EFFECT OF MONTELUKAST AND HYDROCORTISONE ON THE LIVER FUNCTION, LIVER ANTIOXIDANT LEVELS AND HISTOLOGY IN ASTHMA INDUCED SPRAGUE-DAWLEY RATS

BY

EGBORO SARAH TREASURE

BMS1802591

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN, BENIN CITY.

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A PROJECT WORK WRITTEN AND SUBMITTED IN PARTIAL FULFILLMENT ILMENT OF THE REQUIREMENT FOR THE AWARD OF A 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.

OCTOBER, 2023.

CERTIFICATION

This is to certify that this project work on "**EFFECTS OF MONTELUKAST AND HYDROCORTISONE ON THE LIVER FUNCTION, LIVER ANTIOXIDANT LEVELS AND HISTOLOGY IN ASTHMA INDUCED SPRAGUEDAWLEY RATS"** was carried out by **EGBORO SARAH TREASURE,** with matriculation number: **BMS1802591**; in partial fulfillment for the Award of Bachelor of Science (B.Sc.) Degree in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

_____ _____

EGBORO SARAH TREASURE **Date**

Student

_____ _____

DR. (MRS). J. O. NZOPUTAM **Date**

Project Supervisor

_____ _____

PROFESSOR O. K. UCHE **Date**

Head of Department

_____ _____

PROFESSOR V. U. IGBOKWE **Date**

External Examiner

DEDICATION

This project work is dedicated to God Almighty, my strength and sustainer. I also dedicate it to my family for their encouragement and support during the process of my undergraduate study.

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I wish to acknowledge Dr. Mrs. J.O NZOPUTAM, my supervisor for her patience and diligence in supervising me through this research and my course mates who supported one way or the other.

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ABBREVATIONS

AP-1: Activator protein-1

CAT: Catalase

COPD: Chronic obstructive pulmonary disease Cu/Zn-SOD: Copper/Zinc-Superoxide dismutase
CysLT: Cysteinyl leukotriene

DNA: Deoxyribonucleic acid

FDA: Food and Drug administration

FeNO: Exhaled nitric oxide

Fe-SOD: Iron-Superoxide dismutase

FEV1: Forced expiratory volume in one minute FVC: Forced vital capacity

GPX: Glutathione peroxidase

GR: Glucocorticoid receptor

GSH: Glutathione

ICS: Inhaled corticosteroids

IgE: Immunoglobulin E

IN: Intravenously

IP: Intraperitoneally

LABA: Long-acting beta agonists

LTRA: Leukotriene-receptor antagonist

LTs: Leukotrienes

MDA: Malondialdehyde

Mn-SOD: Manganese-Superoxide dismutase NF-KB: Nuclear factor-KappaB

OVA: Ovalbumin

PEF: Peak expiratory flow

PFT: Pulmonary function test

ROS: Reactive oxygen species

RSVs: Respiratory syncytial virus

SABAs: Short-acting beta-agonists

SC: Subcutaneously

SD: Sprague-Dawley

SOD: Superoxide dismutase

Th2: T-helper type 2

ABSTRACT

Asthma is a long-term inflammatory condition of the airways that causes a variety of symptoms due to the irritation and hyperresponsiveness of the airways. The aim of this study is to determine the effects of Montelukast and hydrocortisone on liver function, liver antioxidant levels, and histology, in order to determine which medication may have a more favorable effect on the liver. Methodology: Sprague-Dawley rats weighing between 180-250g were divided into two main groups; the Control group and Test group. The test group was further divided into three subgroups treated with anti-asthmatic drugs. The control group received normal rat chow and water throughout the experiment 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 hydrocortisone and montelukast. All experimental groups (2, 3, and 4) were sensitized 1mg OVA and 200mg aluminum hydroxide dissolved in 0.9 saline on day 0 and 7. After confirmation of asthma in all test groups, treatment began with 5mg/kg hydrocortisone (i.p) and 10mg/kg Montelukast. They were challenged with OVA (1% w/v, dissolved 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 70cm in diameter and 40cm in length connected to a Medel family nebulizer with aerosol delivery of 0.28ml/min. At the end of drug administration, all animals were euthanized, blood and tissue samples collected for biomarker assay and histology. All data obtained from the experiments are expressed as mean ± Standard Error of Mean (SEM) and statistical analysis performed by one way analysis of variance (ANOVA) for assessing differences amongst multiple groups, followed by Tukey's test using Graphpad Prism 10.0.3 software. The result of the experiment showed that there were statistically significant differences in liver enzymes and antioxidant levels among Montelukast and hydrocortisone treatment on asthma induced spraguedawley rats.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Asthma is a chronic respiratory disorder marked by inflammation and constriction of the airways (Nakagome *et al.*, 2011). It causes symptoms like wheezing, coughing, tightness in the chest, and shortness of breath. It has been proposed that environmental factors, such as allergens, pollutants, and respiratory infections, interact intricately with genetic predisposition to develop asthma (Togias *et al*., 2010). When the immune system reacts to these triggers, airways become inflamed, which results in the typical asthma symptoms. When T-helper 2 (Th2) cells are activated, they create cytokines such interleukins (IL)-4, IL-5, and IL-13, which are indicative of an immunological response in asthma. These cytokines cause the airways to produce more mucus and eosinophilic inflammation, which makes the airways more sensitive (Kabesch *et al.*, 2006). Cysteinyl leukotrienes are one of the proinflammatory mediators that have an essential role in the pathophysiology of asthma and are responsible for mediating bronchoconstriction and allergic airway inflammation (Holgate *et al.*, 2003). Inflammatory cells in the airways emit cysteine leukotrienes, which cause smooth muscle proliferation, mucus secretion, bronchoconstriction, and increased vascular permeability (Dahlen *et al*., 1980). By acting on Cysteinyl leukotriene-1 and 2 receptors, the leukotriene (LT) antagonist montelukast plays a therapeutic function in the treatment of asthma (Mechiche *et al.*, 2003). It is primarily suggested for the prophylaxis and treatment of asthma, particularly the prevention of exercise-induced bronchoconstriction, with an oral dosage for adults of 10 mg once daily (Leff *et al.,* 1998). Montelukast, has been shown to improve symptoms and lung function within 15 minutes of administration in the case of chronic asthma, with an effect lasting for at least 24 hours (Price *et al*., 2006). Glucocorticoids are used as anti-inflammatory and immunomodulatory agents in a wide variety of diseases. Glucocorticoids (GCs) are broadly used in the treatment of inflammation and in suppressing hyperactivity of the immune system expressed in allergies, asthma, autoimmune diseases and sepsis (Franco *et al.*, 2019). Their physiological effects may be accomplished largely by modulating the expression of many cytokine genes, such as IL-1, IL-2, TNF-α, interferon-β, interferon-γ, and monocyte chemotactic and activating factor (Peppel *et al*., 1991). Hydrocortisone is one of the most frequently used GCs and is considered as one of the safest. The majority of anti-asthma drugs have a low incidence of hepatotoxicity, but it is viable, especially with anti-leukotrienes. The

liver serves as the primary organ for the metabolism of both xenobiotics and endogenous chemicals (Muriel, 2007). Our bodies contain antioxidants, which are also found in many foods. Antioxidants are substances that, in small amounts, may inhibit or delay the oxidation of a substrate that is susceptible to oxidation (Halliwell, 1990). An imbalance between the generation of free radicals (FR) and the antioxidant defenses is referred to as oxidative stress (Betteridge, 2000).

1.2 JUSTIFICATION OF STUDY

There is a critical need in the modern world for asthma to be treated more successfully, to contribute to the understanding of the potential hepatotoxicity and protective effects of Montelukast and hydrocortisone in the context of asthma treatment, providing valuable insights for the development of safer and more effective therapeutic strategies.

1.3 AIM

To compare the effects of Montelukast and hydrocortisone on liver function, antioxidant levels, and histology, in order to determine which medication may have a more favorable effect on the liver in asthma-induced Sprague-Dawley rats.

1.4 STATEMENT OF PROBLEM

Although hydrocortisone and monteulukast are commonly used to treat asthma, little is known about how they specifically affect the liver and antioxidant system. This lack of knowledge makes it more difficult to fully comprehend how the drugs work and how they might affect antioxidant defence and oxidative stress in the setting of asthma. Therefore, when these rats were treated with hydrocortisone and montelukast, a comparative examination of the antioxidant profiles in their blood and liver was necessary.

1.5 RESEARCH QUESTIONS

1. Does Montelukast affect the liver function and histology in asthma induced Spraguedawley rats?
2. Does hydrocortisone affect the liver function and histology in asthma induced Spraguedawley rats?

3. Does Montelukast affect the levels of liver antioxidant in asthma induced Spraguedawley rats?
4. Does hydrocortisone affect the levels of liver antioxidant in asthma induced Spraguedawley rats?

1.6 SPECIFIC OBJECTIVES

1. To investigate the impact of Montelukast and hydrocortisone on liver function in asthma-induced Sprague-Dawley rats by measuring liver enzyme levels, such as alanine transaminase (ALT) and aspartate transaminase (AST).
2. To assess the effect of Montelukast and hydrocortisone on liver antioxidant levels by measuring the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), as well as levels of oxidative stress markers, like malondialdehyde (MDA).
3. To evaluate the histological changes in the liver tissue using staining techniques to observe for potential hepatotoxicity or protective effects.

CHAPTER TWO

LITERATURE REVIEW

2.1 ASTHMA

Asthma is a chronic inflammatory disorder of the airways that results in a number of symptoms, due to the irritation and hyperresponsiveness of the airways. In western nations, asthma is the most prevalent chronic pediatric disease, and over the past few decades, its prevalence has been steadily increasing (Braman, 2006). Asthma is associated with a chronic inflammation of the airway mucosa, involving a complex interaction between T-lymphocytes, neutrophils, eosinophils, epithelial cells and mast cells (Frieri, 2005). These inflammatory cells release cytokines and other mediators like leukotrienes, platelet-activating factor, and histamine, and these intricate interactions between cells and mediators result in structural and physiological changes as well as exposed parasympatic nerve endings (Holgate, 2008). Sustained bronchoconstriction, mucus secretion, and oedema in the airways are all caused by CysLTR1 (Peters-Golden and Henderson, 2007). Initial research revealed that leukotriene D4 (LTD4) activation of CysLTR1 results in G protein activation and the production of various second messengers, including calcium ions, inositol phosphates, and diacylglycerol (Drost *et al*., 2012). PKC is then activated as a result of these second messengers. Mutations in the PKC phosphorylation site inhibited LTD4's ability to mobilize calcium, internalize it, or produce phosphatidylinositol (Ng *et al*., 2012). CysLTR1 expression has been shown to be closely associated with smooth muscle contraction, vascular permeability, and plasma leakage (Di Gennaro and Haeggstrom, 2012). Additionally, the asthmatic effects of CysLTR1 are blocked by selective CysLTR1 antagonists that have been approved for use in treating asthma (Gauvreau *et al*., 2016). The physiological consequence of these processes is airway hyperreactivity, which leaves the asthmatic patient's airways primed for a variety of triggers that could cause further airway blockage and, clinically, asthma exacerbations (Werner, 2001). The clinical manifestation of the illness and its accompanying phenotypes, including bronchial hyperresponsiveness, atopy, and increased IgE, are known to be influenced by a variety of genetic and environmental variables (Anderson, 2010). Viral airway infections, particularly rhinoviruses, are the main trigger of asthma in children and young adults. In addition, asthma attacks (acute in chronicum) can be triggered by allergens, tobacco smoke, environmental irritants, exercise, stress, and gastric reflux, either alone or in combination (Sykes and Johnston,

2008). Numerous studies have demonstrated a direct link between smoking and a greater risk of developing asthma (Flodin *et al.*, 1995).

In an asthma attack, mast cell degranulation and the release of histamine, leukotrienes, and other mediators exacerbate the chronic inflammation, leading to mucosal vasodilatation and oedema, increased mucous secretion, and smooth muscle contraction, especially in the medium- and small-sized airways (Werner, 2001). As a result, the airway lumen gets smaller, which increases airflow resistance, especially near the end of expiration when lung volume is low. Premature airway closure will also result from the significant airflow restriction. In order to make up for this, the patient increases functional residual capacity (FRC), which causes pulmonary hyperinflation and air trapping (Werner, 2001). Airway obstruction, hyperinflation and air trapping may lead to ventilation/perfusion mismatch and hypoxemia. Varying airflow obstruction leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough (Horwitz and Busse 1995). Cardiac function may be impeded by increased airflow resistance, pulmonary hyperinflation, greater work of breathing, and changes in the acid/base balance. The negative intrapleural pressure will increase during a severe asthma episode, raising the left ventricular afterload and pulmonary oedema risk (Stalcup and Melling, 1977). The patient's risk of developing pulmonary oedema will increase due to fluid overload caused by excessive hydration during treatment or fluid retention caused by inappropriate antidiuretic hormone secretion (Baker et al. 1976). An asthma exacerbation is not clearly defined. However, it is often described in clinical trials as a necessity for systemic corticosteroids or for hospitalization (Sears, 2008). Exacerbations of asthma that are mostly ascribed to inflammatory processes may take some time to develop and resolve, and as a result, symptoms usually worsen and get better rather slowly. In these circumstances, airway narrowing may mainly be caused by inflammatory changes, and there may be an accompanying down-regulation of β-receptors (Kaza *et al.*, 2007). In contrast, allergy-induced episodes may manifest very quickly, with bronchoconstriction predominating in their pathology, thereby, also reacting rapidly to bronchodilator medications (Kaza *et al.*, 2007). Asthma mortality in children is uncommon and on the decline, despite the fact that severe asthma exacerbations are very prevalent (Wijesinghe *et al.,* 2009). In contrast, the economic costs associated with asthma have significantly increased. However, rather than hospitalization, the primary financial impact of childhood asthma is related to indirect costs, ongoing monitoring, and medication (Braman, 2006).

2.1.1 Clinical Diagnosis

The diagnosis of asthmatic pathology has long relied on bronchial provocation tests, bronchodilator responses, and assessments of airflow limitation (Wang *et al*., 2021). Other markers include forced vital capacity, forced expiratory volume in one second, fractional exhaled nitric oxide (FeNO), peripheral blood eosinophils, and others. However, because this disease has so many different pathomechanisms, it is now very challenging to develop more customized and precise diagnostic methods (Guilleminault et al., 2017). The establishment of societies and working groups producing updated guidelines in recent years has improved asthma strategy and clinical care. Skin prick tests (SPT), the detection of total and specific IgE (sIgE), and an allergy-specific nasal or bronchial challenge test to ascertain whether the patient has an atopic condition are all included in this (Bernstein *et al.*, 2008). Even if there is no gold standard test and spirometry may not be sufficient to make the diagnosis, it is nevertheless important. The following spirometry parameters are FEV1 and FVC, and they depend on factors like age, height, weight, sex, and ethnicity (van den Berge *et al.*, 2011). Variations in peak expiratory flow (PEF) and FEV1 within a single patient during flare-ups and steady periods are also frequent. Because variability decreases as lung function improves, spirometry with bronchodilator testing and PEF assessment should be performed before beginning medication (Reddel, 2006). Spirometry can be difficult to perform because it necessitates a lot of patient effort and the capacity to adhere to medical professionals' directions (Schneider *et al.*, 2009). Exhaling forcefully through a mouthpiece is not a natural breathing manoeuvre, which can make it challenging to obtain accurate results (Motamedi-Fakhr *et al*., 2017). Other tests that do not rely on such effort-dependent exercises should be taken into consideration in order to get around the limits of maximal forced breathing manoeuvres. These include impulse oscillometry (IOS) and forced oscillation techniques (FOT) (Dubois *et al.*, 1956). The FOT was first developed in the 1950s and uses sinusoidal pressure oscillations, the IOS uses the same concept but uses square wave pressure oscillation (‘pulses’) of multiple frequencies of oscillation at the same time (Komarow *et al*., 2011). Both FOT and IOS are easy to perform, but result in complex measurements, describing lung impedance from which lung reactance and resistance are derived (Oostveen *et al.*, 2003). Airflow blockage, reversibility, unpredictability, and bronchial hyperresponsiveness are the main alterations in asthma. Bronchial challenge with direct (methacholine) may be utilized to confirm the diagnosis when spirometry results are abnormal (FEV1/FVC > 0.7, FEV1 rise 12%), as well as Nitric oxide in

exhaled air testing (FeNO > 40ppb), or examination of induced sputum (eosinophil counts, eosinophil cationic protein levels, and Creola body counts) (Saito *et al*., 2004). Imaging studies like thorax radiography can rule out other respiratory illnesses, including those with symptoms of air embolism, atelectasis, or bronchial thickness [GIF, 2022].

2.1.2 Treatment of Asthma

There are several drug types for the management of asthma, they include Inhaled Corticosteroids (like Fliticasone, Dexamethasone, Budesonide, and mometasone), Long-acting beta agonist (LABAs which includes drugs like Salmeterol and formoterol), Leukotriene modifiers (like Montelukast and Zafirlulast), Short-acting beta agonists (SABAs such as albuterol and levalbuterol). There are two main classes of asthma medications: quick-relief drugs (known as rescue medications) and long-term control medications. Quick-relief medications like short-acting beta agonists provide immediate relief during an asthma attack, while long-term control medications like inhaled corticosteroids work to reduce inflammation and prevent future attacks (Sobieraj *et al*., 2019). For patients with chronic asthma, inhaled corticosteroids are one of the first line of treatment (NIH, 1998). ICS improve disease control and reduce asthma exacerbations (Juniper *et al.*, 1990). These medications have been shown to be effective in treating asthma, enhancing lung function, decreasing asthma attacks, and having established anti-inflammatory effects on the lungs (Keatings *et al*., 1997). Although ICS can also have undesirable side effects, including osteoporosis, osteomyelitis, hoarseness, oral infections, and suppression of the hypothalamic-pituitary-adrenal axis (Philip, 2014). Oral corticosteroids (OC) can also be used to treat asthma in addition to inhaled corticosteroids (ICs), they are more efficient than ICs at reducing inflammation, but they also have more side effects including diabetes, sleep apnea, cataracts, hyperglycemia, weight gain, loss of bone density, and others (Sullivan and Baker, 2018). ICS and LABA (long lasting β-agonists) have consistently been shown to prevent exacerbations (Papi *et al*., 2007). The combination of budesonide and formoterol significantly lowers asthma exacerbations compared to ICS alone in patients with poorly controlled asthma and a history of prior asthma exacerbations (Pauwels *et al*., 1997). Despite this, a lot of patients on inhaled corticosteroids still complain of asthma symptoms, presumably because the drugs only partially prevent the synthesis and release of cysteinyl leukotrienes in the lungs (Dworski et al., 1994). Antileukotriene drugs enhance lung function, reduce β agonist use in patients with persistent asthma, and minimize triggered asthmatic responses (Drazen *et al.*, 1999). CysLT1 receptor antagonist montelukast has been proven to decrease eosinophilic inflammation in the airways of individuals with persistent

asthma (Pizzichini *et al.,* 1999). Tiotropium is an anticholinergic that has been given FDA approval for long-term maintenance treatment of patients with persistent asthma who are unable to control their condition with ICS plus one or more controllers and who are 6 years of age and older (Kerstjens *et al.*, 2012). Tiotropium lowers the frequency of asthma attacks. Asthma can also be treated using Immunomodulators such as Anti-IgE (omalizumab). Omalizumab is a humanized monoclonal antibody directed against IgE and reduces the risk for asthma exacerbations in allergic asthmatic patients (Busse *et al*., 2001). The goal of asthma treatment is to reduce symptoms associated with the disease in order to lower future risks caused by the symptoms severity, and variability.

2.2 MONTELUKAST

Montelukast, a CysLT1R-specific antagonist, is an effective treatment for asthma (Barnes *et al.*, 2005). Antileukotriene drugs enhance lung function, reduce β agonist use in patients with persistent asthma, and significantly reduce triggered asthmatic responses (Drazen *et al.*, 1999). The CysLTR1 antagonist that is most usually given to asthmatic patients is montelukast (Hoxha et al., 2017). Another benefit is that it lessens kidney damage and has antioxidant properties (Dong *et al.*, 2018). Recent research has demonstrated the effectiveness of montelukast in treating autoimmune illnesses and its strong ability to prevent chemotaxis (Lee *et al.*, 2015). Montelukast, a cysteinyl leukotriene type 1 (CysLT1) receptor antagonist, has been found to reduce airway eosinophilic inflammation in patients with chronic asthma (Pizzichini *et al.*, 1999). It is an effective leukotriene D4 selective antagonist at the cysteinyl leukotriene (cysLT) type 1 receptor, which is present in human respiratory epithelial cells and is made by cells such as macrophages and eosinophils (Back *et al.*, 2013). As an important anti-inflammatory modulator that is frequently used to treat allergic rhinitis and asthma, montelukast is also well known for having anti-oxidant activities in numerous tissues and organs (Dong *et al.*, 2018). Monteleulast is indicated for asthma prophylaxis and chronic treatment in adults and pediatric patients 12 months of age and older (Sánchez and Buitrago, 2018). The amount of eosinophils in bronchoalveolar lavage fluid, peripheral blood, bronchial mucosa, and sputum in asthmatic subjects as well as in experimental allergic asthma is decreased by montelukast and other CysLT1R-specific antagonists like pranlukast (Minoguchi *et al.*, 2002). It is recommended to prevent exercise-induced bronchoconstriction (EIB) in patients (de Benedictis *et al.*, 2008). Patients taking montelukast have reported experiencing neuropsychiatric problems. Adults, adolescents, and younger individuals have all experienced these experiences, including, among others: anxiety, sadness, hostility, agitation, poor

concentration and memory, aggressiveness, somnambulism, abnormal dreams, sleeping disorders, seizures, paresthesia, hypoesthesia, and suicidal thoughts and actions. While undergoing treatment, young patients may require careful supervision by a mental health nurse (Schwimmbeck *et al*., 2021). Clinically apparent liver damage caused by montelukast is rare, although in clinical trials, mild elevations in serum aminotransferase levels were found in 1% to 2% of patients taking montelukast chronically (Sass et al., 2003). Nevertheless, the rare instances of hepatotoxicity attributed to montelukast may be caused by formation of toxic or immunogenic intermediates during its metabolism (Lewis, 2013).

2.3 HYDROCORTISONE

The diverse cellular mechanisms that underlie the actions of glucocorticoids make them good candidates for testing their effectiveness in a range of physiological processes, including apoptosis and cell proliferation, as well as their function in pathological conditions like inflammation (Payne and Adcock, 2001). When Cortisol is administered as a medication, it is referred to as hydrocortisone (Becker, 2001). Hydrocortisone is an adrenocortical steroid that inhibits accumulation of inflammatory cells at inflammation sites, phagocytosis, lysosomal enzyme release and synthesis, and release of mediators of inflammation while preventing or suppressing cell-mediated immune reactions and decreasing or preventing tissue response to inflammatory processes (H, 2007). Hydrocortisone is an important drug of chronic asthma management and it is an effective therapy in maintaining asthma control through anti-inflammatory effects on the airway while also reducing morbidity and mortality from asthma (Raissy *et al.,* 2013). It has immuno-modulatory effects by influencing B cells and T cells via various molecular pathways. They influence B cells by downregulating Toll-like receptor 7 signaling and upregulating the anti-inflammatory cytokine interleukin-10 (IL-10). By controlling the expression of their cytokines and the signaling of their T cell receptors, GCs influence T cells (Franco *et al*., 2019). Pro-inflammatory cytokines like IL-1 and IL-6, prostaglandin E2 and histamine, as well as their synthesis, can all be inhibited by GCs. Common side effects of hydrocortisone could include, 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 bruisability, immunosuppression etc (Williams, 2018).

2.4 THE LIVER

The liver is the largest organ in the body and is responsible for performing several crucial biological functions such as metabolism, detoxification, and protein production (Crispe, 2009). Interestingly, the liver is also highly immune active due to its function as a systemic barrier to gut-draining pathogens and toxins, in addition to its role in maintaining a local immunoregulatory environment that promotes tolerance to frequently encountered antigens (Robinson *et al*., 2016). The liver performs a vast number of functions, including protein synthesis, glucose and lipid metabolism (gluconeogenesis, glycogen storage, lipogenesis, fatty acid b-oxidation, and lipoprotein metabolism), detoxification, and bile production (Crispe, 2009). In order to keep the organism's metabolic balance, the liver serves as the primary organ for the metabolism of both xenobiotics and endogenous chemicals. Consequently, a variety of assaults that target the liver can cause dysregulation of hepatic homeostasis and hepatic diseases (Muriel, 2007). The liver is composed of the following cell types: hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, pit cells, and hepatic stellate cells (HSC) (Muriel and Arauz, 2012). Liver diseases are a worldwide medical problem because the liver is the principal detoxifying organ and maintains metabolic homeostasis. Chronic liver diseases leading to organ failure account for approximately 2 million global deaths annually (Asrani *et al.*, 2019). The most significant liver disease, cirrhosis, is marked by the buildup of extracellular matrix proteins, such as collagens I, III, and IV, as well as distortion of the hepatic architecture (Bataller and Brenner, 2005). Cirrhosis is the final stage of progressive fibrosis and is brought on by liver damage from a number of etiological factors (Reyes-Gordillo *et al.*, 2008).

2.5 LIVER ANTIOXIDANTS

Antioxidants are substances that, in small amounts, can stop or delay the oxidation of a substrate that is susceptible to oxidation (Halliwell, 1990). The liver metabolizes various compounds that produce free radicals (FR). Antioxidants, however, neutralise FR and keep the liver's oxidative/antioxidative balance in check. When the liver oxidative/antioxidative balance is disrupted, the state is termed oxidative stress (Betteridge, 2000). Oxidative stress triggers the development of fibrosis, which leads to cirrhosis (Muriel, 1997). Therefore, restoring antioxidants is essential to maintain homeostasis. FR are defined as atoms or molecules with one or more unpaired electrons and are usually unstable and highly reactive because they can react with nearby molecules and abstract electrons (Ternay and Sorokin, 1997). Oxidative

stress is an important contributor to the pathophysiology of a variety of pathological conditions including cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases (Aruoma, 1998). Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) are the first line of defense against reactive oxygen species (ROS) and are generally referred to as primary antioxidant (Birben *et al.*, 2012).

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

3.1 EXPERIMENTAL ANIMALS

This study involved the use of 20 female Sprague-Dawley rats. They all received proper animal care in line with international guidelines for experimental animal handling. Ethical approval obtained from the College of Medical Sciences ethics board. The Sprague-Dawley rats were housed in a clean, cool and sterile environment at 22ºC room temperature, they were kept in cages, where they had access to food and water ad libitum throughout the period of the experimental process.

3.2 MATERIALS

- Plastic cages.
- Dissection materials.
- Feed (top grower mash).
- Clean drinking water
- Feeding and drinking plates
- Syringes
- Cotton wool
- Montelukast
- Weighing balance
- Chloroform
- Nebulizer
- Gloves
- Plain bottles
- Saline solution
- Mortar and pestle.
- Hydrocortisone

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 treated with anti-asthmatic drugs. All the groups consist 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 hydrocortisone and montelukast.

3.4 EXPERIMENTAL PROTOCOL/DESIGN

This experiment was carried out in phases:

Phase 1

Rats were acclimatized into their new environment for two (2) weeks after which they were divided into four (4) groups.

GROUP 1: Control

GROUP 2: Asthmatic and not treated.

GROUP 3: Asthmatic and treated with hydrocortisone.

GROUP 4: Asthmatic and treated with montelukast.

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 was 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 & Pretorius, 2008) and 10 mg/kg Montelukast.

Phase 3

At the end of drug administration, all animals were euthanized. Blood and tissue samples collected for biomarker assay and histology.

Phase 4

TNF-α, endothelin-I, adrenergic beta 1 and beta 2 receptor, and STAT gene expressed proteins were assayed in blood plasma

Day 28 (last day) of treatment

3.5 HISTOLOGICAL ANALYSIS

Dissected heart, aorta, portal vessels and kidney tissues were washed with normal saline, immersed in 10% (v/v) formaldehyde solution, and embedded in paraffin. Tissue specimens were sectioned and stained with haematoxylin and eosin (H & E) dye. Images of selected sections captured at 10X magnifications using a zoom digital camera (Thakur *et al.,* 2019).

3.6 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 was stored and frozen for the later quantitative determination of some biomarkers (Thakur et al., 2019).

3.7 STATISTICAL ANALYSIS

All the data obtained from the experiments is expressed as mean ± Standard Error of Mean (SEM). Statistical analysis performed by one way analysis of variance (ANOVA) for assessing differences amongst multiple groups, followed by Tukey's test using Graphpad Prism 10.0.3 software (Graphpad, San Diego, CA). $P < 0.05$ were considered statistically significant.

CHAPTER FOUR

RESULTS

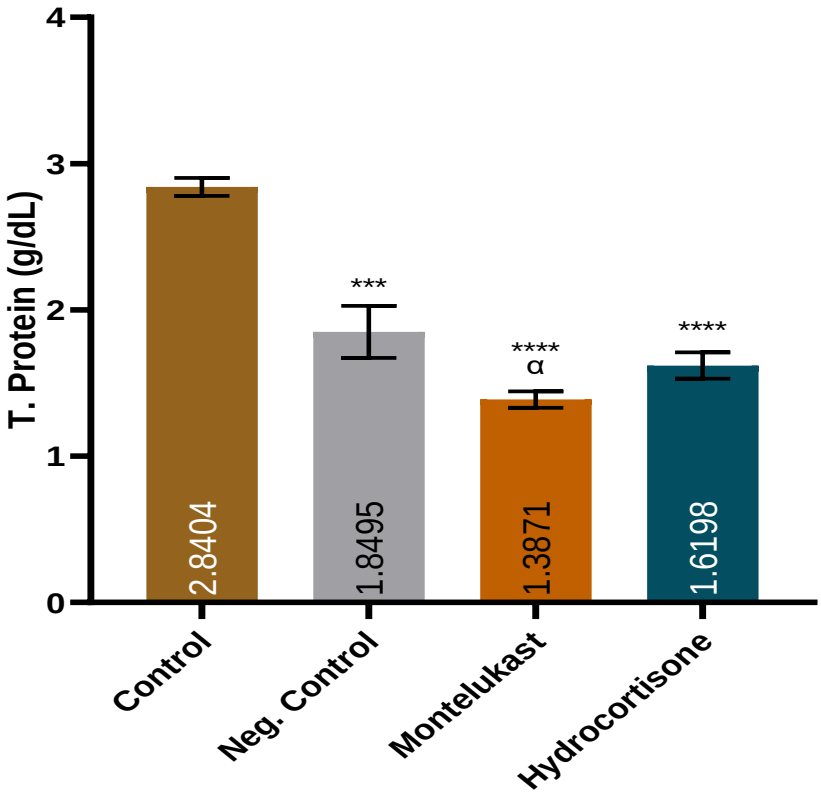

Fig. 1: chart show effect of montelukast and hydrocortisone on total protein in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in total protein $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

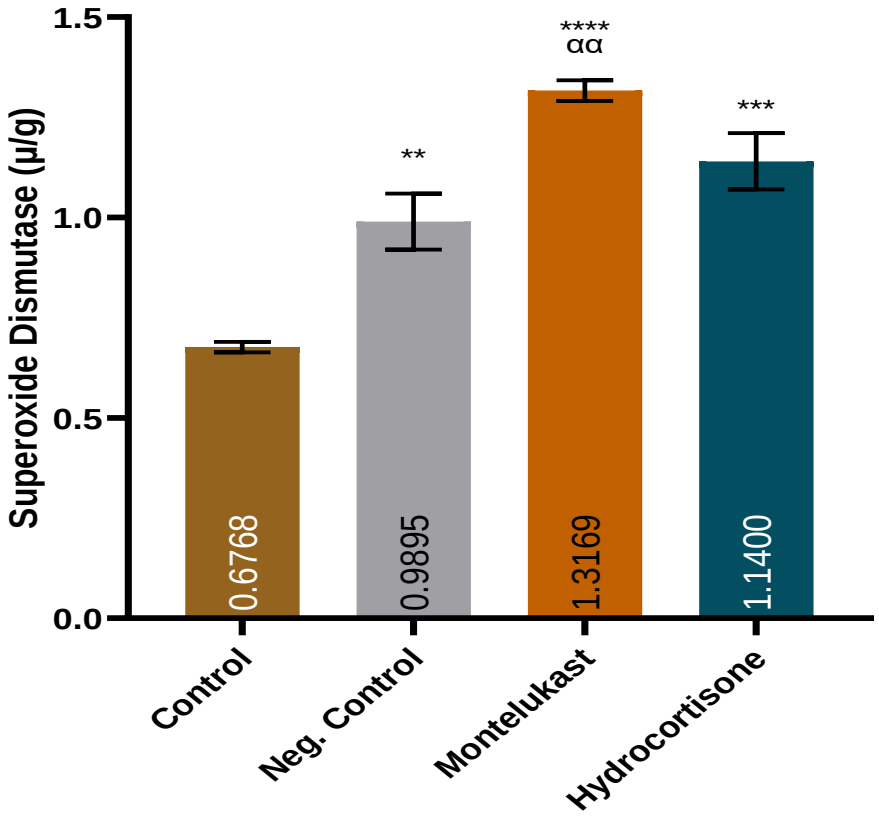

Fig. 2: chart show effect of montelukast and hydrocortisone on superoxide dismutase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in superoxide dismutase $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

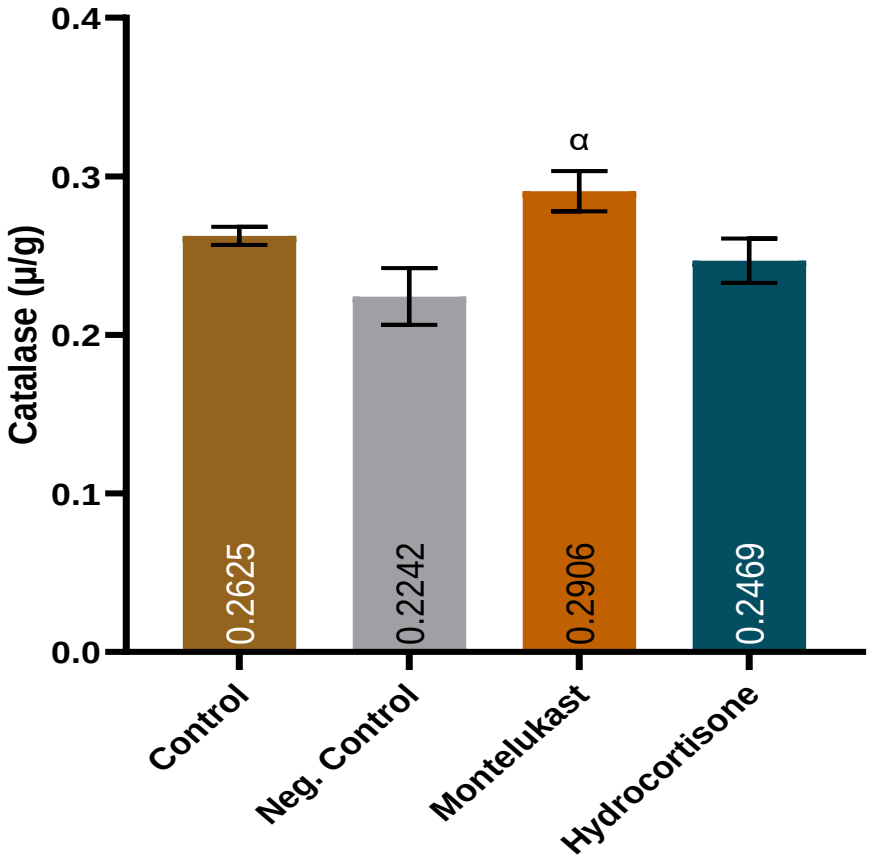

Fig. 3: chart show effect of montelukast and hydrocortisone on catalase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in catalase $p<0.05$

*$p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

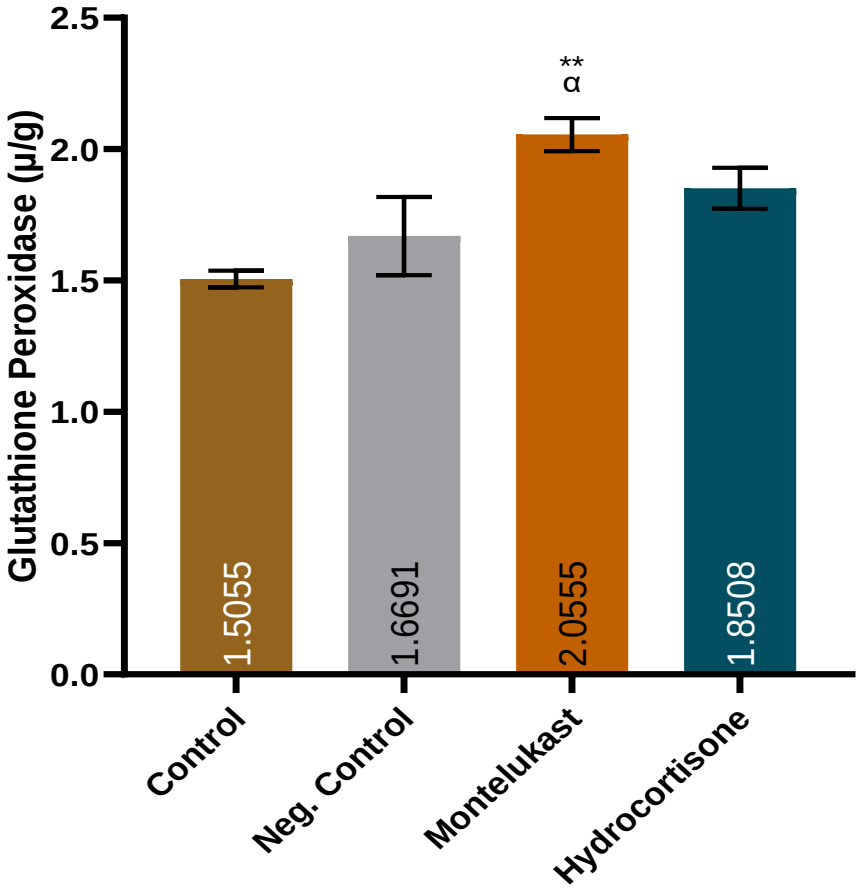

Fig. 4: chart show effect of montelukast and hydrocortisone on glutathione peroxidase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in glutathione peroxidase $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

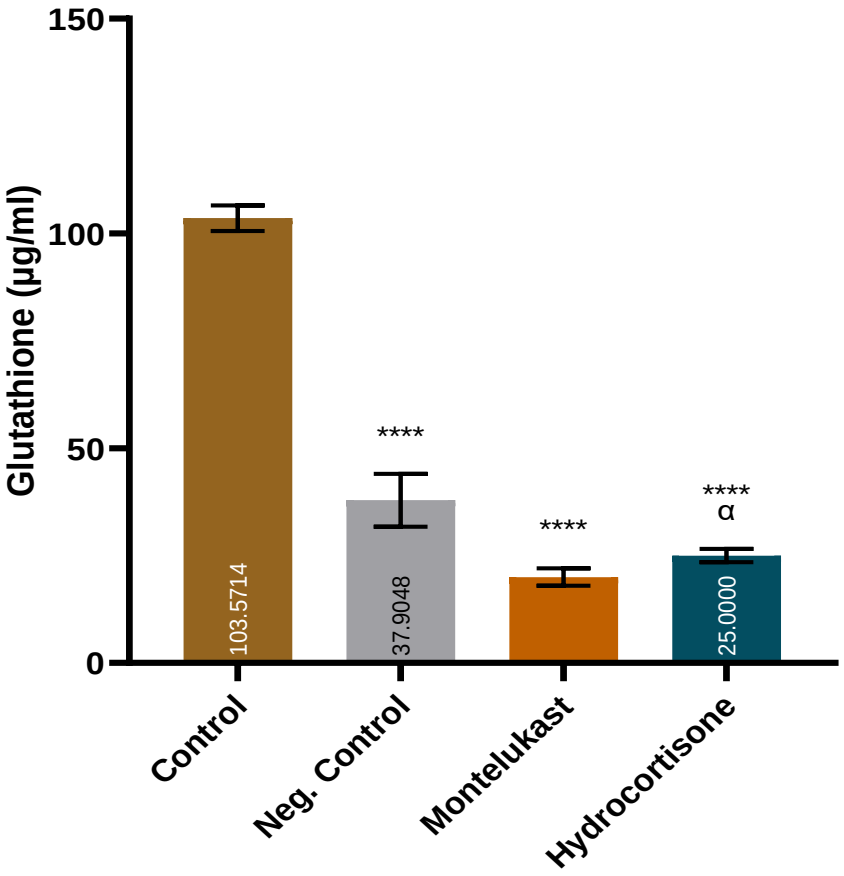

Fig. 5: chart show effect of montelukast and hydrocortisone on glutathione in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in glutathione $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

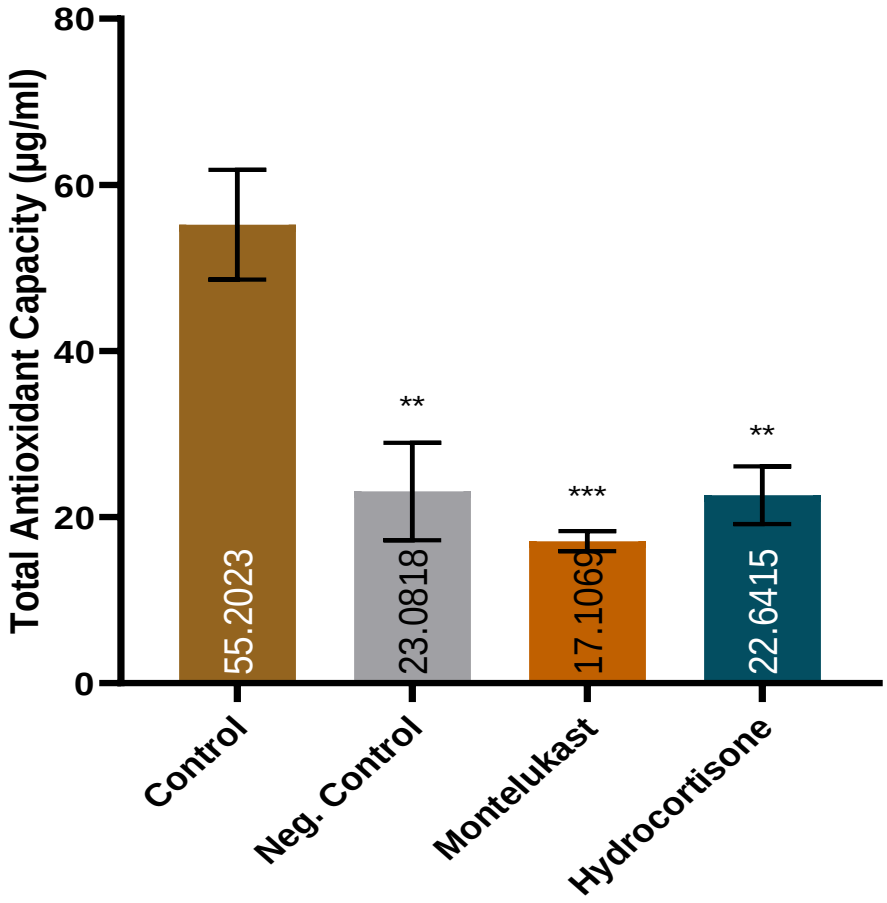

Fig. 6: chart show effect of montelukast and hydrocortisone on total antioxidant capacity in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in total antioxidant capacity $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

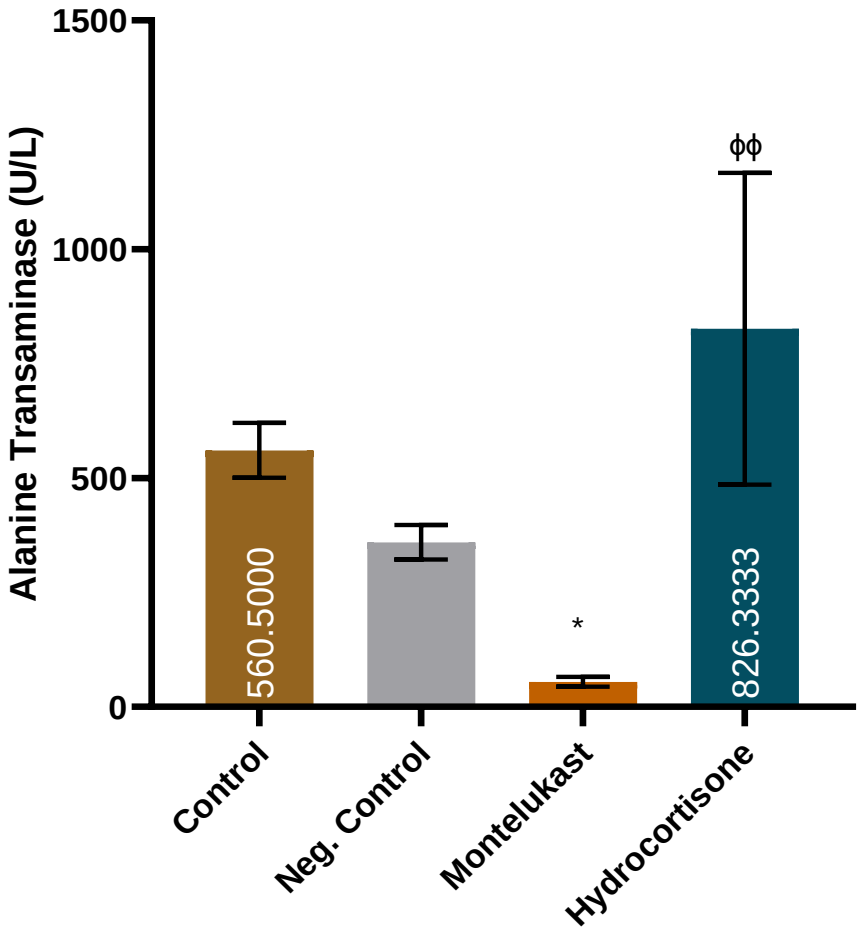

Fig. 7: chart show effect of montelukast and hydrocortisone on alanine transferase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in alanine transferase $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

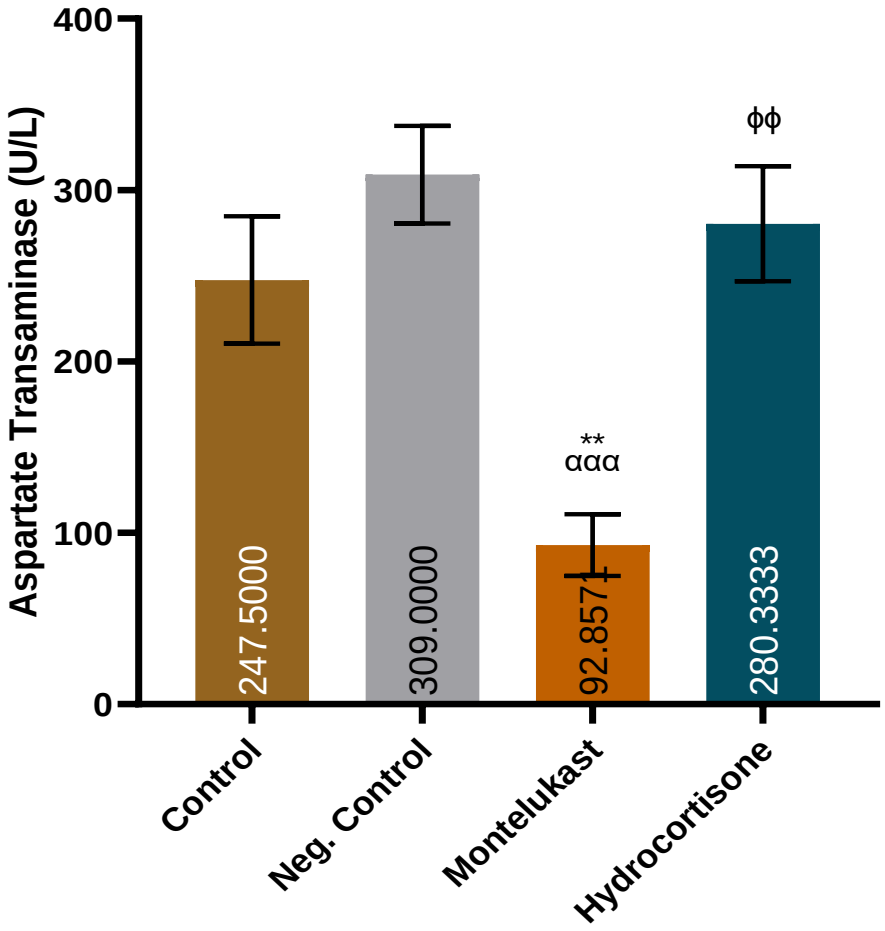

Fig. 8: chart show effect of montelukast and hydrocortisone on aspartate transaminase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in aspartate transaminase $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

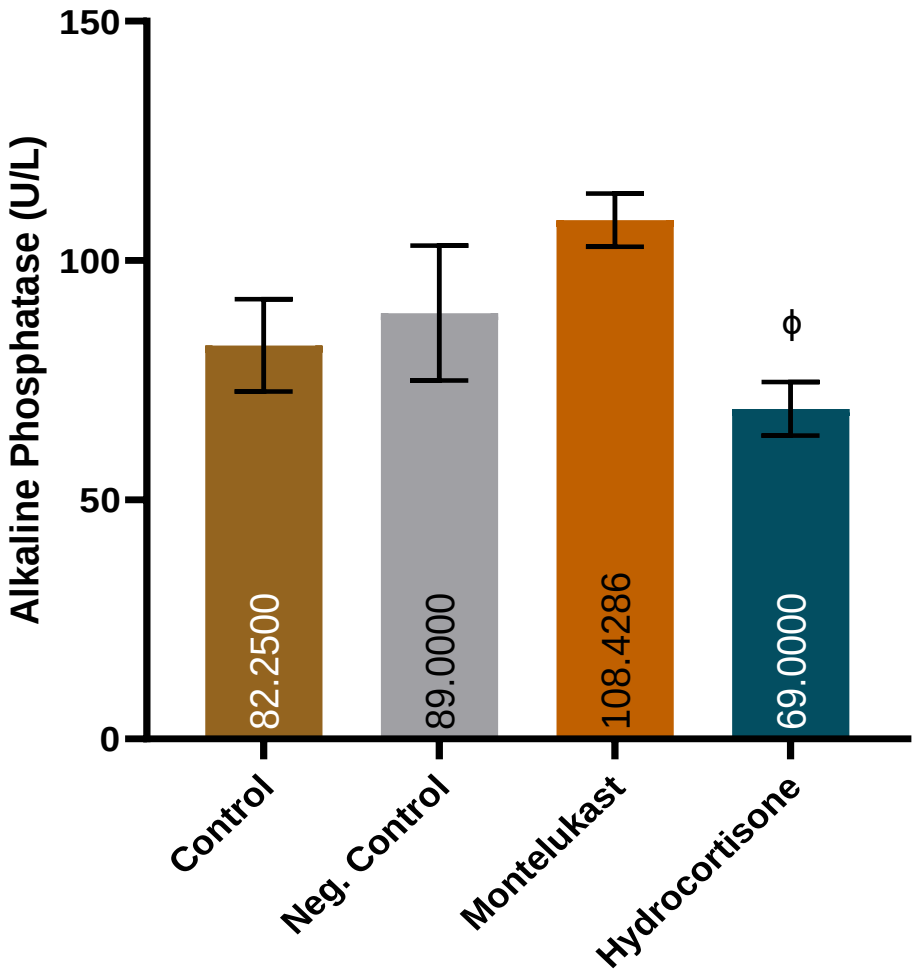

Fig. 9: chart show effect of montelukast and hydrocortisone on alkaline phosphatase in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in alkaline phosphatase $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

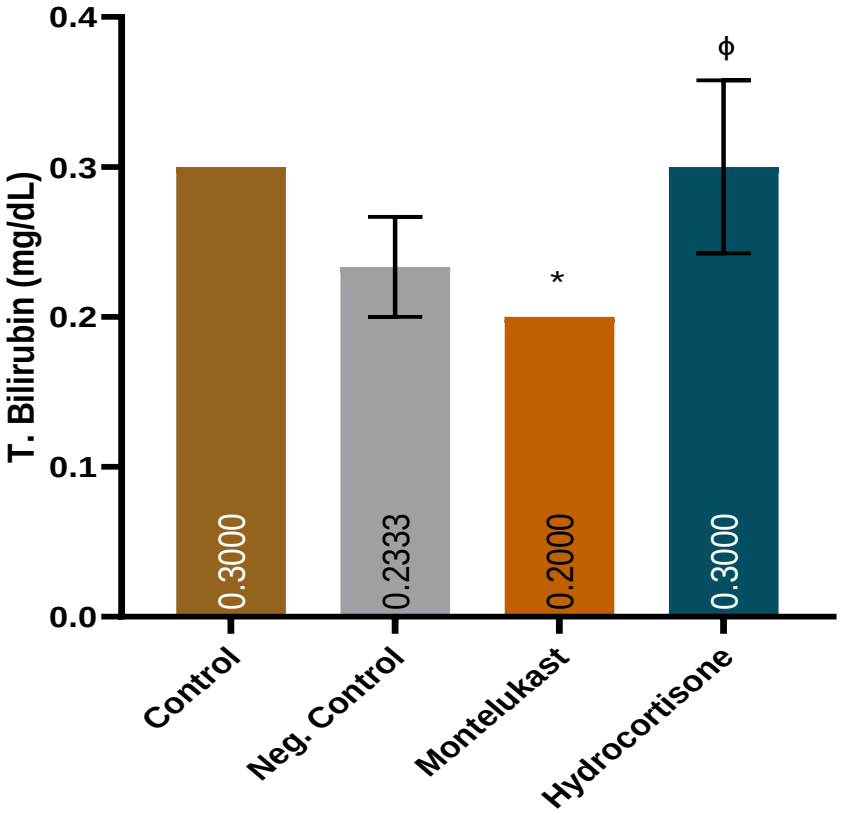

Fig. 10: chart show effect of montelukast and hydrocortisone on total bilirubin in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in total bilirubin $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

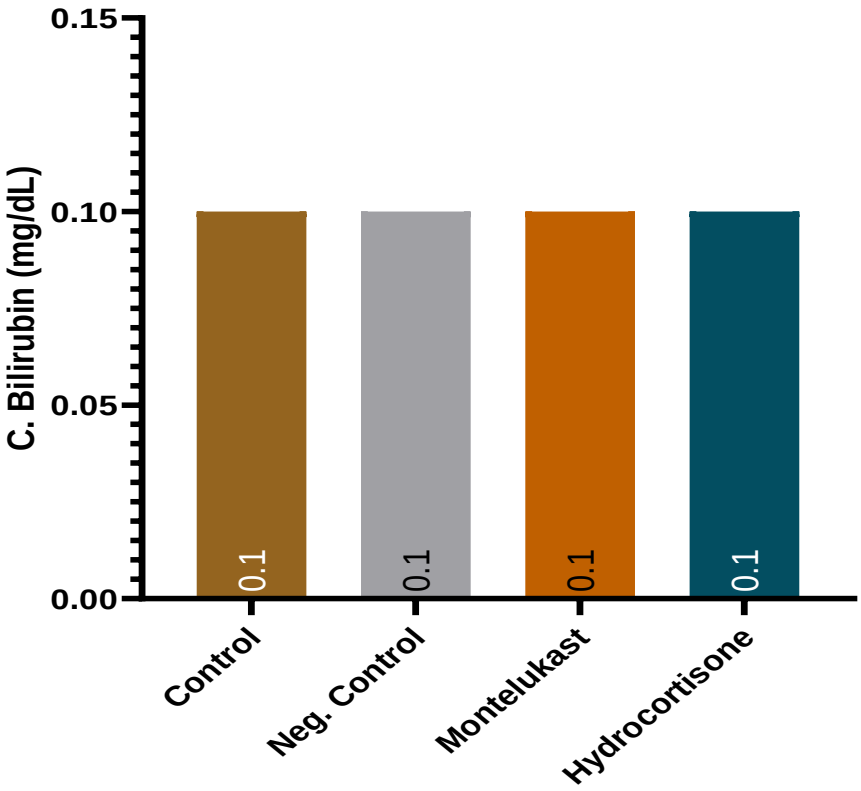

Fig. 11: chart show effect of montelukast and hydrocortisone on conjugated bilirubin in asthma induced Sprague Dawley rats. Result shows no statistically significant difference in conjugated bilirubin $p>0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

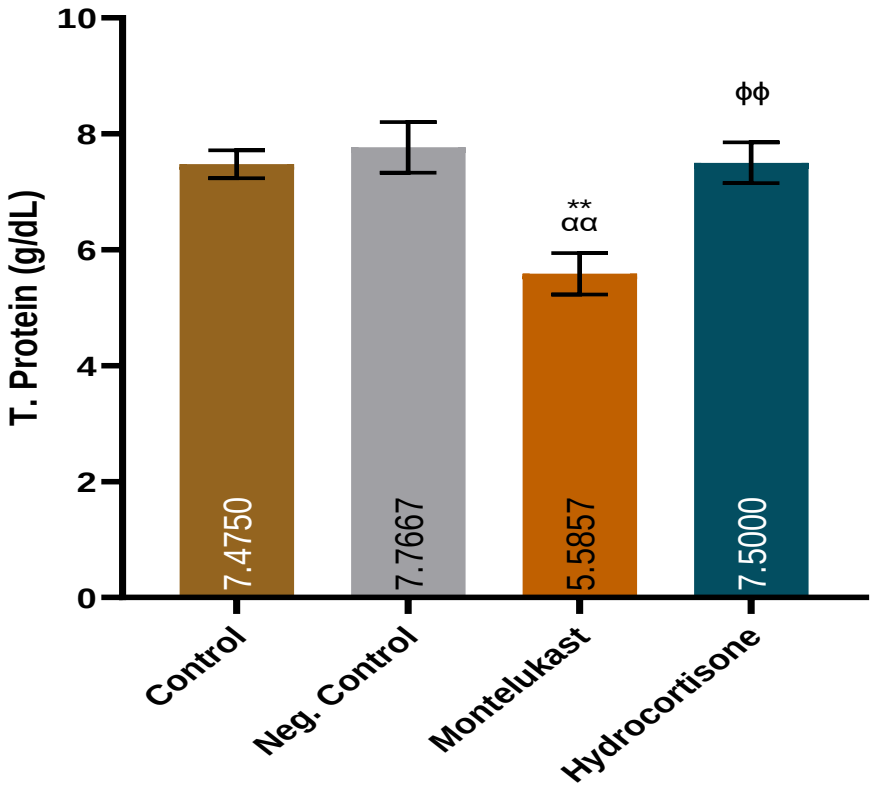

Fig. 12: chart show effect of montelukast and hydrocortisone on total protein in asthma induced Sprague Dawley rats. Result shows a statistically significant difference in total protein $p<0.05$

$^{*}p<0.05$ compared to control

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

$^{\phi}p<0.05$ compared to montelukast

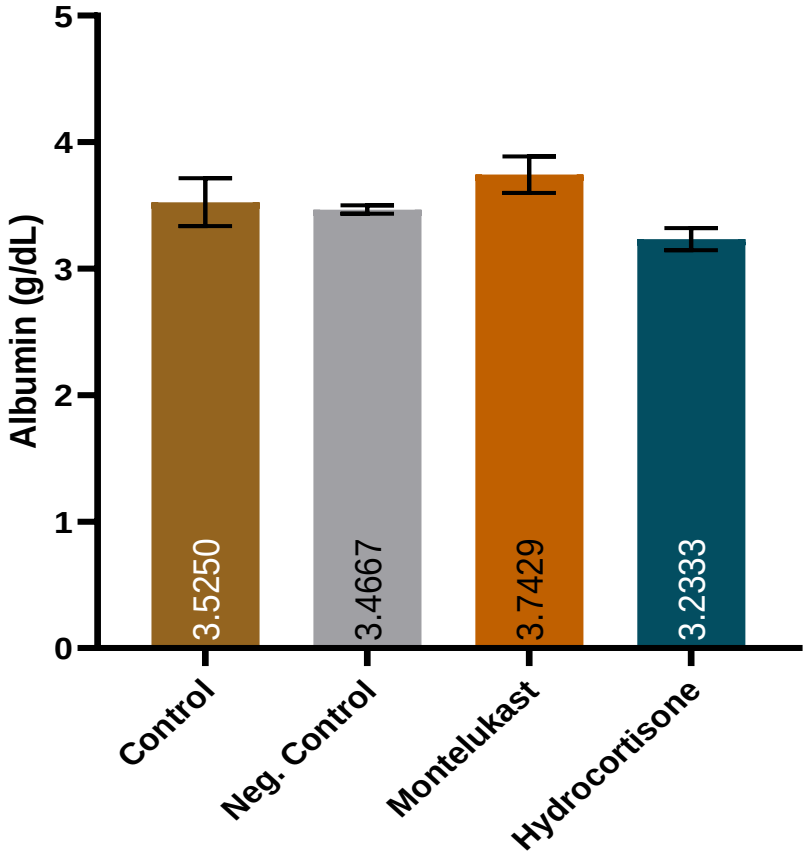

Fig. 13: chart show effect of montelukast and hydrocortisone on albumin in asthma induced Sprague Dawley rats. Result shows no statistically significant difference in albuminp>0.05

*p<0.05 compared to control

αp<0.05 compared to negative control

ϕp<0.05 compared to montelukast

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Asthma is one of the most common respiratory disease that affects both children and adults worldwide, with diverse phenotypes and underlying pathogenetic mechanisms poorly understood. Montelukast and hydrocortisone are anti-asthmatic drugs that have shown therapeutic effects in the treatment of asthma.

The results from this experiment suggest that the use of montelukast and hydrocortisone has significant effect on antioxidant levels and liver enzymes which includes the Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). This is seen in the P values obtained.

The findings showed that, when compared to blood, the total protein levels of all groups (control, negative control, montelukast, and hydrocortisone) decreased statistically significantly. This reduction could be a sign of asthma-related inflammation, which raises vascular permeability and causes protein to seep into the lung tissue. When montelukast and hydrocortisone are given to rats that have been given asthma, the inflammation in their airways is reduced, which in turn reduces vascular permeability in the lungs. Prior studies on the management of asthma have demonstrated the efficacy of leukotriene receptor antagonists, like montelukast, and corticosteroids, like hydrocortisone, in lowering airway inflammation.

Superoxide dismutase (SOD) levels in the lungs of all groups were much higher than in the blood, which may indicate an adaptive reaction to mitigate the oxidative damage brought on by asthma is a positive result. Superoxide radicals are an important antioxidant enzyme that contribute to oxidative stress and inflammation. SOD neutralises these radicals. It appears that both hydrocortisone and montelukast improve this response, either by increasing SOD activity or by decreasing the generation of superoxide radicals.

On the other hand, a possible or adaptive response to lessen oxidative stress and inflammation can be seen by the statistically significant rise in catalase (CAT) levels of all groups (control, negative control, montelukast, and hydrocortisone) when compared to blood ($p<0.05$). The ability of CAT to convert hydrogen peroxide into oxygen and water shields cells from oxidative damage. The elevated CAT levels in asthma-induced rats may be a defence mechanism against increased oxidative damage. It also suggests a stronger antioxidant defence system. This may be related to the use of hydrocortisone and montelukast, which by encouraging the activity of

these enzymes, may have decreased oxidative stress. This is consistent with their putative antioxidant and anti-inflammatory qualities.

The administration of montelukast and hydrocortisone greatly increased the antioxidant levels in asthma induced spraguedawley rats

5.2 CONCLUSION

With the data obtained from this research, the administration of Montelukast and Hydrocortisone indicated favorable results in enhancing antioxidant defense mechanisms, reducing oxidative stress, and normalizing certain markers of oxidative stress, in asthma induced spraguedawley rats.

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