THE EFFECTS OF MONTELUKAST AND PREDNISOLONE ON THE HISTOLOGY OF THE HEART, LUNGS AND AORTA IN ASTHMA INDUCED SPRAUE-DAWLEY RATS

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ABSTRACT

Asthma is a prevalent respiratory condition that is characterized by chronic airway inflammation and bronchoconstriction, often accompanied by systemic inflammation. Pharmacological interventions such as Montelukast and Prednisolone are commonly used to manage asthma, but their impact in extra-pulmonary tissues still remains less explored. This study is aimed to investigate the effect of Montelukast and Prednisolone on the histology of the heart, lungs and aorta in asthma induced Sprague-dawley rats. To achieve this, a total of 80 Sprague-dawley rats were used for this study, which were divided into two (2) main groups (control and test groups). Group 1 control - not induced with asthma, Group 2 negative control - induced with asthma but not treated. While the test groups were divided into: Group 3 (asthma induced and treated with montelukast) and Group 4 (asthma induced and treated prednisolone), with 20 rats per group. Asthma was induced by sensitizing all experimental groups (2, 3, and 4) with 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 with a Medel family of nebulizer. During the period of challenged, the tested groups were being treated with 10mg/kg of montelukast and 3mg/kg of prednisolone (oral) and at the end of the experiment, the heart, lungs and aorta were harvested and fixed in 10% formaldehyde solution, embedded in paraffin and then subjected to histopathological study. Results revealed that negative control group showed congestion of the interstitium with chronic inflammatory cells in the lungs, and presence of acute inflammatory cells in montelukast and prednisolone lung tissue groups. Moreover, examination of the heart and aorta proved minimal histological alterations in all groups, indicating that Montelukast and Prednisolone treatment did not induce noticeable extra-pulmonary effects. In conclusion, these findings suggest that Montelukast and Prednisolone exert distinct ameliorate and immunomodulatory effect on the lung tissue.

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CHAPTER 1

INTRODUCTION

BACKGROUND OF STUDY

Asthma represents a significant global health issue that impacts up to 235 million individuals on a global scale (WHO, 2017). It is a prevalent, non-communicable, and fluctuating chronic condition that can lead to intermittent or enduring respiratory symptoms (such as breathlessness, wheezing, chest constriction, and cough) as well as restricted airflow. The airflow limitation is primarily caused by bronchoconstriction, thickening of airway walls, and heightened mucus production (Papi *et al.,*2020). At the population level, some individuals with asthma experience a faster decline in lung function throughout their lives at the population level (Lange *et al.,* 1998), which in severe chronic disease, it presents as fixed airflow obstruction, especially in late-onset asthma (Porsbjerg *et al.,* 2015). Asthma's development and severity are influenced significantly by both genetic and environmental factors. While many asthma cases start in childhood due to sensitivity to common environmental allergens mediated by IgE, asthma can also emerge later in life (Custovic, 2015). The disease frequently co-occurs with other medical conditions, encompassing multi-organ allergies such as allergic rhinitis, conjunctivitis, atopic dermatitis, and food allergies, as well as non-allergic

disorders like obesity, gastroesophageal reflux, and psychiatric conditions (Ledford *et al.,* 2013). Asthma can experience sudden worsening episodes, referred to as exacerbations, which can be triggered by factors such as viral infections, allergen exposure, air pollutants, and specific medications like aspirin and other NSAIDs (Custovic *et al.,* 2013). Additionally, particular asthma variants can spontaneously improve, leading to symptom-free periods, especially during late childhood and adolescence (Fu *et al.,* 2014). Furthermore, some types of asthma may respond positively to allergen-specific immunotherapy by developing immunological tolerance (Soyka *et al.,* 2014).

Histamine has long been recognized as a prominent chemical mediator released from mast cells during immediate allergic reactions and has been attributed a significant role in asthma pathophysiology (white, 1990). In response to inhaled allergens and direct bronchoscope contact, histamine is released onto the airway's surface and can be detected in Broncho-alveolar lavage fluid (BALF). In the same conditions, additional chemical mediators are also discharged from mast cells, including 9α, 11β-prostaglandin F2-α, and tryptase, and are likewise recoverable in BALF. Notably, research has shown that the concentration of histamine in the BALF of asthma patients is notably higher than in individuals with allergic rhinitis (Casale, 1987). Additionally, Tomioka *et al*. have reported a greater presence of

mast cells in the BALF of asthmatic patients compared to control subjects (Tomioka *et al.,* 1984).

Traditionally, asthma classification in both adults and children has been based on either the severity of symptoms or the level of disease control achieved through a stepwise management approach. Under this system, patients are placed into one of four or five categories, guiding the prescription of appropriate controller medications. These medications encompass inhaled corticosteroids (ICSs), long-acting β2-adrenergic receptor agonists (LABAs), long-acting muscarinic antagonists, leukotriene receptor antagonists (LTRAs), and, in the case of the most severe cases, the IgE-specific monoclonal antibody omalizumab (GINA, 2015).

Montelukast, an FDA-approved medication, is available in multiple oral formulations, including film-coated tablets, chewable tablets, and oral granules. This versatile drug is vital for managing chronic asthma, prophylactic treatment, and the prevention of exercise-induced bronchoconstriction. Its various administration options and proven efficacy make it a promising candidate for inclusion in respiratory disease management projects (Wermuth *et al.,* 2023). It is also a highly selective antagonist of leukotriene receptors, binds strongly to the cysteinyl leukotriene receptor for leukotrienes D4 and E4. These specific leukotrienes, which originate from various cells like mast cells, are implicated in the inflammatory processes responsible for the symptoms of asthma and allergic

rhinitis.When montelukast binds to these leukotriene receptors, it effectively blocks the physiological effects of leukotrienes, including airway edema, smooth muscle contraction, and disruption of normal cellular functions. Importantly, montelukast accomplishes this without exhibiting any agonist activity. In individuals with asthma, even at low doses such as 5 mg, montelukast significantly reduces bronchoconstriction caused by leukotriene D4. This mechanism is pivotal in the management of asthma symptoms (Wermuth *et al*., 2023). Prednisone is an FDA-approved corticosteroid with delayed-release properties. It is prescribed as an anti-inflammatory or immunosuppressive treatment. Its mechanism involves reducing inflammation by inhibiting the movement of polymorphonuclear leukocytes and reversing heightened capillary permeability. Additionally, it modulates the immune system by diminishing both its activity and volume (Puckett *et al.,* 2023).

1.1 **Justification of the Study**

Numerous prior studies have explored the impact of Montelukast and Prednisolone on asthma management. However, there remains a significant gap in our

understanding regarding their specific effects on the histology of vital organs, such as the aorta and heart, when subjected to treatment with Montelukast and Prednisolone. This research project is justified by the imperative to uncover and elucidate the histological outcomes resulting from the administration of these two therapeutic agents, as it holds substantial importance in advancing our knowledge of their potential implications for asthma care and cardiovascular health within the field of physiology.

1.2 **Aim of the Study**

The aim of the study is to investigate the effect of Montelukast and Prednisolone on the histology of the Heart, Aorta and Lungs in asthmatic induced Sprague-dawley rats.

1.3 **Research Question**

- Does Montelukast influence the histology of the lungs, aorta and heart of the female Sprague-dawley rat?
- Does Prednisolone influence the histology of the lungs, aorta and heart of the female Sprague-dawley rat?

1.4 Specific Objectives

The objective of this study is to investigate the effects of:

- Montelukast on the histology on the lungs, heart and aorta.
- Prednisolone on the histology on the lungs, heart and aorta.

Chapter 2

Literature Review

2.1 **Asthma**

Asthma can be described as a persistent or chronic inflammatory condition affecting the air passages. This chronic inflammation is linked to heightened sensitivity of the airways, resulting in an excessive narrowing response when exposed to specific triggers like viruses, allergens, or physical exertion bronchodilators (Global initiative for asthma, 2017). This, in turn, leads to recurring instances of wheezing, shortness of breath, chest tightness, and/or coughing, which can fluctuate in frequency and severity over time. These episodes of symptoms typically coincide with varying levels of airflow obstruction throughout the lungs, but this obstruction is often reversible either on its own or through the use of appropriate asthma management, including rapid-acting bronchodilators (Global initiative for asthma, 2017). Furthermore, it holds the distinction of being the most prevalent chronic condition among children. While asthma is commonly viewed as a condition primarily affecting the lungs, contemporary research suggests that it might be a constituent of a broader airway ailment encompassing the entire respiratory system. This assertion is substantiated by the frequent occurrence of asthma alongside other allergic conditions, notably allergic rhinitis (Akinbami *et al.,* 2009). Asthma is a prevalent condition with

varying degrees of severity, spanning from infrequent mild wheezing to severe, potentially life-threatening airway constriction. Typically, it manifests during childhood and is often accompanied by other allergic tendencies, including conditions like eczema and hay fever (Lee *et al.,* 2018). Asthma is a very common childhood illness leading to multiple hospital admissions and increased healthcare costs. The key feature is airway hyper-responsiveness, which can be triggered by many factors. If not treated promptly, asthma has a high mortality (Scirica and Celedon, 2007).

2.1.1 **Classification of Asthma**

Asthma represents a diverse respiratory condition impacting over 300 million individuals worldwide (Brusselle and Koppelman, 2022). It is characterized by fluctuating airway obstruction and increased airway reactivity, resulting in sporadic and reversible bronchoconstriction. This condition is marked by an exaggerated response to various environmental triggers, including allergens. Traditionally, asthma is categorized into two primary groups: allergic asthma, also referred to as extrinsic asthma, primarily induced by allergens and associated with abnormal T helper type 2 (Th2) inflammation; and intrinsic asthma, which is precipitated by a range of factors such as aspirin, pulmonary infections, physical exertion, cold temperatures, stress, and obesity, among others (Brusselle and Koppelman, 2022). In recent times, asthma classification has evolved to consider

the status of Th2 inflammation, resulting in a division into two categories: Th2-high and Th2-low asthma. Th2-high asthma is distinguished by eosinophilic inflammation in the airways, which is linked to elevated blood eosinophil levels or increased fractional exhaled nitric oxide (FeNO) levels (Fahy, 2015).

2.1.2 **Pathologic Mechanism of Asthma**

While asthma is traditionally categorized into Th2-high and Th2-low asthma, it's important to recognize that the disease can also result from mixed airway inflammation (Habib *et al.,* 2020). Patients may initially exhibit Th2-high asthma and transition to Th2-low asthma at a later stage, or the reverse may occur. Additionally, some individuals can concurrently experience both Th2-high and Th2-low asthma (Habib *et al.,* 2020).

Mechanisms of Asthma

Th2 cells represent a unique subset of CD4+ effector T cells responsible for the secretion of interleukin (IL)-4, IL-5, IL-13, and IL-9. Notably, around 50% of individuals with mild-to-moderate asthma and a substantial proportion of those with severe asthma experience inflammation that is driven by Th2-dependent mechanisms (Brusselle and Koppelman, 2022).

Th2 inflammation comprises two primary phases:

1. **Sensitization (Phase 1):** When allergens enter the lower airways, antigen-presenting cells process and present these allergens to Th2 cells, which subsequently secrete Th2 cytokines, including IL-5, IL-4, and IL-13. IL-4 and IL-13, in turn, activate B cells, prompting them to produce IgE antibodies that bind to the FcεRI receptors on mast cells (Brusselle and Koppelman, 2022).

2. **Challenge (Phase 2):** Upon re-exposure to the same allergens, these allergens bind to IgE antibodies, triggering mast cells to release various mediators like leukotrienes (LTs), histamine, and ILs. Additionally, allergens interact with cholinergic nerves, leading to the release of acetylcholine. These mediators and neurotransmitters collectively irritate airway smooth muscles, resulting in bronchoconstriction (Fahy, 2015). Furthermore, IL-5 plays a crucial role in eosinophil production, maturation, and their recruitment to the lungs (Pelaia *et al.,* 2019). Eosinophils also release mediators, including major basic protein (MBP), which stimulates mast cells to release histamines and LTs. MBP additionally inhibits M2 receptors while promoting acetylcholine release from cholinergic nerves, inducing bronchospasm (Drake *et al.,* 2021). Furthermore, IL-13 directly enhances airway smooth muscle contraction, stimulates epithelial cells to secrete mucins, and induces fibrosis.

2.2 **HISTOLOGY OF THE HEART**

The heart, comprising four chambers, plays a crucial role in circulating blood throughout the body. It receives deoxygenated blood from the body, directs it to the lungs, receives oxygenated blood from the lungs, and subsequently distributes this oxygen-rich blood throughout the entire body (Arackal and Alsayouri, 2019). The heart's fundamental structure is composed of three main components: the fibrous skeleton, cardiac muscle, and the impulse conduction system. Located at the heart's base is the dense fibrous or cardiac skeleton, which serves several vital functions. These functions encompass providing a robust framework for cardiomyocytes, securing the valvular leaflets in place, and serving as electrical insulation, effectively separating the conduction processes between the atria and ventricles (Saremi *et al.,* 2017). The heart's wall comprises three distinct layers: the epicardium, myocardium, and endocardium. Interestingly, these three heart layers correspond, in terms of embryological origin, to the three layers found in blood vessels: the tunica adventitia, tunica media, and tunica intima, respectively (Rodriguez and Tan, 2017). Enveloping the heart is a double-layered, fluid-filled sac referred to as the pericardium. This pericardium consists of two layers: the outer fibrous or parietal pericardium and the inner serous or visceral pericardium. Notably, the epicardium forms the visceral pericardium, which rests atop fibro-elastic connective tissue and adipose tissue (Arackal and Alsayouri, 2019). Below the epicardium, one can find coronary arteries, veins, lymphatic vessels, and nerves.

The endocardium, on the other hand, consists of the endothelium and the underlying subendothelial connective tissue layer. Sandwiched between the endocardium and myocardium is the subendocardium, housing the impulse-conducting system (Rodriguez and Tan, 2017).

2.3 **HISTOLOGY OF THE LUNGS**

The lungs, found in the thoracic cavity alongside the mediastinum, serve as the primary organs for respiration. They are enveloped by a delicate, double-layered serous membrane known as the pleura. The respiratory system can be divided into two main components: the conducting portion and the respiratory portion. The conducting portion is responsible for transporting air from the external environment to the site of respiration. In contrast, the respiratory portion plays a crucial role in facilitating the exchange of gases and oxygenating the blood (Khan and Lynch, 2023).

The conduction segment of the lung spans from the trachea to the terminal bronchioles and includes components outside the lungs such as the nasal cavities, nasopharynx, larynx, and trachea. Within the lungs, this conducting system begins with the paired main bronchi, branching out further into lobar (secondary) bronchial branches and subsequently into segmental (tertiary) bronchi. These tertiary bronchi further divide into smaller bronchioles, marking a

histological transition where cartilage is no longer present. The conduction portion concludes at the terminal bronchioles, which serve as the entry point to the respiratory bronchioles (Murray, 2010). The conducting segment of the respiratory system serves as a route for guiding and preparing the incoming air. Specialized cells work together to accomplish tasks like warming, humidifying, and filtering out particles from the air. These tasks are carried out by the respiratory epithelium, which spans the entire respiratory tree. The majority of the respiratory epithelium consists of ciliated pseudostratified columnar epithelium and comprises five distinct cell types:

1. Ciliated cells

2. Goblet cells

3. Basal cells

4. Brush cells

5. Neuroendocrine cells (Khan and Lynch, 2023).

Ciliated cells, the most abundant among these cell types, play a crucial role in orchestrating the actions of the mucociliary escalator (Ganesan *et al.,* 2013), a primary defense mechanism of the lungs responsible for debris removal. While goblet cells produce mucus that entraps inhaled particles, cilia actively beat to

propel this material toward the pharynx, where it can be either swallowed or expelled through coughing. Goblet cells, named for their goblet-like shape, contain mucin granules at their apical surface, with the nucleus situated closer to the basal layer (Khan and Lynch, 2023). As the respiratory tree narrows, the number of goblet cells diminishes and is eventually replaced by club cells (formerly known as Clara cells) once the respiratory bronchioles are reached. Basal cells serve as the attachment layer for ciliated and goblet cells, connecting them to the basement membrane. Functionally, they can be likened to the stem cells of the respiratory epithelium, as they retain the capacity to give rise to both ciliated and goblet cells (Evans *et al.,* 2001). Brush cells, sometimes referred to as type III pneumocyte cells, are sparsely distributed throughout the respiratory mucosa. These cells may appear columnar or flask-like and are characterized by their short microvilli-covered apical layer, resembling a brush. Although their precise function remains undetermined, it is hypothesized that they may serve as chemoreceptors, monitoring air quality due to their association with unmyelinated nerve endings (Brody, 2005). Within the bronchial mucosa, a small cluster of neuroendocrine cells, also known as Kulchitsky cells (Drozdov *et al.,* 2009), can be found. These cells contain neurosecretory-type granules and have the capacity to secrete various substances, including catecholamines and polypeptide hormones like serotonin, calcitonin, and gastrin-releasing factors (bombesin). However, they constitute only

a small fraction, approximately 3%, of the mucosal epithelium. The bronchial submucosa contains submucosal glands, composed of a mixture of serous and mucinous cells, resembling salivary gland tissue. These glands secrete substances that are emptied into ducts and eventually reach the bronchial mucosa (Drozdov *et al*., 2009).

Alveoli: Alveoli initially appear as scattered outpockets within the respiratory bronchioles, extending from their inner surfaces. These respiratory bronchioles extend for significant distances and progressively acquire more alveoli as they branch into alveolar ducts, which in turn become densely lined with alveoli. Typically, there are between two and eleven ducts branching off from each bronchiole. These ducts ultimately lead to five or six alveolar sacs, where clusters of alveoli are connected (Spencer, 1996).

2.4 **HISTOLOGY OF THE AORTA**

The aorta is the largest vessel within the human body. It originates from the left ventricle of the heart anterior to the pulmonary artery before arching posteriorly and descending along the posterior mediastinum (Shahoud *et al*., 2023). The aorta, an elastic artery, is highly flexible. It comprises various components like smooth muscle, nerves, cells, and an intricate extracellular matrix. The vascular wall

divides into three layers: tunica externa, tunica media, and tunica intima. The aorta is nourished by tiny blood vessels known as vasa vasora, supplying the outer layers (Ritman and Lerman, 2007). In the tunica media, smooth muscle and the extracellular matrix make up the largest portions, and they are organized concentrically as musculoelastic layers known as elastic lamella in mammals. The elastic lamella, composed of both smooth muscle and elastic matrix, can be regarded as the primary structural unit of the aorta. It consists of elastic fibers, primarily type III collagens, proteoglycans, and glycoaminoglycans (*Tsamis et al*., 2013).

2.6 **EFFECTS OF ASTHMA ON THE HEAR**T

Several studies have reported a heightened risk of cardiovascular mortality in severe asthmatic patients (Toren and Lindholm, 1996; Musk *et al*., 1987). Occupational asthma has also been linked to an increased risk of coronary heart disease (CHD). Notably, prior research has identified associations between asthma and cardiovascular outcomes. For instance, in a study involving Swedish severe asthma patients receiving daily oral steroid treatment for over a year, there was an elevated mortality rate due to ischaemic heart disease (Toren and Lindholm, 1996). In Western Australia, a cohort of hospitalized patients diagnosed with asthma

experienced a significant rise in deaths attributed to ischaemic heart disease (Musk *et al*., 1987). Furthermore, a study involving Canadian workers with occupational asthma found an increased risk of hospital admission for CHD (Liss *et al*., 2000). Lastly, a cohort study of 2242 subjects aged 16–64 years admitted for asthma revealed that failure to prescribe inhaled steroids upon discharge was associated with subsequent deaths from asthma.

2.7 **EFFECT OF ASTHMA ON THE AORTA**

In a comprehensive research, it was observed that individuals diagnosed with mild asthma exhibited elevated levels of arterial inflammation when compared to a group without asthma (Vijayakumar *et al*., 2013).

2.7 **EFFCT OF ASTHMA ON THE LUNGS**

Asthma is a heterogeneous disorder of the conducting airways involving chronic airway inflammation, airway function and tissue remodeling (Murdoch and Lloyd, 2010). Clinically presents as a physiological dysfunction of the lungs characterized by breathlessness, wheeze and a variable airflow obstruction (Bousquet *et al.,* 2000).

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

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. 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 22^0C room temperature, they were kept in cages, where they had access to food and water *ad libitum* throughout the period of the experimental process.

3.2 **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 were further divided into three (3) subgroups 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 prednisolone.

3.3 **Experimental protocol**

Experiment were carried out in phases

Phase 1

Rats were acclimatized into their new environment for two (2) weeks after which they wers 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 prednisolone

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 with 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 10 mg/kg montelukast and 3 mg/kg prednisolone (oral) (Pourmehdi *et al*., 2020).

Phase 3

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

3.4 **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).

3.5**Statistical analysis**

All the data obtained from the experiments were 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). $P < 0.05$ will be considered statistically significant.

CHAPTER 4
RESULTS

Plate 4.1 Shownig the histology of the heart

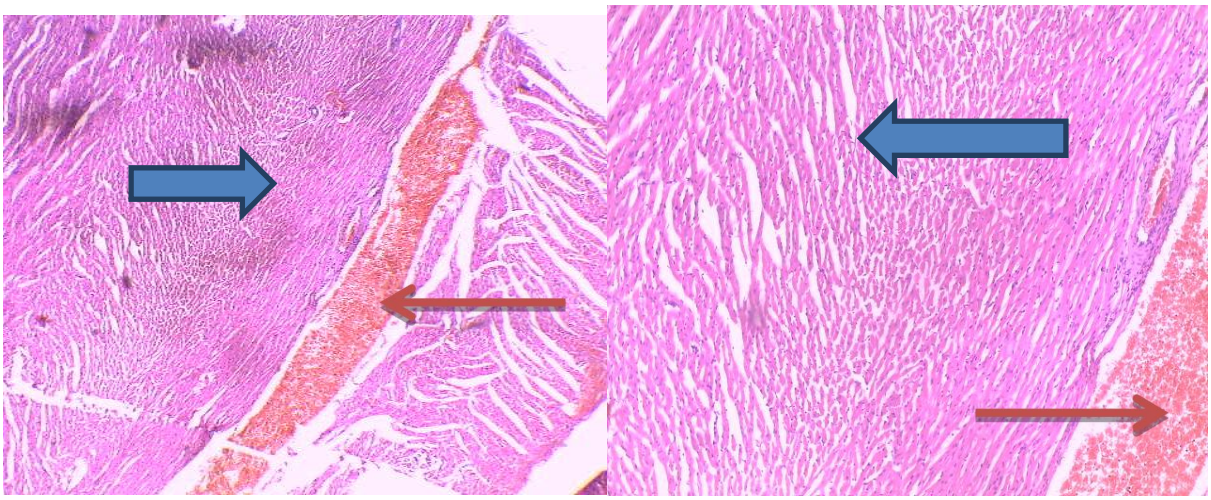

Plate 1. Control heart

Heart: sections of heart showing normal heart muscles (blue arrow) and congested blood vessel (red arrow)

Plate 4.2 Showing the histology of the heart

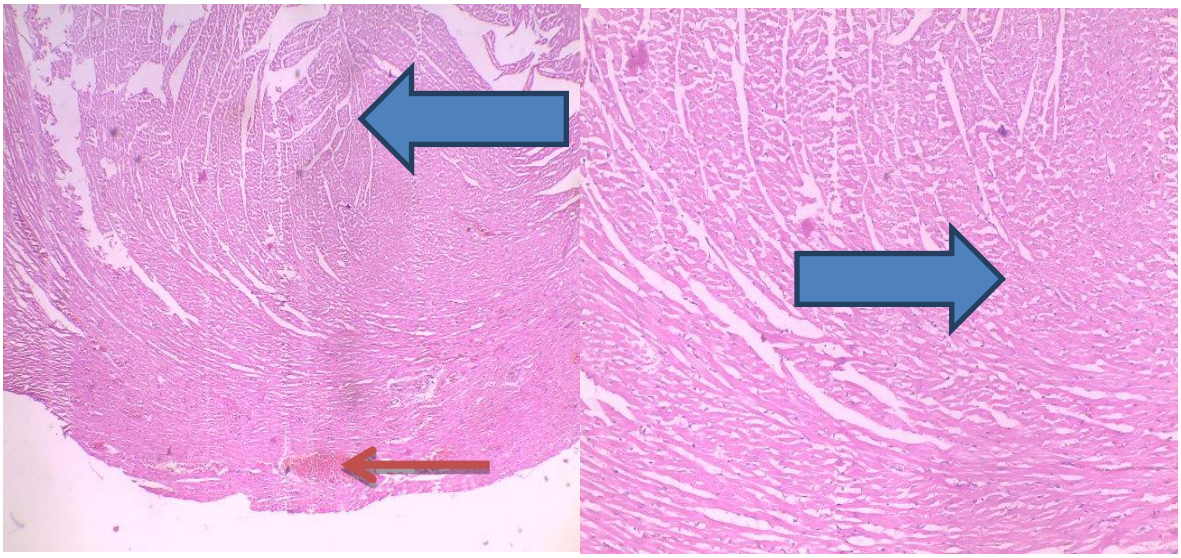

Plate 2. Negative control heart

Heart: sections of heart showing normal heart muscles (blue arrow) and blood vessel (red arrow)

Plate 4.3 Showing the histology of the heart

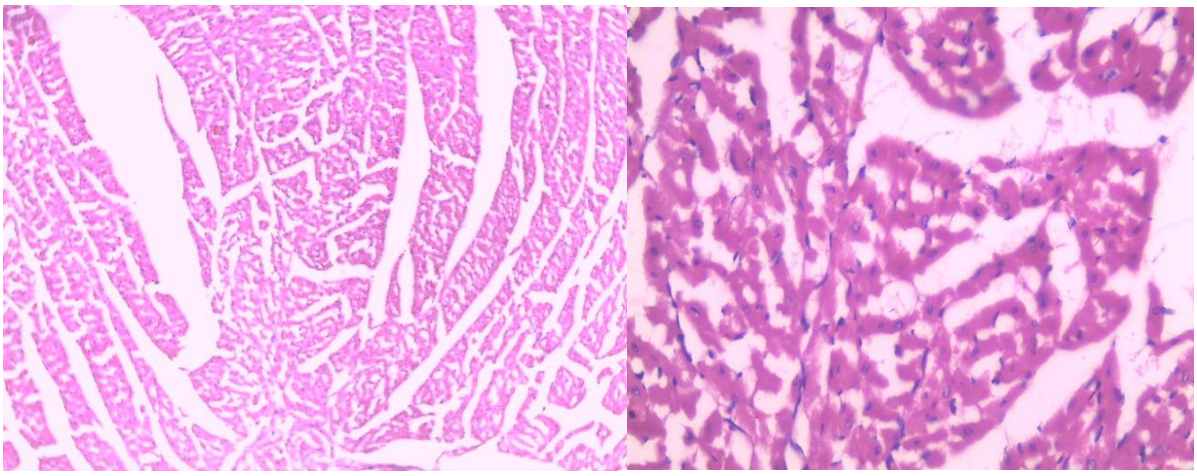

Plate 3. Montelukast heart

Heart: normal heart muscle

Plate 4.4 Showing the histology of the heart

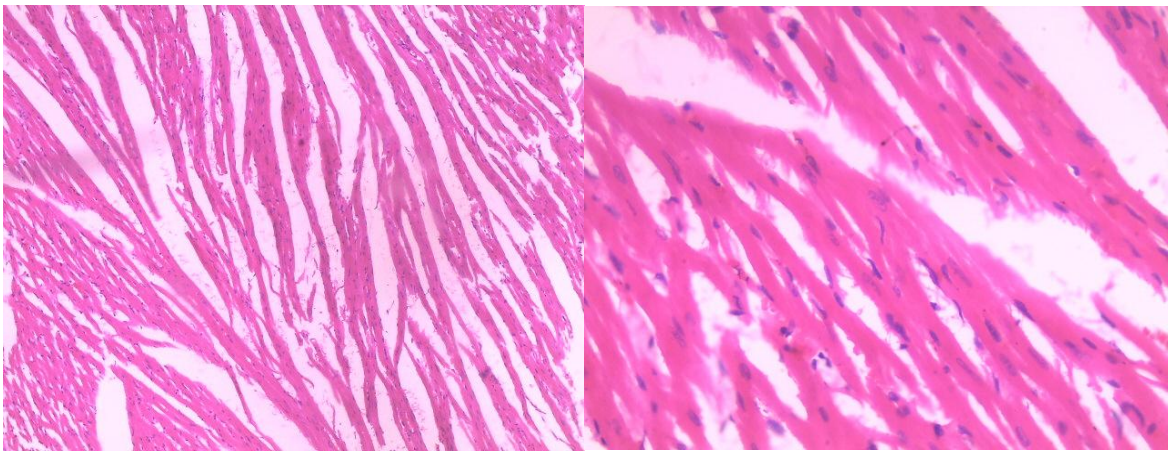

Plate 4. prednisolone heart

Heart: normal heart muscle

Plate 4.5 Showing the histology of the Aorta

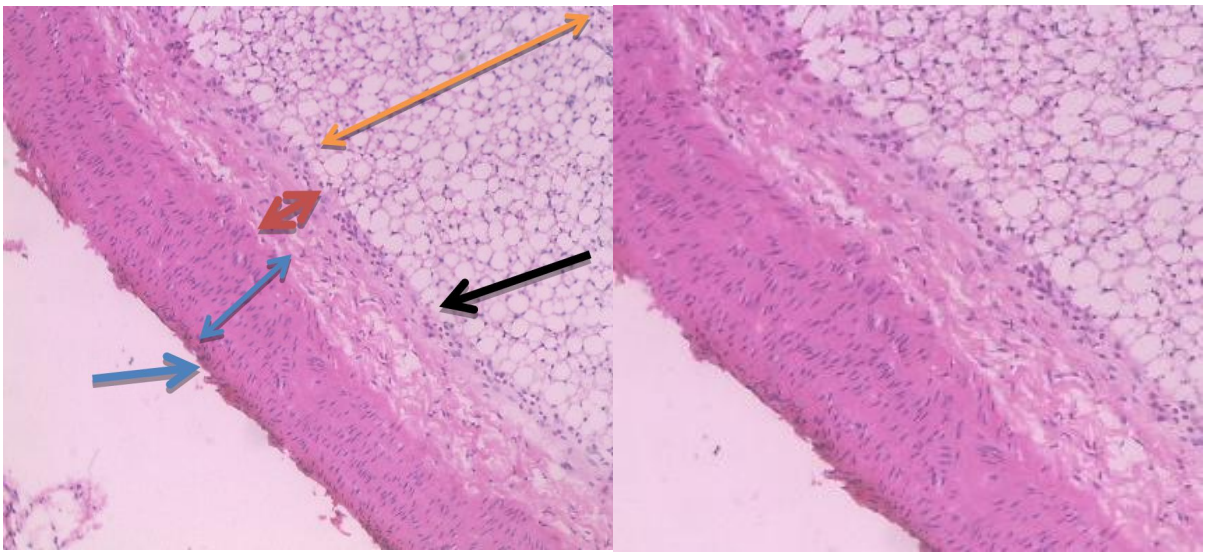

Plate 5. control aorta

Aorta: normal aortic valve consisting of tunica intima (blue arrow) tunica media containing smooth muscle cells (blue double arrow), tunica adventitia (red double arrow), interstitial cells (blackarrow), fat (orange double arrow).

Plate 4.6 Showing the histology of the Aorta

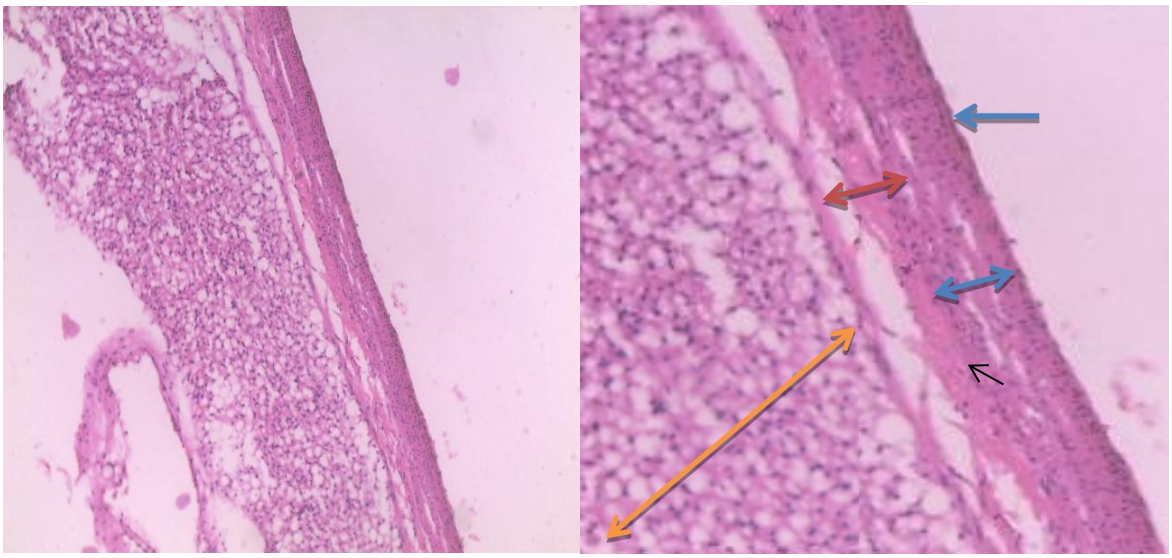

Plate 6. negative control aorta

Aorta: normal aortic valve consisting of tunica intima (blue arrow) tunica media containing smooth muscle cells (blue double arrow), tunica adventitia (red double arrow), interstitial cells (blackarrow), fat (orange double arrow).

aorta: normal aortic valve

Plate 4.7 Showing the histology of the Aorta

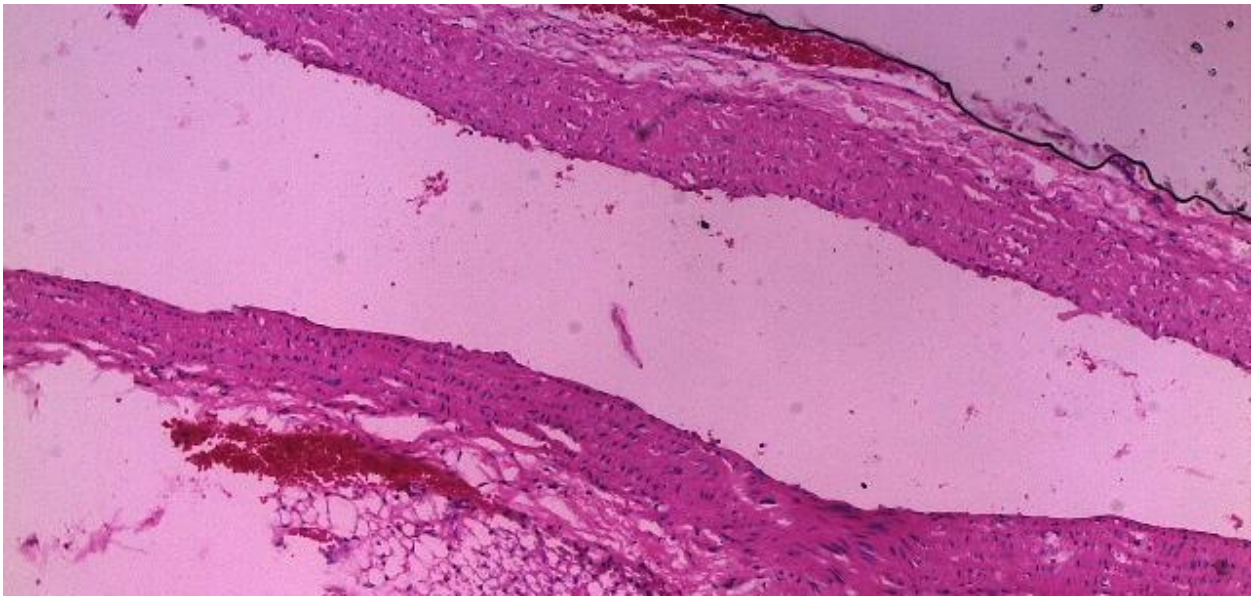

Plate 7. Montelukast aorta

Aorta: normal aorta

Plate 4.8 Showing the histology of the Aorta

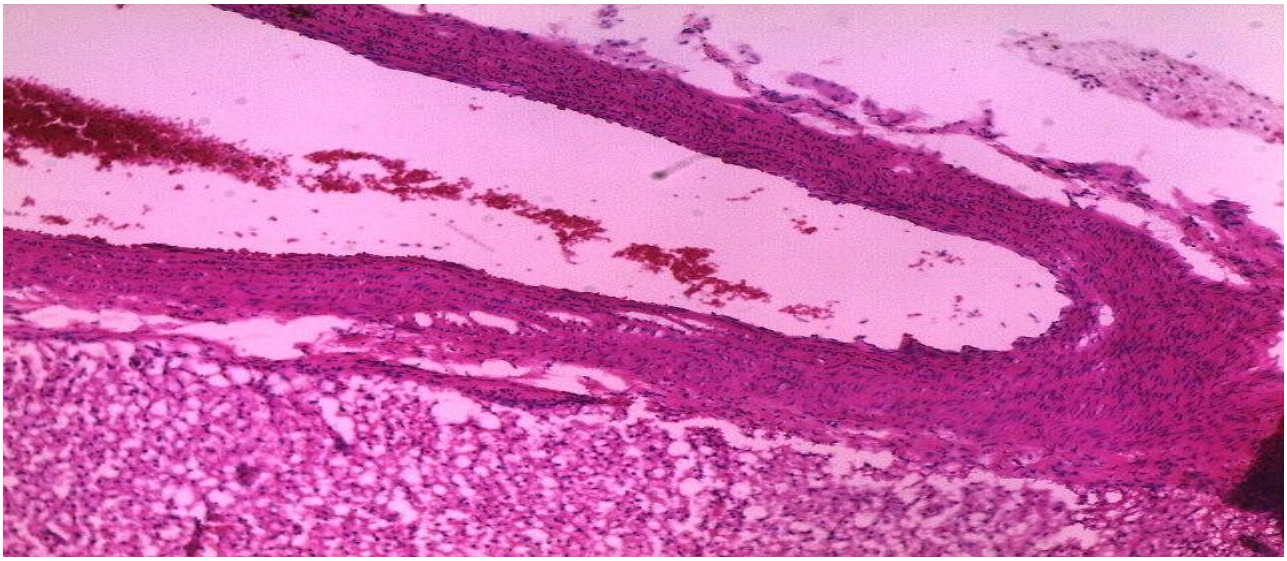

Plate 8. prednisolone aorta

Aorta: Normal aorta

Plate 4.9 Showing the histology of the Lungs

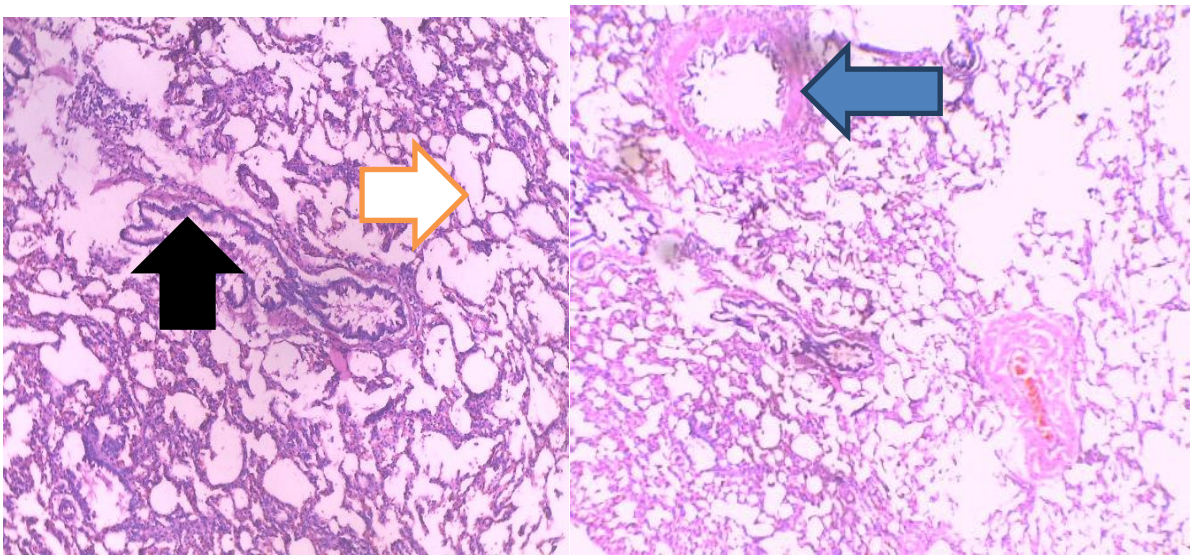

Plate 9. Control lungs

Lungs: sections of the lungs showing normal aveolar sac (white arrow), bronchiole (blue arrow), aveoli (black arrow)

Plate 4.10 Showing the histology of the Lungs

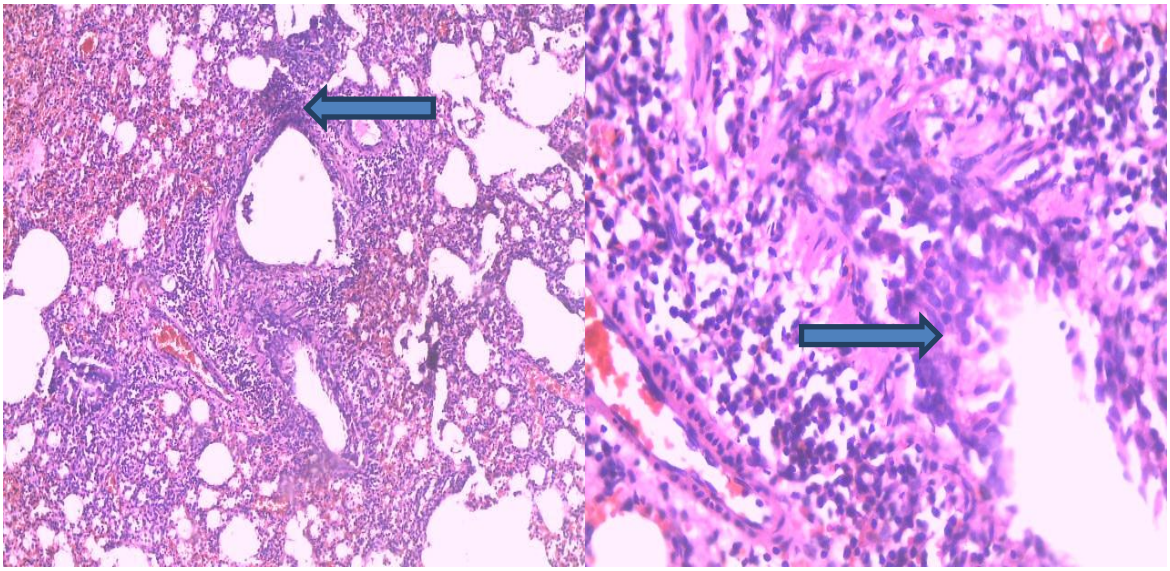

Plate 10. Negative control lungs

Lungs: congestion of the interstitium with chronic inflammatory cells (chronic intestitial pneumonitis)

Plate 4.11 Showing the histology of the Lungs

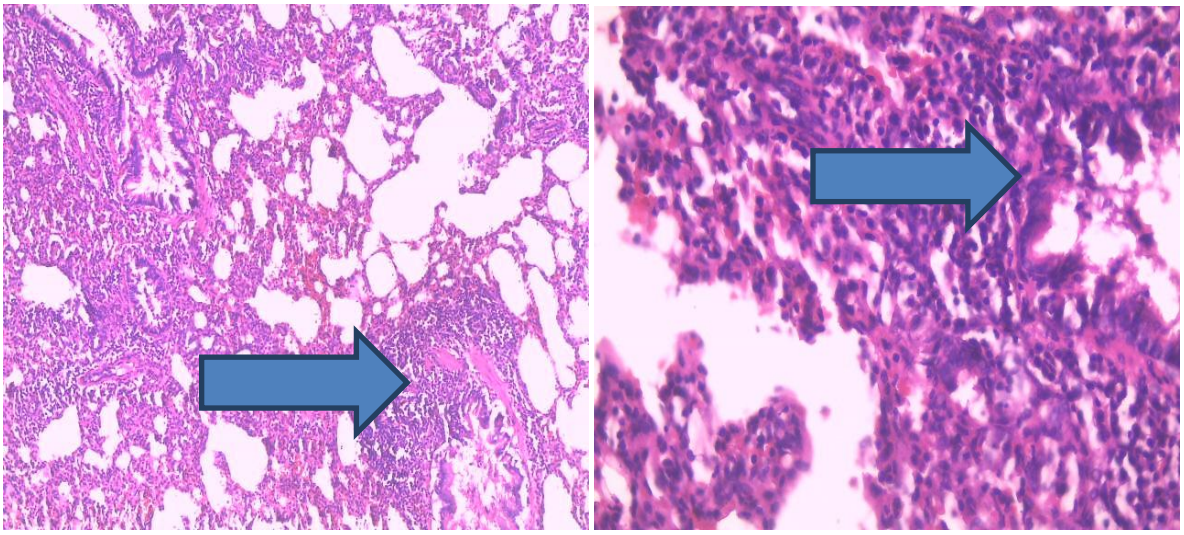

Plate 11. Montelukast lungs

Lungs: shows heavy presence of acute inflammatory cells in the interstitium around the bronchiole (interstitial pneumonitis)

Plate 4.12 Showing the histology of the Lungs

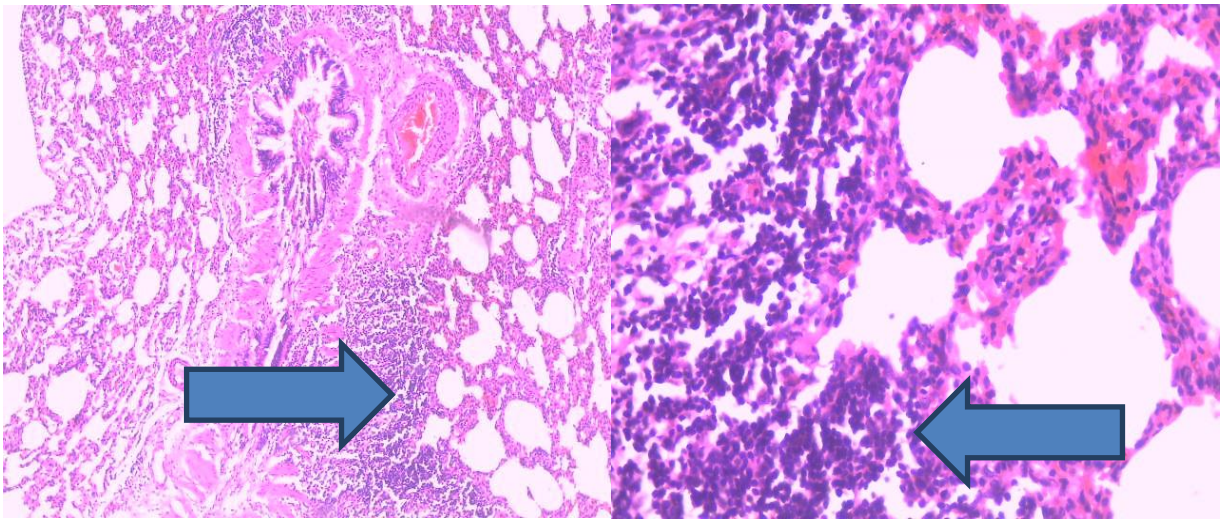

Plate 12. Prednisolone lungs

Lungs: shows acute inflammatory cells in the interstitium (interstitial pneumonia)

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 **DISCUSSION**

5.1.1 **HEART**

In this study, histological analysis revealed vascular congestion within the cardiac tissue of the control group. Congestion is defined as the accumulation of fluid in the intravascular compartment and the interstitial space. This could have resulted from increased cardiac filling pressures caused by maladaptive sodium and water retention by the kidney (Martens *et al*., 2015).

While no discernible histological alterations were observed in any other cardiac group under investigation. This may be as a result of short administration time in the inducement of asthma using ovalbumin.

5.1.2 **AORTA**

In the aortic study, throughout all the groups, there were no histological alterations. This may have been due to duration of asthma inducement, individual variability of animal model.

5.1.3 **LUNGS**

In this study, chronic inflammatory cells were found in the lungs of negative control. Recent studies have demonstrated a variety of cellular inflammatory phenotype associated with asthma. An eosinophilic or neutrophilic infiltrate is a

common feature of allergic airway inflammation and this has been clinically correlated with Airway Hyper-responsiveness (Murdoch and Lloyd, 2010).

In the rats treated with montelukast and prednisolone, there were reductions from chronic inflammatory cells to acute inflammatory cells. Previous studies have shown how montelukast, a selective cysteinyl-leukotriene receptor antagonist, and prednisolone, an anti-inflammatory glucocorticoid have been widely used to treat asthma. Their mechanism involves:

1. Prednisolone decreasing inflammation through the suppression of the migration of polymorphonuclear leukocytes and reversing increased capillary permeability (Puckett *et al.,* 2023).
2. Montelukast binding to the cysteinyl-leukotriene receptors inhibiting leukotriene physiological effects without exhibiting any agonist effect (Wermuth *et al.,* 2023).

5.2 **CONCLUSION**

In this study, montelukast and prednisolone have been shown to have an ameliorative effect in the reducing of the inflammatory cells during the period of the challenge with ovalbumin.

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