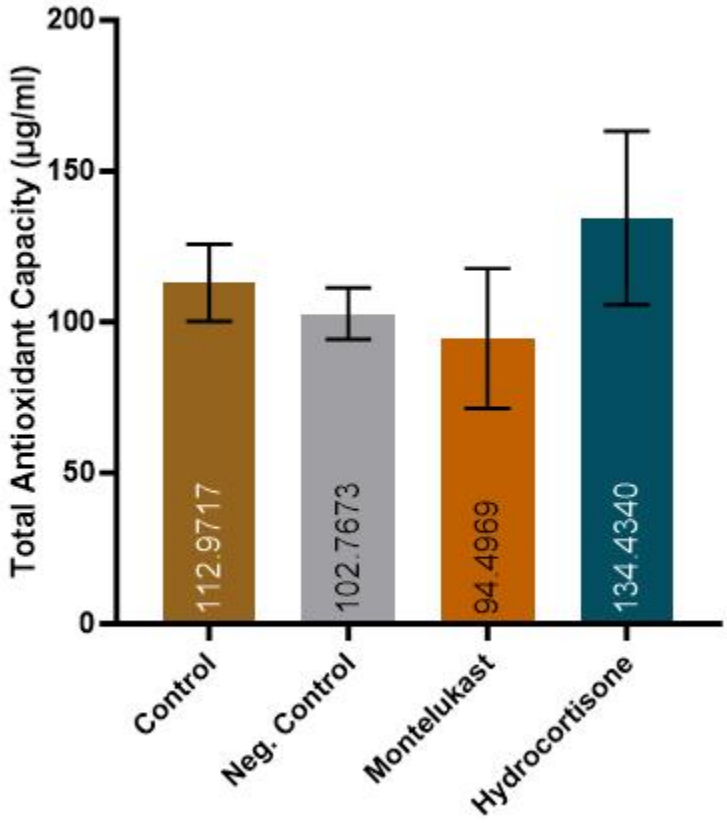

Fig. 6: chart show effect of montelukast and hydrocortisone on total antioxidant capacity in asthma induced Sprague Dawley rats

Result shows no statistically significant difference in total antioxidant capacity $p>0.05$

*$p<0.05$ compared to control

α$p<0.05$ compared to negative control

ϕ$p<0.05$ compared to montelukast

CHAPTER FIVE 5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Fig 4.2. Result shows no statistically significant difference in superoxide dismutase $p>0.05$
*$p<0.05$ compared to control
α$p<0.05$ compared to negative control
ϕ$p<0.05$ compared to montelukast

In the research project examining the effect of Montelukast and Hydrocortisone on antioxidant enzyme levels in asthma-induced Sprague Dawley rats, the statistical analysis revealed that there was no statistically significant difference in superoxide dismutase (SOD) levels ($p>0.05$) compared to the control group. Additionally, there were comparisons made against the negative control (α$p<0.05$) and Montelukast (ϕ$p<0.05$). The lack of statistical significance ($p>0.05$) in SOD levels compared to the control group suggests that neither Montelukast nor Hydrocortisone had a significant effect on the SOD enzyme levels in asthma-induced Sprague Dawley rats in this study. SOD is a critical antioxidant enzyme responsible for scavenging superoxide radicals and plays a role in protecting cells from oxidative stress. Several factors could contribute to these results and lack of significance. Firstly, the dosages of Montelukast and Hydrocortisone administered in the study might not have been sufficient to induce a significant change in SOD levels. Dosage and duration of treatment are crucial factors in determining the efficacy of a drug.

Secondly, the variability in individual rat responses within the treatment groups could have influenced the overall statistical analysis. Biological variability, inherent differences in individual rat responses, and the complexity of asthma as a disease could contribute to this lack of significance. It's also important to consider the model of asthma induction and how closely it mimics the human condition. The efficacy of these drugs may vary based on the nature and severity of the asthma induction in the rats. While Fig. 4.5 chart show effect of montelukast and hydrocortisone on glutathione in asthma induced Sprague Dawley rats, the study suggests that examining the effect of Montelukast and Hydrocortisone on antioxidant enzyme levels in asthma-induced Sprague Dawley rats, the statistical analysis for glutathione levels yielded different results compared to superoxide dismutase (SOD). The results showed a statistically significant difference in

glutathione levels ($p<0.05$) compared to the control group, as well as significant differences compared to the negative control ($\alpha p<0.05$) and Montelukast ($\phi p<0.05$).

This finding indicates that both Montelukast and Hydrocortisone had a significant impact on the levels of glutathione in the rats with asthma. Glutathione is a crucial endogenous antioxidant that plays a key role in protecting cells from oxidative damage and maintaining redox homeostasis. The significant increase in glutathione levels compared to the control group suggests that both Montelukast and Hydrocortisone may have a positive effect on the antioxidant defense system in asthma-induced rats. This increase in glutathione could be seen as a beneficial response since higher levels of glutathione are associated with better oxidative stress protection and reduced cellular damage. The significant differences compared to the negative control indicate that the observed effects were not merely due to the presence of asthma alone but were indeed influenced by the administration of Montelukast and Hydrocortisone. This suggests that these drugs may have a protective or modulatory role in enhancing the antioxidant capacity of the rats in this study. The significant difference compared to Montelukast ($\phi p<0.05$) is particularly interesting, as it implies that Hydrocortisone might be more effective at increasing glutathione levels in this specific experimental context. This could lead to further investigations comparing the mechanisms of action and therapeutic potential of Montelukast and Hydrocortisone in asthma management.

5.2 CONCLUSION

In conclusion, the significant increase in glutathione levels in response to Montelukast and Hydrocortisone treatment suggests a potential positive impact on the antioxidant defense system in asthma-induced Sprague Dawley rats. These findings support the idea that these drugs may help mitigate oxidative stress, which is often associated with asthma. while Montelukast and Hydrocortisone did not significantly affect superoxide dismutase levels, they showed promising potential in increasing glutathione levels, emphasizing their potential therapeutic value in combating oxidative stress in the context of asthma. Further investigations are vital to fully comprehend their mechanisms and optimize their use in asthma management.

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