EFFECTS OF SALBUTAMOL, MOTELUKAST AND HYDROCORTISONE ON LUNG HISTOLOGY AND ANTIOXIDANTS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS

BY

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CERTIFICATION

This is to certify that this project work on "**EFFECT OF SALBUTAMOL, MONTELUKAST AND HYDROCORTISONE ON LUNG HISTOLOGY AND ANTIOXIDANTS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS"** was carried out by **JOSHUA PRAISE TOSIN**, with the matriculation number **BMS1902406**; in partial fulfillment of the requirement of 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, Edo State, Nigeria.

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DEDICATION

This project work is dedicated to God almighty, my parents, the department of physiology and all asthmatic patients.

ACKNOWLEDGEMENT

My profound appreciation goes to the Almighty God for keeping me in sound health, in body and mind, and seeing me through this project work. I appreciate my amazing parents, **MR & MRS JOSHUA KOLAWOLE** for their relentless support in every aspect of life. I sincerely appreciate my project supervisor, **DR. (MRS) O. J. NZOPUTAM** for her motherly support throughout the course of this project. I also also appreciate my lecturer, **MR O. E. ALOAMAKA** for his support and accessibility through the tough times in the course of this project. I thank my friends, **JAMES, TOBE** and **CEPHAS** for their timely support and encouragement, and my project team mates for being supportive during the course of this project.

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ABSTRACT

The aim of this study is to understand the significance of montelukast, hydrocortisone and salbutamol on the lung histology and antioxidant levels in asthma induced Sprague Dawley rats. Chronic asthma is a respiratory disease characterized by oxidative stress and inflammation of the airways. Montelukast, hydrocotisone and salbutamol are drugs that are often used to treat asthma. Their impact on endogenous antioxidant levels in asthmatic conditions are yet to be clearly defined. Medication for asthma might include corticosteroids (like hydrocotisone), leukotrine receptor antagonists (like Montelukast) and Beta 2-adrenergic receptors (like salbutamol). Free radicals may be neutralized by antioxidants, which also lessen oxidative stress in the body. As a selective antagonist of the leukotriene D4 (LTD4) receptor, montelukast acts by preventing the body's production of leukotrienes, which are substances that promote inflammation and constriction of the airways when they come into contact with allergen. Other classes of drugs also prove useful in bronchodilation. Five (5) primary groups of Sprague Dawley rats were grouped (control, negative control and test groups). Group 1 control was not induced with asthma, Group 2, negative control was induced with asthma but not treated. These two groups make up the control group. Group 3 was induced with asthma and treated with salbutamol, Group 4 was induced with asthma and treated with montelukast, while Group 5 was induced with asthma and treated with Hydrocortisone. These three groups make up the test group, five rats in each group. The rats were sensitized to 1mg ovalbumin and 20mg Aluminium hydroxide dissolved in 0.9 saline, and then they were challenged with ovalbumin 1 % w/v adsorbed in 0.9 saline, twice weekly for four weeks (28 days), using a Medal family nebulizer. This caused the rats to develop asthma. After the Conclusion of treatment, the rats were sacrificed and their lungs were extracted for histological assay, while 1ml of blood is extracted for measurement of antioxidants using the spectrophotometric method, following reagent manufacturers guidelines. Measurements were made of the amounts of endogenous antioxidants, such as glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH). The findings demonstrated that there was statistically significant increase in superoxide dimutase and malondialdehyde levels in the negative control in comparison to the control group, while there was a statistically significant decrease in catalase and glutathione levels in the negative control group in comparison to the control group. Super-Oxide Dismutase was considerably increased after treatment with all classes of drugs. There was no statistically significant variance in catalase level noticed among the test group. Glutathione peroxidase was only significantly in the group treated with salbutamol, it showed no significant variance in other drug administration. There was significant increase in malondialdehyde in all groups except salbutamol. All test groups had considerably lower glutathione levels than the control group. As a result, the research concludes that some antioxidant levels (except glutathione) can be significantly increased with the given drugs, reducing oxidative stress in lung tissues.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Asthma is a chronic inflammatory disease of the airways that has caused a dreary state on the global health care system. Regardless of the medical advancement made in its respect, asthma is still a disease which is not optimally controlled in many patients (Chaudhari *et al.,* 2014).

Asthma is a clinical condition of uncertain etiology and is characterized by three components primarily:

I. Intermittent episodes of obstruction of the airway, which can either occur spontaneously or as a result of medication.

II. Exaggerated response of broncho-constrictor to stimuli that have almost no effect in normal or non-asthmatic subjects. This is also called airway hyper responsiveness.

III. Inflammation of the airways, resulting from varying factors (Naveen *et al.,* 2011).

In the developed and developing countries of the world, asthma continues to gain prevalence. About 18-20 million people in the United States and over 250 million people in the world suffer from this disease (Ronald, 2008; Sneha *et al.,* 2016).

Severity of asthma symptoms can either increase or decrease with time, depending on environmental factors, patients' adherence to treatment schedule and other factors (Mohamed *et a.l*, 2016).

The treatment of asthma majorly involves the use of allopathic drugs such as: sympathomimetics, anticholinergics, corticosteroids, methylxanthines and mast cell stabilizers. Although, along with therapeutic effects, these drugs also have quite a number of side effects.

Herbal treatment has been considered safer, compared to the conventional treatment for asthma (Bajani *et al.*, 2009).

Some classes of drugs used overtime to treatment asthma are:

• Corticosteroids/ glucosteroids: They have anti-inflammatory properties and are often used to treat conditions like asthma, allergic reactions, and inflammatory disorders. Examples are budesonide and hydrocortisone

• Leukotriene modifiers: It is commonly used in the treatment of asthma and allergic rhinitis. It acts by blocking leukotriene receptors which are inflammatory mediators. An example of this drug is montelukast.

• Short-acting beta-2 agonists: It is a quick bronchodilator commonly used to relieve symptoms of asthma and chronic obstructive pulmonary disease (COPD). An example of this drug is Salbutamol.

Possible effects of salbutamol, montelukast and hydrocortisone on antioxidants and lung histology:

Possible effect of montelukast on antioxidants and lung histology:

Montelukast's anti-inflammatory properties may indirectly contribute to a reduction in oxidative stress (Fatih *et al.,* 2015). Treatment with montelukast results in a significant decrease in deposition of collagen (29 %) and a reduction in hydroxyproline content by 32 % (Olfat *et al.,* 2011).

Possible effect of salbutamol on antioxidants and lung histology:

Salbutamol's ability to relax bronchial smooth muscle may indirectly contribute to the reduction of oxidative stress and also modulates oxidative stress markers (Suleyman *et al.,* 2010). Salbutamol reduces the infiltration of polymorphonuclear neutrophil leukocytes, perivascular/ intra alveolar haemorrhage and interstitial edema (Sema *et al.,* 2012).

Possible effect of hydrocortisone on antioxidants and lung histology:

By suppressing inflammatory processes, hydrocortisone may indirectly contribute to a reduction in oxidative stress. Corticosteroids like hydrocortisone may influence the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Costa *et al.,* 2022). The use of hydrocortisone results in increase in diameter of the alveoli and cause thinning of the interalveolar space (Michael *et al.,* 2002).

1.1 Aim of study

The aim of this study is to understand the effect of montelukast, salbutamol and hydrocortisone on lung histology and antioxidants in asthma-induced Sprague Dawley rats.

1.2 Research question

This research aims at answering the following questions

1. What is the effect of asthma on lung histology?

2. What are the effects of Salbutamol, motelukast and hydrocortisone on antioxidant levels and lung histology?

1.3 Specific objectives

1. To determine, through research and experimental analysis, the effect of asthma on lung histology in asthma induced Sprague Dawley rats.

2. To determine the effects of montelukast, hydrocortisone and salbutamol on lung histology and antioxidant levels in asthma induced Sprague Dawley rats.

1.4 Justification of study

A number of studies have been done to determine the specific effects of respiratory drugs on lung histology. Although remarkable progress has been made, some mechanisms of these drug actions are yet to be specified (Higgins *et al.,* 1991), hence the need for further research.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF ASTHMA

Chronic asthma is a respiratory disease that causes airway inflammation and airway constriction, which may result to wheezing, dyspnea, intrathoracic pressure and severe coughing that happens often. It is one of the most common chronic illnesses that afflict people of all ages, globally. A person's quality of life may be greatly impacted by asthma, which can range in intensity from moderate to severe symptoms (Lee and Mcdonald, 2018). Asthma prevalence varies across nations and populations, with an estimated population of over 250 million worldwide, with industrialized nations showing greater prevalence rates. Asthma can affect both adults and children, however it usually begins in childhood and can last until old age. Asthma is a disease of complicated etiology that combines environmental and hereditary variables. Asthmatics experience inflammation and hypersensitivity of the airways in response to various kinds of stimuli, including exercise, allergies, respiratory infections, and irritants like smoke and pollution (Tesfaye *et al.,* 2018). As a result of this inflammation, the lungs show altered epithelial cells, mucus cell hyperplasia and airway constriction. As a consequence of these modifications, there is less airflow, which exaggerates asthma symptoms, which vary in the frequency, severity, and duration in different individuals. Common symptoms include chest tightness, wheezing (a whistling sound made during inhalation), coughing, especially at night or in reaction to triggers, and shortness of breath. According to Salo *et al*. (2018), asthma symptoms may vary from mild, sporadic episodes to more severe, chronic symptoms that need constant care. A patient's medical history, physical examination, lung function tests (such spirometry), and symptom evaluation are all used in the diagnosis of asthma. In order to manage symptoms, avoid exacerbations, and preserve normal lung function, asthma patients are usually treated with a combination of prescription based medicines, such as bronchodilators, which relax airway muscles, and anti-inflammatory agents, which reduce inflammation in the airways. An individual's daily activities, sleep patterns, and general quality of life may be greatly altered by asthma. Uncontrolled asthma may result in recurrent hospitalization, ER visits, and an unbalanced social life. If asthma is not well controlled, long-term consequences may also occur, such as decreased lung function and a higher risk of respiratory infections (Piloni *et al.,* 2019).

2.1.1 EFFECT OF ASTHMA ON THE LUNGS

Asthma attacks occur when the airways of the lung become narrowed causing an obstruction in airflow. Symptoms of asthma include coughing, wheezing, breathlessness and tightness in the chest. The larger airways of the lungs have cartilage in their walls to prevent them from collapsing, but the smaller bronchi and bronchioles do not have this support. These smaller airways are muscular tubes and they are lined with many mucus secreting cells (Pascual and Peters, 2005).

During an asthma attack the muscle walls contract and tighten, and the lining of the airways becomes swollen and inflamed. As a result of these changes, the airways become narrowed, which is further aggravated by an increased secretion from the mucus membrane, which may actually block the smaller airways. All these cause an obstruction to airflow. This results in a significant increase in the effort needed to move air in and out of the lungs, giving rise to breathlessness and wheezing (Peden, 1996).

Although asthma primarily affects the lungs, it's effect can still extend beyond the lower respiratory tract into other parts of the respiratory system. Other parts which may be affected include:

(I) Trachea (windpipe): Asthma may result in inflammation of the trachea

(II) Larynx (voice box): Although vocal cord dysfunction and asthma are two different conditions, asthmatics could experience vocal cord dysfunction.

(III) Pharynx (throat): The pharynx or throat could often be dry, itchy and clogged.

(IV) Nose: Asthmatics could experience runny nose and nasal congestion.

2.2 EFFECT OF MONTELUKAST ON LUNG HISTOLOGY

Montelukast is a cysteinyl leukotriene receptor antagonist which is used as a preventive treatment for persistent asthma in patients > or =2 years of age. Montelukast was first used in children aged 2 to 14 years in clinical trials for asthma (Muijsers and Noble, 2002). In a research carried out by Reiss *et al,* (1997), in individuals with mild to moderately severe asthma, the acute effects of montelukast (MK-0476), a strong, oral, selective cysteinyl leukotriene receptor antagonist, on airway obstruction were evaluated. It was concluded that, Single oral doses of montelukast 100 mg and 250 mg provided significant increases in FEV1 regardless of the contemporaneous use of inhaled corticosteroids in asthmatic individuals with airflow limitation.

The prevention of viral induced asthma (PREVIA) research sought to determine the role of montelukast, a leukotriene receptor antagonist, in preventing viral-induced asthma exacerbations in children aged 2 to 5 years with a history of intermittent asthma symptoms. Montelukast reduced the rate of asthma exacerbations by 31.9 % over a 12-month period when compared to placebo. The average number of exacerbation episodes per patient was 1.60 per year on montelukast, compared to 2.34 on placebo. Montelukast effectively reduced asthma exacerbations in 2- to 5-year-old patients with intermittent asthma over a 12-month therapy period and was generally well tolerated (Bisgaard *et al.,* 2005).

Patients with moderate asthma usually experience just exercise-induced bronchoconstriction, which is an indication of insufficient asthma management. Investigation shows the ability of montelukast, a leukotriene receptor antagonist, to protect such patients from exercise-induced bronchoconstriction (Leff *et al.,* 1998). It also has the ability to cause a significant improvement in chronic asthma at an oral dose as low as 10mg, once daily in the evenings, as proven by Altman *et al.,* (1998).

2.3 EFFECT OF SALBUTAMOL ON LUNG HISTOLOGY

Salbutamol is a bronchodilator and reliever that acts quickly. It is a member of the group of drugs known as short-acting beta-2 adrenergic agonists. Salbutamol is used to treat bronchospasm and asthma symptoms. Salbutamol is also used to treat other chest conditions like COPD, which can cause symptoms like coughing, shortness of breath, wheezing, and tightness in the chest (Dodson *et al.,* 2011).

In asthma and chronic bronchitis, bronchial reactivity to agonist such as histamine has been observed. According to a research carried out by Higgins *et al* in 1991, it was observed that acute administration of salbutamol reduces bronchial reactivity in asthmatics.

Further research was carried out in 1995, which compared the effects of uniphyl, salbutamol and a combination of both on a group of 32 patients with moderately severe, chronic asthma, maintained on moderately high doses of corticosteroids. The results were effective in showing the improvement in Mean FEV1 and maximum FEV1 on administration of salbutamol compared with placebo. Salbutamol was generally observed to significantly improve pulmonary function and asthma symptoms in patients who were treated with high doses of corticosteroids and rightly needed beta agonists.

2.4 EFFECT OF HYDROCORTISONE ON LUNG HISTOLOGY

Steroid medications like hydrocortisone are used to treat a wide range of ailments, including inflammatory bowel diseases, arthritis, blood or bone marrow abnormalities, asthma, and allergic responses. It functions by reducing inflammation, calming down an overactive immune system, or replenishing the cortisol that the body naturally produces. Bronchopulmonary dysplasia, a major complication of extreme prematurity, has few treatment options. The use of postnatal steroid is quite controversial, but the use of low-dose hydrocortisone might prevent the effects of inflammation on the premature/developing lung.

A research study was carried out in 2016 to ascertain whether low-dose hydrocortisone improves survival without bronchopulmonary dysplasia in extremely preterm infants, it was observed that Low-dose hydrocortisone treatment improved survival without bronchopulmonary dysplasia in extremely preterm infants, with 60 % surviving without bronchopulmonary dysplasia compared to 51 % with placebo (Baud *et al.,* 2016).

Hydrocortisone is a cell division modulator that has been found to increase the replicative in vitro life span of human embryonic lung fibroblasts. The results show that hydrocortisone reduces the interdivision time (IDT) of a subset of the population's cells, and that this impact is exacerbated by continuous hydrocortisone exposure. Hydrocortisone does not appear to enhance the number of initial dividers in the population, but it does boost growth rates in the early stages of the culture period. Analysis of mother-daughter IDT pairings indicates that hydrocortisone affects IDT individually for each cell (Absher and Cristofalo, 1984).

The effect of stress doses of hydrocortisone on the duration of vasopressor therapy in human septic shock was investigated. It was recorded that with the administration of stress doses of hydrocortisone on 20 patients, 18 of them achieved shock reversal in comparison to 16 out of 20 patients in placebo. Hydrocortisone significantly reduced the time to cessation of vasopressor support (Briegel *et al.,* 1999).

2.5 ANTIOXIDANTS

An antioxidant is a chemical that, when present in small amounts, inhibits or delays a substrate's oxidation. The chemical methods by which antioxidant chemicals work include single electron transfer (SET), hydrogen atom transfer (HAT), and the capacity to bind transition metals. Antioxidant compounds can generally respond through a single mechanism or through several different ways. Understanding the antioxidant response mechanism is made possible by the substance's chemical structure (Santos-Sánchez *et al.,* 2019).

The clinical and nutritional literature frequently discusses antioxidants and free radicals. Antioxidants are necessary to stop reactive oxygen and nitrogen species from forming and to counteract their effects. These species are produced in vivo and can harm proteins, lipids, DNA, and other macromolecules. Antioxidants produced from diet are necessary for preserving health since endogenous antioxidant defenses (superoxide dismutases, H2O2-removing enzymes, and metal binding proteins) are not sufficient to entirely prevent harm (Halliwell, 1996). Antioxidants protect the body from oxidative stress-mediated pathology and oxidative deterioration (Gulcin, 2020).

Some examples of antioxidants are:

2.5.1 Superoxide dismutase (SOD)

A study found that the primary physiological function of superoxide dismutase is to protect oxygen-metabolizing organisms from the potentially harmful effects of the superoxide free radical, a biologically produced intermediate caused by the univalent reduction of molecular oxygen (McCord *et al.,* 1971).

Superoxide dismutase limits interactions involving O2- and adrenaline while catalyzing the dismutation of superoxide radicals (McCord and Fridovich, 1969).

2.5.2 Catalase (CAT)

Catalase is an enzyme that may break down hydrogen peroxide or oxidize tiny substrates like ethanol, methanol, or elemental mercury (Hg0). Native catalase has been shown to peroxidatically oxidize bigger organic molecules (e.g., L-dopa), and catalase maintained at alkaline pH for varying amounts of time increases peroxidase activity when guaiacol is used as a substrate. It was demonstrated, using two different techniques of introducing H2O2 to measure peroxidase activity, that native catalase has no peroxidatic activity toward these bigger organic compounds (Sp and Al, 1986).

2.5.3 Glutathione peroxidase (GPx)

Glutathione peroxidases prevent oxidative damage, guard against hydroperoxides, and may perform cell- and tissue-specific roles in metabolic control, and also play a role in leukotriene formation (Brigelius-Flohé, 1999; Chambers *et al.,* 1986).

2.5.4 Malondialdehyde (MDA)

Malondialdehyde is a harmful byproduct of lipid oxidation which stiffens and inhibits collagen remodelling in the cardiovascular system in diabetes mellitus (Slatter *et al.,* 2000). Treatment with Malondialdehyde causes a conformational shift in hemoglobin A, boosting its oxygen affinity while decreasing mechanical stability, potentially affecting its function and stability in erythrocytes (Kikugawa *et al.,* 1984).

2.5.5 Glutathione (GSH)

Glutathione is a tripeptide thiol which is present in almost all cells and is essential for metabolism, transport, and cellular defense. It conjugates with both endogenous and foreign chemicals and takes part in the reduction of disulfides and other molecules. It shields cells from the damaging effects of free radicals and reactive oxygen intermediates (Meister, 1983).

2.6 ANTIOXIDANTS AND ASTHMA

Several investigations have found that reactive oxygen species (ROS) play an important role in the start and amplification of inflammation in asthmatic airways. Excessive ROS production in asthma alters essential enzymatic and nonenzymatic antioxidants such as glutathione, vitamins C and E, beta-carotene, uric acid, thioredoxin, superoxide dismutases, catalase, and glutathione peroxidases, resulting in an oxidant-antioxidant imbalance in the airways. An oxidant-antioxidant imbalance causes pathophysiological consequences linked with asthma, including increased vascular permeability, mucus hypersecretion, smooth muscle contraction, and epithelium shedding. Epidemiological findings reinforce the scientific evidence of an oxidant-antioxidant imbalance in asthmatics. As a result, antioxidant supplementation to raise endogenous antioxidants or scavenge excess ROS generation could be used to reduce/prevent asthmatic inflammation by restoring oxidant-antioxidant balance (Nadeem *et al.,* 2008). One major factor in the pathophysiology of asthma is oxidative stress. The impact of various antioxidants on asthma has been the subject of recent research by numerous researchers. Some antioxidants, such as catalase and superoxide dismutase, can be used as innovative treatment agents for asthma because they lower hyperresponsiveness and airway inflammation (Park and Lee, 2006).

2.6.1 Effect of montelukast on antioxidant levels

Montelukast, a strong leukotriene receptor antagonist, improves asthma management and quality of life in chronic asthma patients as doses increase (Noonan *et al.,* 1998). Montelukast lowers exhaled nitric oxide levels in people with mild asthma, which is compatible with its anti-inflammatory activity (Sandrini *et al.,* 2003).

2.6.2 Effect of hydrocortisone on antioxidant levels

Endogenous hydrocortisone regulates lymphocyte activity in both asthmatic patients and healthy controls, which may have an impact on asthma symptoms (Weller *et al.,* 1986). When treating acute asthma, hydrocortisone does not immediately alleviate symptoms when given at the dosages and methods that are used (McFadden *et al.,* 1976).

2.6.3 Effect of salbutamol on antioxidant levels

Salbutamol and fenoterol, two beta 2-agonists, had no effect on glutathione peroxidase activity or plasma selenium levels in asthmatic patients, indicating that they are unlikely to be the cause of low selenium status (Burgess *et al.,* 1994). Administration of Nebulized salbutamol (5.0 mg) given before GSH challenge showed hindrance of GSH-induced bronchoconstriction in patients with mild asthma (Marrades *et al.,* 1997).

2.7 ANIMAL MODEL: SPRAGUE DAWLEY RATS

Asthma is a chronic disease of airway inflammation with extensive airway remodeling. The ethical requirements associated with studies in asthmatic patients demand a development of animal models in asthma studies. Animal models have been developed in rats and mice, considering their physiological similarities with man and their response to allergic asthma (Piechuta *et al.,* 1979).

However, compared with mice, rats are easier to mimic primary symptoms of asthma such as airway hyperresponsiveness and airway inflammation. Rats are also easier to handle and their size allows for collection of higher number of samples.

For this study, Sprague Dawley rats were used. The Sprague Dawley rat's reproductive efficiency is especially valuable because it makes it possible to gather enough data in short period of time. Also, they are just as susceptible to asthma-induced conditions as man, making it a very relevant model. The Sprague Dawley rat develops respiratory impairment after sensitization and challenge with aerosolized antigen (Homle and Pechuta, 1981). An experimental procedure was developed to evaluate the effect of antigen aerosol challenge on sensitized conscious rats. The challenge resulted in significant changes in respiratory pattern and lung structure alterations (Piechuta *et al.,* 1979).

Sprague Dawley rats showed significant improvement in goblet cell hyperplasia and airway hyperresponsiveness with administration of corticosteroids and Beta 2- adrenergic agonists (Kamachi *et al.,* 2001).

In conclusion, the animal model, Sprague Dawley rat, offers a reliable platform for examining the complex interactions between the administration of corticosteroids (Hydrocortisone), Beta 2- adrenergic agonist (Salbutamol) and leukotriene receptor antagonist (Montelukast) on lung histology and antioxidant levels brought about by asthma.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

3.0. Materials

Materials used for this study include

- Feed
- Clean water
- Plastic cages
- Chlorofom
- Dissection materials
- Aluminum Hydroxide
- Weighing balance
- Syringes
- Gloves
- Cotton wool
- Montelukast
- Hydrocortisone
- salbutamol
- Saline solution.

3.1. Experimental animals

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

3.2. Experimental Design

Sprague-Dawley rats weighing between 180-250 g were divided into two (2) main groups; the Control group and Test group. The test groups were further divided into three (3) subgroups treated with anti-asthmatic drugs. All the groups consisted of twenty (25) rats in total, with each group having (n=5) number of rats. The control group received normal rat feed and water throughout the experimental period while the test groups were exposed to concentrations of Ovalbumin (OVA, egg albumin grade l) and aluminum hydroxide to induce asthma after which they were treated with Salbutamol, Montelukast and hydrocortisone.

3.3. Duration of Study

This study was conducted for a period of six (6) weeks. During which there was two (2) weeks of acclimatization and four (4) weeks of drug administration.

Experimental protocol/design

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 five (5) groups of twenty (25) rats with five (5) rats in each group.

Test groups

GROUP 1: Control comprised rats fed with regular feed and water ad

libitum

GROUP 2: Asthma-induced, not treated (negative control)

GROUP 3: Asthma-induced and treated with Salbutamol

GROUP 4: Asthma-induced and treated with montelukast

GROUP 5: Asthma-induced 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 (3, 4 and 5) were sensitized with 1 mg OVA and 200 mg aluminum hydroxide dissolved in 0.9 saline on day 0 and 7 and 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).

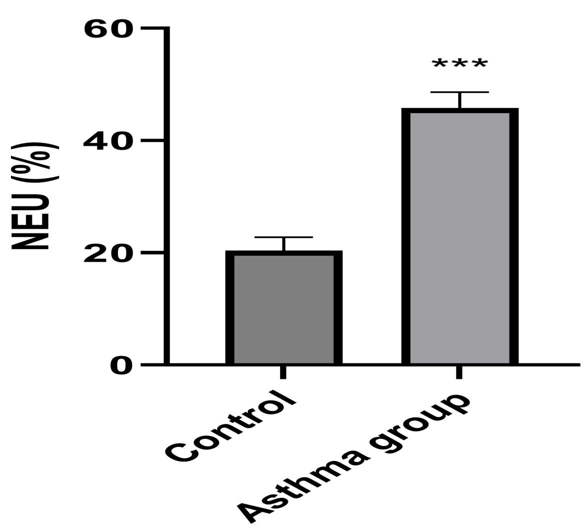

Fig.3.1: chart showing the effect of asthma on neutrophil count in asthma induced Sprague-Dawley rats

Result shows a statistically significant increase in neutrophil count in ovalbumin induced Sprague-Dawley rats compared to control (p<0.05)

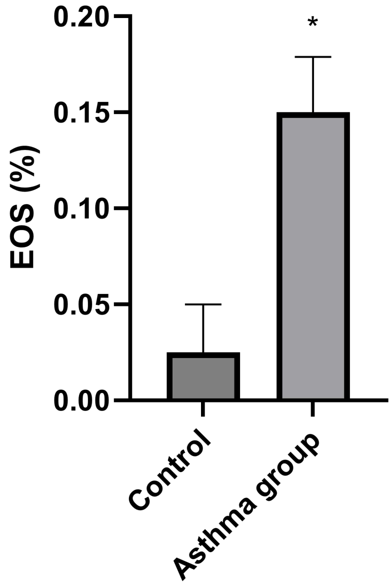

Fig.3.2: chart showing the effect of asthma on eosinophil count in asthma induced Sprague-Dawley rats

Result shows a statistically significant increase in eosinophil count in ovalbumin induced Sprague-Dawley rats compared to control (p<0.05)

Phase 2

After confirmation of asthma in all test groups, treatment began with 3 mgkg Salbutamol (oral), 10 mg/kg Montelukast (oral) and Hydrocortisone (Intraperitoneally).

Phase 3

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

Phase 4

Total Cholesterol, triglycerides, high-density lipoproteins and low-density lipoproteins were assayed in blood plasma.

3.4. Blood Sampling and Serum Isolation

Blood was collected from abdominal aorta 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 pm (rounds per minute) for 15 min, and serum isolated by aspiration. The separated serum was stored at frozen state for the later quantitative determination of some biomarkers (Thakur et al., 2019).

3.5. 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 Prism8.1 software (Graphpad, San Diego, CA). $P<0.05$ were considered statistically significant.

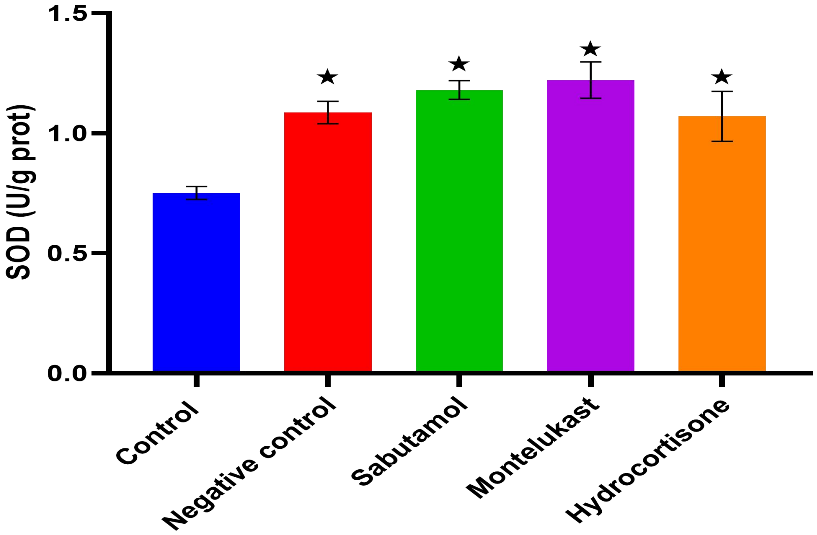

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Fig. 4.1: The effects of salbutamol, montelukast and hydrocortisone on superoxide dismutase in asthma induced Sprague-Dawley rats.

There were significant increases in negative control, salbutamol group, Montelukast and hydrocortisone groups compared with control.

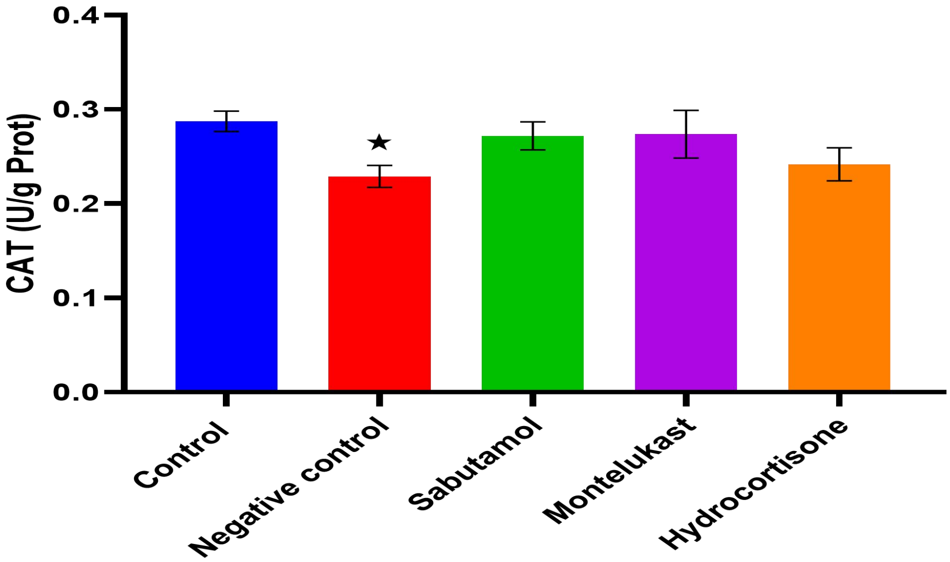

Fig. 4.2: The effects of salbutamol, montelukast and hydrocortisone on catalase in asthma induced Sprague-Dawley rats.

There was a significant decrease in negative control group compared with control, though there were no significant changes in salbutamol group, Montelukast and hydrocortisone groups compared with control.

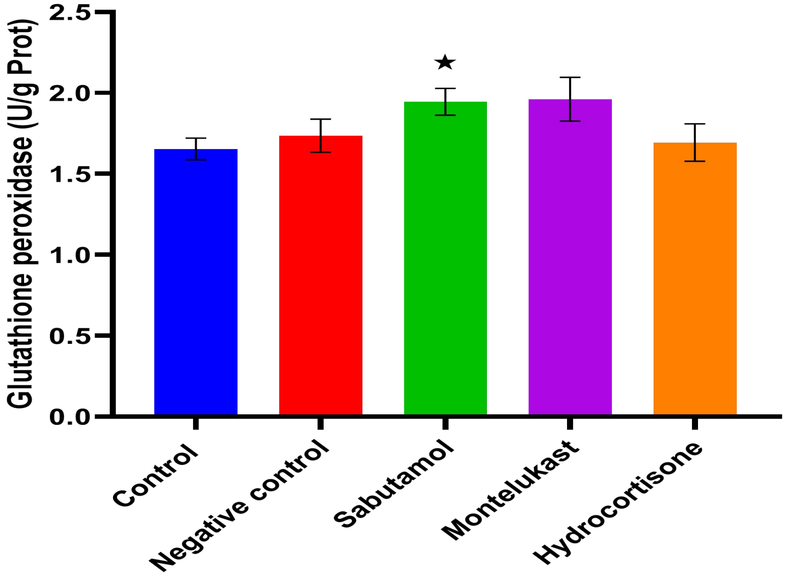

Fig. 4.3: The effects of salbutamol, montelukast and hydrocortisone on glutathione peroxidase in asthma induced Sprague-Dawley rats.

There was a significant increase in salbutamol group compared with control, though there were no significant changes in negative control group, Montelukast and hydrocortisone groups compared with control.

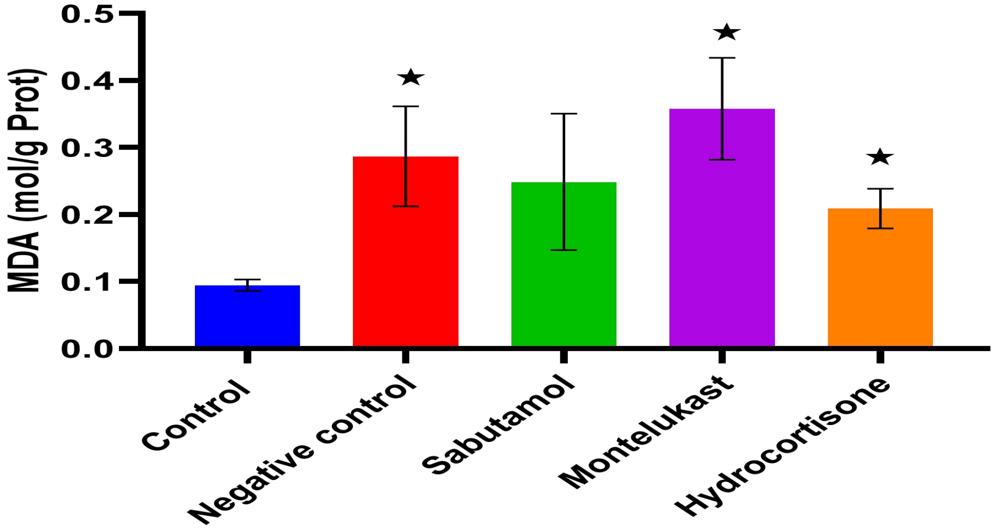

Fig. 4.4: The effects of salbutamol, montelukast and hydrocortisone on MDA in asthma induced Sprague-Dawley rats.

There were significant increases in negative control, Montelukast and hydrocortisone groups compared with control. However, there was no significant change in salbutamol group compared with control.

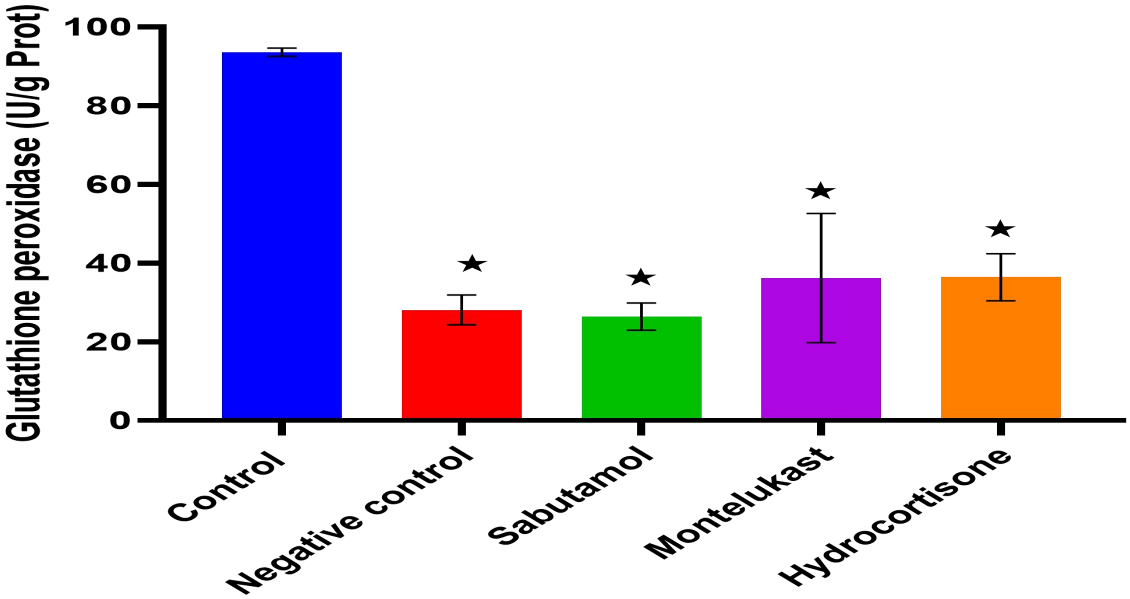

Fig. 4.5: The effects of salbutamol, montelukast and hydrocortisone on Glutathione in asthma induced Sprague-Dawley rats.

There were significant decreases in negative control, salbutamol group, Montelukast and hydrocortisone groups compared with control.

Plate 4.1: Plate showing histology of the lungs

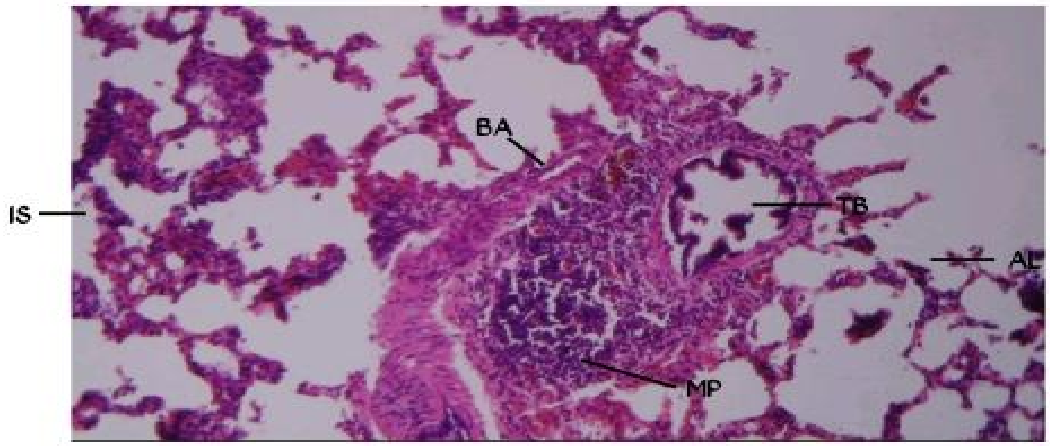

Figure shows rat of Control. Showing tissue architecture (H&E x 100):

AL- alveoli

IS- interstitial space

MP- aggregates of mononuclear phagocyte cells

BA bronchial artery

TB- terminal bronchiole

Plate 4.2: Plate showing histology of the lungs

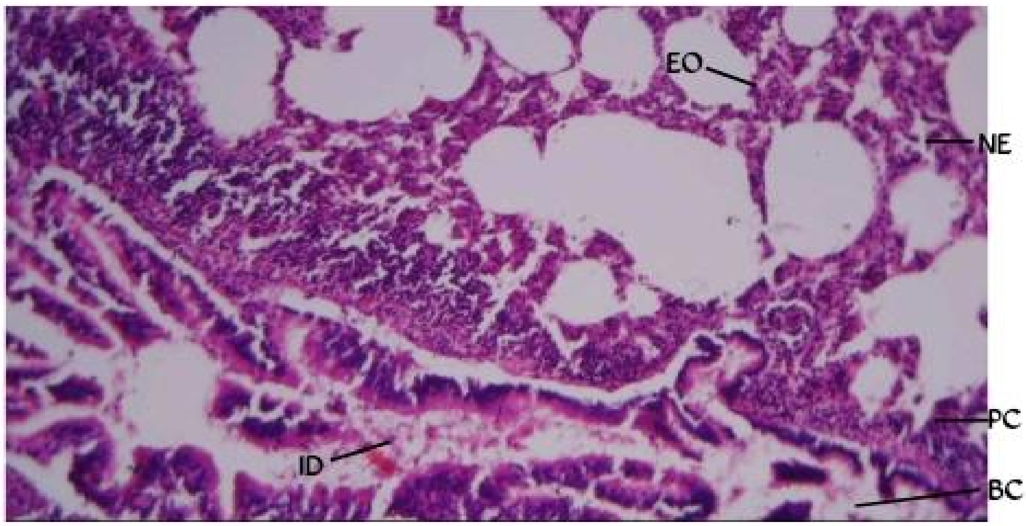

Figure shows rat of induced (Asthmatic). Showing tissue architecture (H&E x 100):

BC- bronchiolar constriction

ID - interluminal debris

NE- interstitial infiltrates of neutrophils

PC- plasma cells

EO- eosinophils

Plate 4.3: Plate showing histology of the lungs

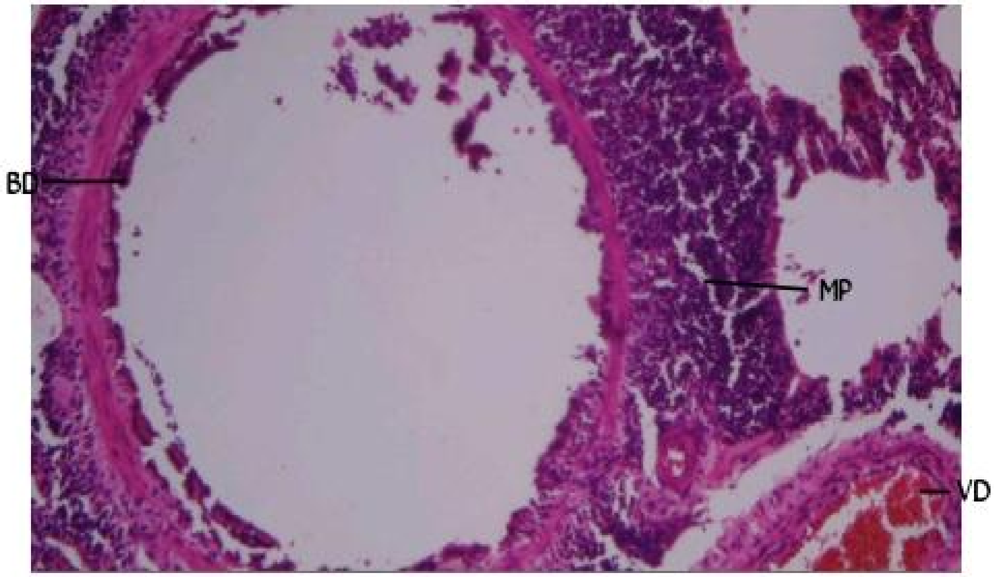

Figure shows Asthmatic rat lungs given Salbutamol showing (H&E x 100):

BD- bronchiolar dilation

VD- vasodilatation

MP- activated cells of the mononuclear phagocyte system

Plate 4.4: Plate showing histology of the lungs

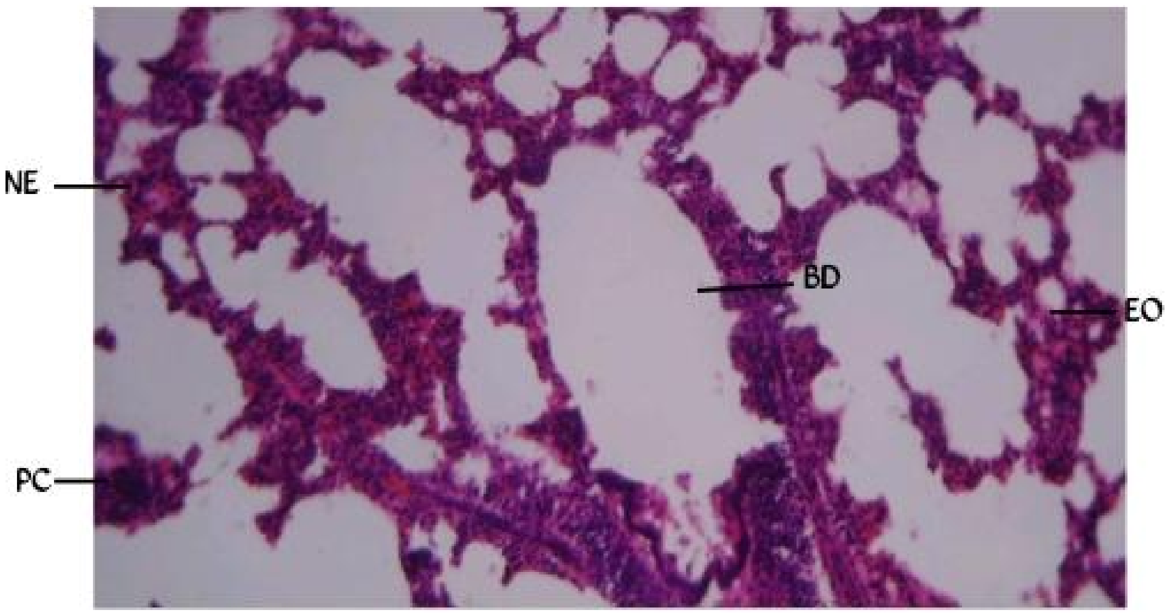

Figure shows Asthmatic rats given Hydrocortisone showing (H&E x 100):

BD- bronchiolar dilation

NE interstitial infiltrates of neutrophils

EO- eosinophils

PC- plasma cells

Plate 4.5: Plate showing histology of the lungs

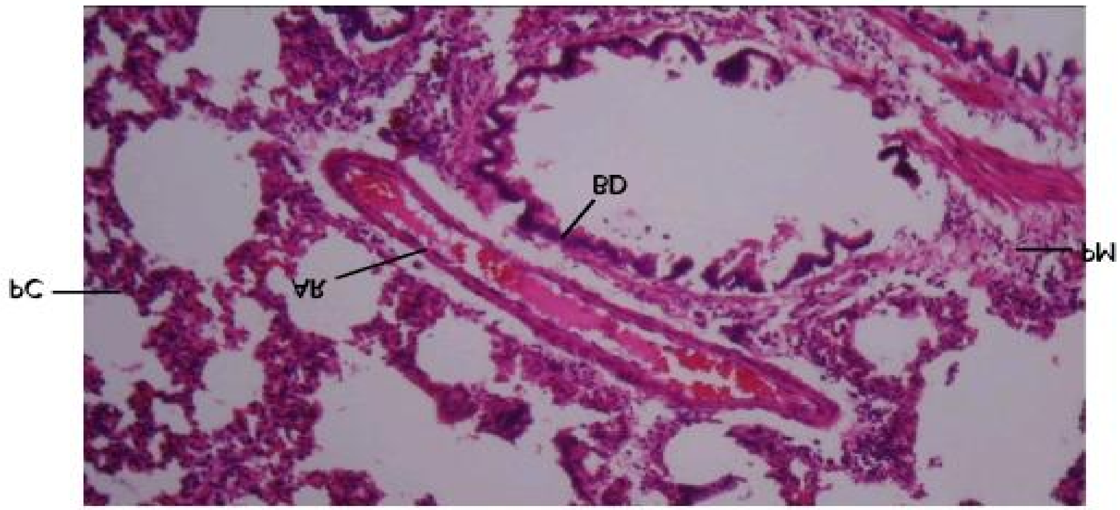

Figure shows Asthmatic rats given Montelukast showing (H&E x 100):

BD- bronchiolar dilation

PM- interstitial infiltrates of polymorphs

PC- plasma cells

AR- normal artery

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

5.1.1. Statistical analysis

Fig. 4.1. Shows the effect of salbutamol, montelukast and hydrocortisone on superoxide dismutase (SOD) in asthma induced Sprague Dawley rats. This result shows that there was a significant increase in SOD levels in negative control and all test groups compared to control group. Increase in SOD is important in neutralizing superoxide radicals which are involved in inflammation and tissue damage. In accordance with this result, a study carried out by Majori *et al.* (1998) showed that corticosteroid-treated asthma patients experienced a drastic fall in superoxide anion which indicates an increase in SOD.

Figure 4.2. Shows the effect of salbutamol, montelukast and hydrocortisone on catalase in asthma. This result shows a significant decrease in catalase in the negative control group compared to control group. In the test groups, no significant variance was recorded. According to a study done by Ghosh *et al.* (2006), catalase activity is reduced by up to 50 percent (%) in asthmatics which contributes to chronic inflammatory state of the airways. Reynaert *et al.* (2007) concluded from research that catalase overexpression failed to attenuate the symptoms of allergic airway responses in mice.

Figure 4.3. The effect of salbutamol, motelukast and hydrocortisone on glutathione peroxide (GPx) levels in asthma induced Sprague Dawley rats. This result shows that

there was a significant increase in GPx in salbutamol group, compared to control group. However, there was no significant variance in negative control, motelukast and hydrocortisone groups. Contrary to this result, a research done by Burgess *et al.* (1994) showed that salbutamol has no effect on glutathione peroxide activity in asthmatic patients.

Figure 4.4. Shows the effect of salbutamol, motelukast and hydrocortisone on Malondialdehyde (MDA) in asthma induced Sprague Dawley rats. This result shows that there was a significant increase in MDA level in negative control, motelukast and hydrocortisone groups, compared to control. However, there was no significant variance in salbutamol group. Increased MDA is an indication of oxidative stress and it suggests severity of the disease. A study carried out by Ozarus *et al.* (2000) measured MDA levels before and after well accepted asthma treatment. It was observed that upon treatment with corticosteroids (hydrocortisone) and Beta 2-agonist (salbutamol), there was a significant increase in MDA compared to control group.

Figure 4.5. Shows the effect of salbutamol, motelukast and hydrocortisone on glutathione in asthma induced Sprague Dawley rats. This result shows a significant decrease in glutathione levels observed in negative control and all test groups as compared with control group. In accordance with this result, Pennings *et al.* (1999) concluded from research that corticosteroids decreased glutathione levels in asthmatic patients.

5.1.2. Histology

The histology of control group shows normal tissue architecture of the lungs in healthy Sprague Dawley rats. Negative control showed altered lung structure with the presence of bronchiolar constriction, interluminal debris, interstitial infiltrates of neutrophils and eosinophils and plasma cells. These abnormalities are a result of the impact of asthma especially left untreated (Bai *et al.,* 2019; Wu *et al.,* 2019; Na *et al.,* 2000). All test groups (salbutamol, motelukast and hydrocortisone) showed bronchiolar dilation after drug administration for four (4) weeks. This is because bronchiolar dilation is a major ameliorating mechanism in asthma attacks. Previous research has proven that the use of leukotriene receptor antagonist, inhaled corticosteroids and adrenergic agonists cause bronchiolar dilation (Bisgaard *et al.,* 2005; Bowman & Raper, 1976; King, 2007). Also, all test groups showed the presence of either eosinophils, plasma cells or cells of mononuclear phagocyte, which is an indicator on inflammation, a common symptom of asthma (Holgate *et al.,* 2015; Hamid, 2012). These defensive cells show an ongoing fight again airway inflammation and tissue injury.

5.2. CONCLUSION

The results of this research show that the administration of salbutamol, motelukast and hydrocortisone are capable of attenuating asthma symptoms such as bronchoconstriction to increase quality of life. The administered drugs also prove to increase antioxidants such as SOD and MDA which help reduce oxidative stress.

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