EFFECTS OF VARYING METHODS OF OVALBUMIN ASTHMA INDUCTION ON RED BLOOD CELL INDICES IN FEMALE WISTAR RATS

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

FEBRUARY, 2025

CERTIFICATION

This is to certify that this project works on the **EFFECTS OF VARYING METHOD OF OVALBUMIN ASTHMA INDUCTION ON RED BLOOD CELL INDICES IN FEMALE WISTAR RATS** by **UWAIFO FAITH UWAILA** with matriculation number **BMS2005130** in partial fulfilment for the Award of Bachelor of Science (B.Sc.) Degree in Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

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DEDICATION

This project is dedicated to **GOD ALMIGHTY** my greatest source of strength. And to my late grandfather, **MR KINGSLEY ODIGIE,** whose love and sacrifice shaped my path. And also to my mentor, **WISDOM GREATNESS, f**or his endless love and support.

ACKNOWLEDGEMENT

I want to express my sincere gratitude to everyone who contributed to the success and completion of this project work. First and foremost, I acknowledge God Almighty for his mercies during his journey. I also want to thank my supervisor, **DR. E.O ALOAMAKA,** for his invaluable guidance, encouragement, and support throughout this project. His expertise and insights have been instrumental in shaping the direction of my work. I am also profoundly grateful to my Family, especially my mom, **MRS DORIS OBAZEE** and my mentor, **WISDOM GREATNESS**, for their support and encouragement. They kept me going through the challenges I encountered. Also, thank you to my roommates for their support during this journey.

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ABSTRACT

Asthma is a chronic inflammatory disorder of the airways, characterized by recurring episodes of wheezing, breathlessness, chest tightness, and coughing. Ovalbumin (OVA)-induced asthma models are widely used to study allergic asthma and its systemic effects, including alterations in red blood cell (RBC) indices. This study investigates the impact of varying methods of OVA administration on RBC indices in female Wistar rats. This study involved 42 female Wistar rats weighing 140-180g who were acclimatized for one week. They were divided into six different groups of 7 rats. During the experimental process, all groups (2-6) were sensitized with 1 mg OVA and 20 mg AlOH dissolved in 0.9 saline on days 1, 7 and 14, then challenged with 1 % OVA biweekly for 28 days. Group 1 control; Group 2 sensitised on days 1 and 7; Group 3 sensitized on days 1, 7 and 14; Group 4 sensitised on day 1 and challenged; Group 5 sensitised on days 1 and 7 and challenged; Group 6 sensitised on day 1, 7 and 14 and challenged. All animal were euthanized at the end of 28 days. Statistical analysis was performed using one-way variance analysis (ANOVA). The result showed no statistically significant difference in red blood cell count, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and red cell distribution in all the groups compared to the control ($p>0.05$); also, there was a significant decrease in haematocrit and mean corpuscular volume in the group sensitised on days 1, 7 and 14 compared to control ($p<0.05$). In conclusion, these ovalbumin-induced asthma methods did not affect red cell indices except on haematocrit and mean corpuscular volume in female Wistar rats.

CHAPTER ONE

1.1 INTRODUCTION TO ASTHMA

BACKGROUND OF STUDY

Asthma is a chronic inflammatory disorder of the airways, characterized by recurring episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or early morning. These episodes are associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment (National Heart, Lung, and Blood Institute (NHLBI, 2007). The hallmark of asthma is inflammation of the airways, which leads to airway hyperresponsiveness and airflow limitation. Inflammatory cells, such as eosinophils, T lymphocytes, and mast cells, infiltrate the airway walls, leading to swelling and increased mucus production (Barnes, 2008). Structural changes, such as sub-basement membrane fibrosis and smooth muscle hypertrophy, may also occur in chronic cases (Holgate, 2011). Asthma triggers can be broadly categorized into allergens, irritants, infections, exercise, and emotional stress. Common allergens include pollen, dust mites, mould, pet dander, and cockroach droppings. Irritants such as tobacco smoke, air pollution, and strong odours can also exacerbate asthma symptoms (American Lung Association, 2021). Asthma is diagnosed based on medical history, physical examination, and pulmonary function tests, such as spirometry. Spirometry measures the amount and speed of air a person can exhale, providing critical information about lung function and airflow obstruction (Global Initiative for Asthma (GINA, 2022). The treatment of asthma involves a stepwise approach tailored to the severity and

frequency of symptoms. This typically includes Inhaled short-acting beta-agonists (SABAs) like albuterol, which are used to relieve acute symptoms by relaxing the muscles around the airways.

Inhaled corticosteroids (ICS) are the most effective long-term control therapy to reduce inflammation. Other options include long-acting beta-agonists (LABAs), leukotriene modifiers, and biologics for severe asthma (GINA, 2022).

To better understand asthma's pathophysiology and develop effective treatments, researchers use various models, including The OVA-induced asthma model, which is widely used to replicate features of allergic asthma in rats. In this model, Wistar rats are sensitzsed to OVA through intraperitoneal injection (with or without an adjuvant like aluminium hydroxide) and later challenged via aerosolized OVA to induce airway inflammation, hyperresponsiveness, and remodelling (Nials *et al*., 2008). This model mimics many human asthma characteristics, including increased eosinophil recruitment, elevated IgE levels, and airway hyperreactivity. House dust mite (HDM) extract is another allergen used to induce asthma in Wistar rats. Repeated intranasal or intratracheal administration of HDM induces airway inflammation and remodeling. This model resembles human asthma because HDM is a clinically relevant allergen that does not require sensitisation adjuvants (Johnson *et al*., 2018). Although less common in Wistar rats, some studies have explored genetic predispositions to asthma-like conditions. Wistar rats can be selectively bred or genetically modified to study specific gene-environment interactions (Foster *et al.*, 2000). Non-allergic asthma can be modelled using LPS, which stimulates innate immune responses without prior sensitisation. Intranasal or intratracheal administration of LPS causes neutrophilic inflammation and airway hyperreactivity in rats, mimicking features of

non-allergic asthma (Pichavant *et al*., 2005). Models that combine allergen exposure (e.g., OVA or HDM) with pollutants such as diesel exhaust particles or cigarette smoke are used to study

asthma exacerbations and environmental triggers. These models are valuable for studying the interplay between allergens and environmental factors in asthma (Saglani *et al.*, 2009).

While asthma primarily affects the respiratory system, systemic inflammation and hypoxia can also impact haematological parameters, including red blood cell (RBC) indices. RBC indices, such as red blood cell count (RBC), haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW), provide crucial insights into the physiological alterations associated with asthma (Teodorescu *et al.*, 2010).

RBC count, hemoglobin concentration, and hematocrit levels reflect the blood's oxygen-carrying capacity. Chronic hypoxia in asthma, particularly in severe or poorly controlled cases, may lead to compensatory increases in these parameters (Ali *et al.,* 2019). However, specific asthma phenotypes associated with inflammation may cause anaemia due to increased oxidative stress and cytokine-driven suppression of erythropoiesis (Lee *et al.*, 2016).

Recent studies suggest that red blood cells (RBCs) also play a role in disease pathology (Sangwung *et al*., 2021). RBCs are primarily responsible for oxygen transport but also have immunomodulatory functions that can influence inflammation and oxidative stress (Nader *et al*., 2020). This review explores the relationship between RBCs and asthma, highlighting their role in oxidative stress, gas exchange, and inflammation. Asthma is associated with impaired gas exchange due to airway obstruction and inflammation (Flenaugh *et al*., 2022). RBCs are crucial in transporting oxygen from the lungs to tissues and removing carbon dioxide. However, hypoxia (low oxygen levels) can lead to RBC dysfunction in asthmatic individuals, including altered haemoglobin affinity for oxygen (Bourgeois *et al.,* 2019). Studies have shown that hypoxia-

induced RBC changes can exacerbate asthma symptoms by reducing oxygen availability and promoting oxidative stress (Dinkla *et al.,* 2016). Oxidative stress is a key factor in asthma pathogenesis, and RBCs contribute significantly to this process (Sangwung *et al*., 2021). In asthmatic individuals, RBCs experience increased oxidative damage due to elevated reactive oxygen species (ROS) levels. This damage leads to RBC membrane alterations, reduced deformability, and increased hemolysis, which can exacerbate airway inflammation (Dinkla *et al.*, 2016). Additionally, oxidised RBCs release free haemoglobin, further promoting oxidative stress and lung inflammatory responses (Nader *et al*., 2020). Recent evidence suggests that RBCs play a role in modulating immune responses in asthma. Damaged or dysfunctional RBCs release pro-inflammatory mediators that activate immune cells such as neutrophils and macrophages (Bourgeois *et al*., 2019). Furthermore, the hemolysis-induced iron release can contribute to airway inflammation and remodelling (Flenaugh *et al*., 2022). These findings suggest that RBC dysfunction may not only be a consequence of asthma but also an active participant in disease progression. Targeting RBC dysfunction in asthma could offer novel therapeutic strategies. Antioxidant therapies aimed at reducing oxidative stress in RBCs have shown promise in preclinical studies (Sangwung *et al*., 2021). Additionally, improving RBC deformability and reducing hemolysis through pharmacological interventions may help alleviate asthma symptoms and improve overall lung function (Nader *et al*., 2020).

1.2. Justification of study

The OVA-induced asthma model in Wistar rats is a valuable tool for understanding asthma's systemic effects, including alterations in red blood cell (RBC) indices. This model closely mimics human allergic asthma, making it relevant for studying disease mechanisms and potential treatments. Chronic inflammation, a hallmark of asthma, impacts erythropoiesis and RBC

characteristics, potentially serving as biomarkers for disease severity and progression. Understanding these haematological changes can also shed light on asthma-associated comorbidities and guide improved methods of inducing asthma in the female Wistar rat.

1.3. AIM OF STUDY

The study aimed to examine the effects of varying methods of ovalbumin asthma induction on red blood cell indices in female Wistar rats.

1.4. RESEARCH QUESTION

What are the effects of varying methods of ovalbumin asthma induction on red blood cell indices in female Wistar rats?

1.5. SPECIFIC OBJECTIVE

The specific objective of this study is to determine:

the effects of varying methods of ovalbumin asthma on red blood cell indices in female Wistar rats.

1.6. ETHICAL APPROVAL

Ethical approval was gotten from the college of medicine ethical board with ref number : CMS/REC/2024/711.

CHAPTER TWO

LITERATURE REVIEW

2.0. ASTHMA

Asthma is a prevalent chronic inflammatory respiratory condition affecting millions worldwide and presents substantial challenges in diagnosis and management. This respiratory condition is characterised by airway inflammation, causing intermittent airflow obstruction and bronchial hyperresponsiveness. The hallmark asthma symptoms include coughing, wheezing, and shortness of breath, frequently exacerbated by triggers ranging from allergens to viral infections. A complex interplay between genetic and environmental factors determines the prevalence and severity of asthma. Despite treatment advancements, disparities persist in asthma care, with variations in access to diagnosis, treatment, and patient education across different demographics (Lee *et al*., 2018)

The development of asthma, often presenting in childhood, is associated with other atopic features, such as eczema and hay fever. Severity varies from intermittent symptoms to life-threatening airway closure. Healthcare professionals establish a definitive diagnosis through patient history, physical examination, pulmonary function testing, and appropriate laboratory testing. Spirometry with a post-bronchodilator response (BDR) is the primary diagnostic test. Treatment focuses on providing continued education, routine symptom assessment, access to fast-acting bronchodilators, and appropriate controller medications tailored to disease severity (Tesfaye *et al.*, 2018)

2.1. Animal models of asthma

During the last decades, several studies have been performed using animal models to understand better the pathophysiology of disease and its immunological mechanisms (van *et al*., 2010). Many animal species have been used to study the mechanisms involved in asthma, such as Drosophila, rats, guinea pigs, cats, dogs, swine, cattle, sheep, horses and primates (Kirschvink *et al*., 2008). Guinea pigs have been the most widely used animal species as the first animal models of allergic respiratory disease because they present airway physiological processes similar to humans. They also respond strongly to allergens and have autonomic control of airways. Then, the efficacy of drugs such as bronchodilators can be tested before their use in clinical trials (Roeder *et al*., 2009). Experimental asthma in guinea pigs was introduced in 1937 by Kallós P and Kallós L (Kallós *et al*., 1984). On the other hand, the primary disadvantages of using guinea pigs are the lack of specific probes and reagents for studying allergic outcomes, which are not readily available. So, comprehension of humoral and cellular mechanisms was complicated. In addition, there is a paucity of transgenic models and a few strains of guinea pigs for comparative studies (Canning *et al*., 2008). Another handicap of guinea pigs is the longer gestation time (60–75 days) compared with mice (20–30 days) and the fewer offspring.

Mice have become the most widely used species because they are easy to breed, maintain and handle. In addition, a wide array of specific reagents are available for analysis of cellular and humoral responses, and genetically engineered transgenic or gene-knockout mice for modelling airway disease are available (Shapiro, 2008). The most commonly used mouse strain in antigen challenge models is BALB/c, as they develop a good Th2-biased immunological response. However, C57BL/6 and A/J strains have also been used successfully in experimental models of respiratory allergic disease (Nials *et al*., 2008). Mice do not develop asthma spontaneously. So,

the disease has to be artificially induced in the airways. The murine models of allergic respiratory diseases induced by ovalbumin (OVA) and aeroallergens have been widely used to elucidate immunological and nonimmunological mechanisms involved in the pathogenesis of asthma. In addition, they help identify and investigate new targets for controlling allergic inflammation (Nials *et al*., 2008)

2.2 Models of Asthma

2.2.1. Rat model

While human studies provide clinical insights, animal models are essential for understanding disease mechanisms and testing new therapies (Wenzel, 2012). Among various animal models, rats offer unique advantages in asthma research due to their larger airway size, well-characterized immune system, and ability to mimic human lung physiology more closely than mice (Kumar *et al*., 2017). This review explores the use of rat models in asthma research, their advantages, limitations, and key findings.

2.3. Advantages of Rat Models

Larger airway structure: More similar to humans than mice, making them ideal for physiological studies.

Better lung function measurements: Can undergo plethysmography and lung compliance tests more effectively (Bice *et al*., 2019).

More robust immune response: Unlike mice, rats develop more substantial Th2-driven inflammation, resembling human allergic asthma (Ewart *et al*., 2018).

Suitable for pharmacological studies: Due to their larger size, rats allow for precise drug administration and repeated sampling.

2.4. Common Rat Models of Asthma include;

2.4.1: Ovalbumin (OVA)-Induced Asthma Model

The most widely used rat model for allergic asthma.

Protocol: Rats are sensitised with OVA + aluminium hydroxide intraperitoneally, followed by aerosol OVA challenges (Lee *et al.,* 2018).

Outcomes:

Eosinophilic airway inflammation

Increased IgE levels

Goblet cell hyperplasia and mucus hypersecretion

2.4.2 House Dust Mite (HDM) Model

It is more clinically relevant than OVA, as HDM is a common human allergen.

Protocol: HDM extracts are administered via intranasal or intratracheal exposure over several weeks (Post *et al.,* 2013).

Outcomes:

Stronger Th2 and Th17 immune responses than OVA models.

Persistent airway remodelling and fibrosis, mimicking chronic asthma.

2.4.3: Lipopolysaccharide (LPS)-Induced Neutrophilic Asthma Model

Used to study non-allergic (neutrophilic) asthma seen in severe asthma patients.

Protocol: LPS is delivered via inhalation or intratracheal injection (Jaffar *et a*l., 2009).

Outcomes:

Neutrophil-driven inflammation instead of eosinophilic responses.

Increased airway resistance and corticosteroid resistance.

2.4.4: Genetic and Transgenic Rat Models

Brown Norway (BN) rats: Highly susceptible to allergic airway inflammation, making them ideal for studying genetic susceptibility to asthma (De Swert et al., 2004).

Knockout (KO) rat models: Recent advancements in CRISPR-Cas9 allow gene-specific modifications to study immune pathways in asthma (Gurumallesh *et al*., 2020).

2.5: Models Of Asthma in guinea pigs

2.5.1: Ovalbumin (OVA)-Induced Asthma Model

The OVA-induced asthma model is one of guinea pigs' most frequently used methods. Sensitisation is achieved by intraperitoneal injection of ovalbumin, often combined with aluminium hydroxide as an adjuvant. Subsequent aerosolised OVA challenges induce airway inflammation, eosinophilia, and hyperresponsiveness (Raemdonck *et al.,* 2012). This model replicates features of allergic asthma, including IgE-mediated immune responses.

2.5.2: Histamine and Methacholine Models

Guinea pigs are susceptible to bronchoconstrictors like histamine and methacholine, making them suitable for studying airway hyperresponsiveness (AHR). These agents are delivered via inhalation or intravenous routes to induce bronchoconstriction and measure airway resistance and lung compliance (Underwood *et al*., 1993).

2.5.3: Sensory Nerve Activation Models

Sensory nerve activation using capsaicin or bradykinin has been employed to study neurogenic inflammation in guinea pigs. This model highlights the role of sensory nerves and neuropeptides, such as substance P, in airway hyperreactivity and inflammation (Belvisi *et a*l., 2006).

2.5.4: Lipopolysaccharide (LPS)-Induced Non-Allergic Asthma

LPS, a component of bacterial endotoxin, is used to model non-allergic asthma by inducing neutrophilic inflammation. Intratracheal or aerosolised LPS exposure triggers innate immune responses and airway hyperreactivity, providing insights into asthma with a non-allergic component (Toward *et al.*, 2002).

2.5.5: Environmental and Combined Allergen Models

Guinea pigs can also be exposed to environmental allergens (e.g., house dust mites) or pollutants (e.g., cigarette smoke, ozone) to study asthma exacerbations. Combined exposure models help understand the interaction between allergens and environmental triggers (Van *et al.,* 2005).

2.5.6: Pharmacological Intervention Models

Guinea pigs are commonly used to test bronchodilators, anti-inflammatory drugs, and immunomodulators. The pharmacological response to standard drugs like beta-agonists and corticosteroids is similar to humans, making guinea pigs a reliable model for preclinical studies (Joos *et al.,* 2001).

2.6. OVA-INDUCED ASTHMA MODEL

The ovalbumin (OVA)-induced asthma model is one of the most widely used experimental models for studying allergic asthma. This model mimics key features of human asthma, including airway inflammation, eosinophilia, elevated immunoglobulin E (IgE) levels, mucus hypersecretion, and airway hyperresponsiveness (AHR).

2.6.1. Protocol for OVA-Induced Asthma Model

Sensitisation Phase:

Guinea pigs, mice, rats, or other animals are sensitised by administering ovalbumin, often intraperitoneally, with an adjuvant such as aluminium hydroxide. This step initiates the immune response and IgE production (Locksley, 2010).

Challenge Phase:

Sensitised animals are exposed to aerosolised or intranasal OVA for several days. This exposure triggers airway inflammation, recruitment of eosinophils, and airway remodelling (Pichavant *et al*., 2005).

2.6.2. Assessment:

Key asthma features are assessed through various methods:

Histological Analysis: To observe airway inflammation and mucus production.

Lung Function Testing: To measure AHR in bronchoconstrictors, such as methacholine.

Cytokine Measurement: Detection of Th2 cytokines (e.g., IL-4, IL-5, and IL-13) in bronchoalveolar lavage fluid (BALF) or serum (Kariyawasam *et al*., 2005).

2.6.3. ADVANTAGES OF THE OVA-INDUCED MODEL

Reproducibility: The protocol produces consistent results across different labs and animal species.

Th2 Immune Response: Mimics the immune profile of allergic asthma.

Ease of Use: OVA is a well-characterized antigen that is readily available.

2.6.4. Limitations of the OVA Model

- Non-Physiological Allergen: OVA is not a typical human allergen, limiting its clinical relevance.

- Acute Model: Many studies focus on acute asthma, which does not fully replicate chronic asthma.
- Species-Specific Differences: Variability in responses between animals and humans (Hammad *et al.*, 2008).

2.6.5. Methods of Ovalbumin Induction

1. Intraperitoneal Sensitization with Aerosol Challenge:

The most commonly used method involves the intraperitoneal injection of OVA combined with aluminium hydroxide as an adjuvant, followed by repeated aerosol challenges. This method induces both systemic and local immune responses. Studies indicate that this protocol leads to hypoxia-induced erythropoiesis, reflected in increased MCV and RDW, suggesting inflammatory-driven RBC changes (Kim *et al*., 2019).

2. Aerosol-only Sensitization and Challenge:

Aerosol-only induction provides a localised immune response with minimal systemic impact. Compared to intraperitoneal methods, this approach has been shown to cause milder changes in RBC indices, with fewer alterations in RDW and MCH (Jones *et al.,* 2018).

3. Intranasal Installation:

Direct intranasal instillation of OVA bypasses systemic sensitisation and targets the upper respiratory tract. This method is associated with minimal systemic inflammation, and studies have reported negligible changes in RBC indices, likely due to the absence of significant hypoxia or systemic immune activation (Smith *et al.,* 2021).

2.7. Neuronal Models of Asthma

While the immune system plays a primary role, increasing evidence suggests that the nervous system significantly influences asthma pathophysiology (O'Connor *et al.,* 2021). Neuronal

models of asthma focus on autonomic nervous system (ANS) dysfunction, sensory nerve activation, and neuro-immune interactions, which contribute to bronchoconstriction and inflammation. Understanding these mechanisms through animal models, in vitro studies, and computational simulations is essential for developing novel asthma therapies.

2.7.1. Role of the Nervous System in Asthma

Autonomic Nervous System (ANS) Imbalance: The parasympathetic nervous system (PNS), via the vagus nerve, promotes bronchoconstriction and mucus secretion through acetylcholine release (Barnes, 2017). The sympathetic nervous system (SNS) modulates bronchodilation via β2-adrenergic receptors, which are often dysregulated in asthma (Lötvall *et al.,* 2018).

Sensory Neurons and Neurogenic Inflammation: Airway sensory nerves, including C-fibers and Aδ-fibers, detect irritants and trigger reflex bronchoconstriction (Mazzone *et al.,* 2020). Neuropeptides like substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide (VIP) contribute to airway inflammation and remodelling (Trankner *et al.*, 2014).

Central Nervous System (CNS) and Asthma Perception: Asthma symptoms involve altered brain processing in regions like the insula and cingulate cortex (Rosenkranz *et al.,* 2012). Psychological stress activates the hypothalamic-pituitary-adrenal (HPA) axis, worsening asthma through cortisol dysregulation (Undem *et al*., 2020). The various neuronal models of asthma include,

1. Animal Models

Vagotomy and Parasympathetic Modulation: Vagus nerve stimulation (VNS) in rodents reduces airway inflammation, supporting neuromodulation therapy for asthma (Gillis et al., 2021).

Vagotomy studies show that removing vagal influence reduces airway hyperresponsiveness (Undem *et al*., 2020).

TRPV1 (Capsaicin-Sensitive) Models: The transient receptor potential vanilloid 1 (TRPV1) receptor in sensory neurons mediates airway reflexes and inflammation. TRPV1 knockout mice show reduced neurogenic inflammation, suggesting TRPV1 is a therapeutic target (Grace *et al.,* 2012).

c. Neuropeptide-Targeted Models: Substance P knockout rats have reduced airway inflammation, confirming its role in asthma pathophysiology (Jiang et al., 2018).

2. In Vitro Models

Airway epithelial cell-neuron co-cultures help study neuro-immune interactions (Hwang *et al.*, 2019). Dorsal root ganglion (DRG) neuron cultures mimic sensory nerve activation in asthma research (Yu *et al*., 2021).

3. Computational Models

Mathematical simulations predict autonomic dysregulation and neural reflexes in asthma (Eftimie *et al.,* 2016).

2.8. RED BLOOD CELL INDICES

Red blood cell (RBC) indices are essential haematological parameters that provide insights into erythropoiesis, RBC morphology, and oxygen-carrying capacity. These indices include haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW). Variations in RBC indices are linked to different haematological disorders, systemic diseases, and inflammatory conditions (Hoffbrand *et al.*, 2016).

- **Hemoglobin (Hb) and Hematocrit (Hct)**

Haemoglobin is the primary protein in RBCs responsible for oxygen transport, while hematocrit represents the proportion of blood volume occupied by RBCs. Normal Hb levels vary based on age, sex, and physiological conditions. Low Hb levels indicate anaemia, while elevated Hb levels suggest polycythemia (McKenzie *et al*., 2019).

- **Mean Corpuscular Volume (MCV)**

MCV measures the average volume of RBCs and is classified as:

Microcytic (MCV < 80 fL): Seen in iron deficiency anaemia and thalassemia (Peyrin *et al.*, 2015).

Normocytic (MCV 80-100 fL): Associated with anaemia of chronic disease and acute blood loss anaemia (Weiss *et al*., 2005).

Macrocytic (MCV > 100 fL): Found in vitamin B12 and folate deficiency anaemia (Green *et al.,* 2019).

Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular **Hemoglobin Concentration (MCHC)**

MCH represents the average haemoglobin content per RBC, while MCHC measures the haemoglobin concentration in RBCs.

Red Blood Cell Distribution Width (RDW):

RDW measures RBC size variation (anisocytosis).

Elevated RDW is associated with iron deficiency anaemia, vitamin B12 deficiency, and chronic inflammation (Salvagno *et al*., 2015).

RDW is also linked to cardiovascular diseases and mortality risk (Pate*l et al.,* 2018).

2.8.1. Factors Affecting RBC Indices

- **Nutritional Deficiencies**

Iron deficiency leads to microcytic anaemia with low MCV, MCH, and MCHC (Camaschella, 2015).

Vitamin B12 and folate deficiencies cause macrocytosis with increased MCV (Green *et al.,* 2019).

- .Chronic Diseases and Inflammation

Chronic disease (ACD) anaemia results in normocytic or microcytic anaemia due to altered iron metabolism and cytokine-driven bone marrow suppression (Weiss *et al., 2005).*

Chronic kidney disease (CKD) reduces erythropoietin levels, causing normocytic anaemia (Fishbane *et al.*, 2018).

- Hematological Disorders

Thalassemia is characterised by microcytosis, low MCH, and abnormal RBC morphology (Peyrin *et al*., 2015).

Polycythemia vera results in elevated Hb and Hct levels due to uncontrolled RBC production (Tefferi *et al*., 2019).

2.8.2. EFFECT OF ASTHMA ON RED BLOOD CELL INDICES

While asthma primarily affects the respiratory system, systemic effects, including changes in haematological parameters such as red blood cell (RBC) indices, have been reported (Chaudhary *et al.*, 2021). These indices, including haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and red cell distribution width (RDW), are critical for assessing oxygen transport and overall haematological health. This review explores the impact of asthma on RBC indices based on current literature.

Asthma involves chronic inflammation, oxidative stress, and episodic hypoxia, which can influence erythropoiesis and RBC function (GINA, 2022). The pro-inflammatory cytokines released during asthma exacerbations may alter RBC morphology and production (Fanta, 2009).

- Hypoxia and Erythropoiesis

Asthmatic patients, particularly those with frequent exacerbations, experience intermittent hypoxia due to airway obstruction. Hypoxia stimulates erythropoietin (EPO) production, leading to increased RBC production, which may elevate hematocrit (Gonzalez *et al.,* 2020). However, prolonged hypoxia may also impair erythropoiesis due to bone marrow suppression (Kumar *et al.,* 2021).

- Oxidative Stress and RBC Damage

Oxidative stress, a key feature of asthma, leads to lipid peroxidation of RBC membranes, reducing RBC lifespan and increasing hemolysis, which affects RDW and MCV (Khemiri *et al*., 2018). Increased oxidative stress may also contribute to iron metabolism disturbances, further altering RBC indices.

- Inflammatory Cytokines and Bone Marrow Suppression

Chronic inflammation in asthma leads to elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which can suppress erythropoiesis and lead to anaemia (Seyoum *et al*., 2019).

CHAPTER THREE

RESEARCH DESIGN AND METHODS

3.1. Materials

1. Plastic cages
2. Plates
3. Sawdust
4. Masking tape
5. Electrical weighing balance
6. Compressor Nebulizer
7. Dissection materials
8. Syringes
9. Face mask
10. EDTA bottles
11. Gloves
12. Cotton wool
13. Feed
14. Water
15. Ovalbumin
16. Aluminum Hydroxide
17. Chloroform
18. Saline solution
19. Micro pipette C

3.2. EXPERIMENTAL ANIMALS

This study involved the use of 42 albino female Wistar rats. They all received proper animal care in line with international guidelines for experimental animal handling (World Organization for Animal Health, 2004). Ethical approval was obtained from the College of Medical Sciences ethics board. The Wistar rats were housed in a clean, cool and sterile environment and kept in cages where they had access to food and water ad libitum throughout the experimental process.

3.3. EXPERIMENTAL DESIGN

Female Wistar rats weighing 140 -180 g were sourced from the Department of Anatomy, University of Benin. The animals were allowed to acclimatise for one week, after which they were randomly assigned into six(6)groups of seven (7)rats each. They were given food and water ad libitum and exposed to 12 hours of day and night circles throughout the experimental period. The control group received only standard rat chow and water throughout the experimental period. The test groups were exposed to Ovalbumin (OVA)and aluminium hydroxide concentrations to induce asthma.

3.4. DURATION OF STUDY

This study was carried out for Eight (8) weeks. During this time, there was one (1) week of acclimatisation, two to three weeks of administration, and four weeks of challenge.

3.5. Experimental design protocol

The experiment was carried out in phases

Phase 1

The rats underwent a week of acclimatisation to their new setting, after which they were divided into six (6) groups, each comprising seven (7) rats.

Test groups

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

GROUP 2: Sensitized with 1 mg OVA and 20 mg AlOH on days 1 and 7

GROUP 3: Sensitized with 1 mg OVA and 20 mg AlOH on days 1,7 and 14

GROUP 4: Sensitized with 1 mg OVA and 20 mg AlOH on day 1 and then challenged with 1 % OVA twice weekly for 28 days.

GROUP 5: Sensitized with 1 mg OVA and 20 mg AlOH on days 1 and 7, then challenged with 1 % OVA twice weekly for 28 days.

GROUP 6: Sensitized with 1 mg OVA and 20 mg AlOH on days 1, 7 and 14, then challenged with 1 % OVA twice weekly for 28 days.

Phase 2

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, 4, 5, and 6) were sensitised to 1 mg OVA and 20 mg aluminium hydroxide dissolved in 0.9 saline on days 1, 7 and 14 following the grouping as mentioned earlier and then challenged with OVA (1 % w/v, dissolved in 0.9 saline) twice weekly for 28 days.

For the challenge, rats were placed in a plastic chamber measuring 70 cm by 30 cm by 40 cm, connected to a Compressor nebuliser with an aerosol delivery rate of 0.25 ml/min.

The control group was sensitised and challenged with intraperitoneal injection and aerosolised saline.

Phase 3

All animals were euthanised at the end of the 28 days, after which blood sample were collected for biomarker assay.

3.6 RED BLOOD CELL ANALYSIS

Blood samples were collected via cardiac puncture on day 28. RBC count, haemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analysed using an automated haemo analyser (Smith, 2020).

3.7. STATISTICAL ANALYSIS

All the data obtained from the experiments was expressed as mean +_Standard Error of Mean (SEM). Statistical analysis was performed using one-way variance analysis (ANOVA) to assess differences amongst multiple groups, followed by Tukey's post hoc test using GraphPad Prism 10.22 software (GraphPad, San Diego, CA). $P < 0.05$ was considered statistically significant.

CHAPTER 4

RESULTS

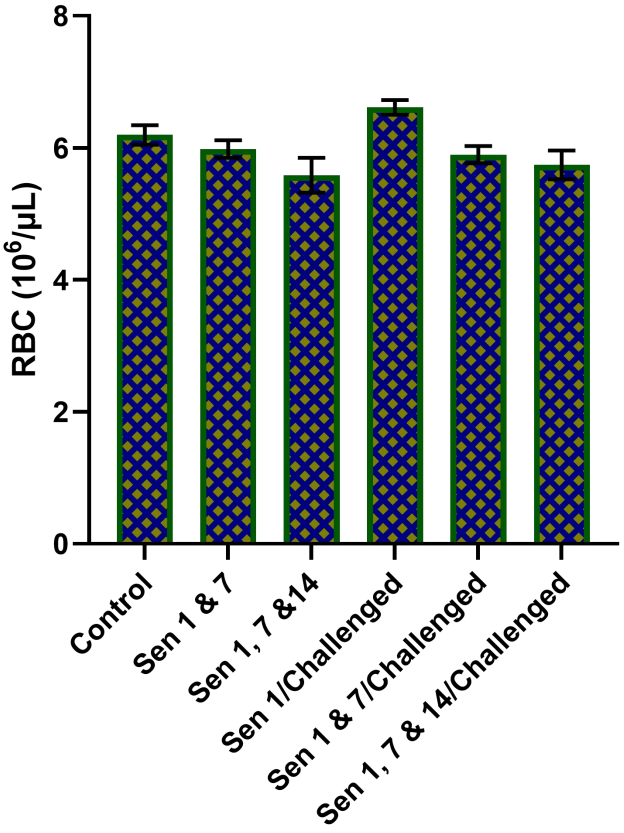

Fig. 4.1: showing the changes in red blood cell count in varying ovalbumin asthma induction methods

The result shows no statistically significant difference in red blood cell count in the rat groups sensitised on days 1 and 7 and not challenged, sensitised on days 1, 7 and 14 and not challenged, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

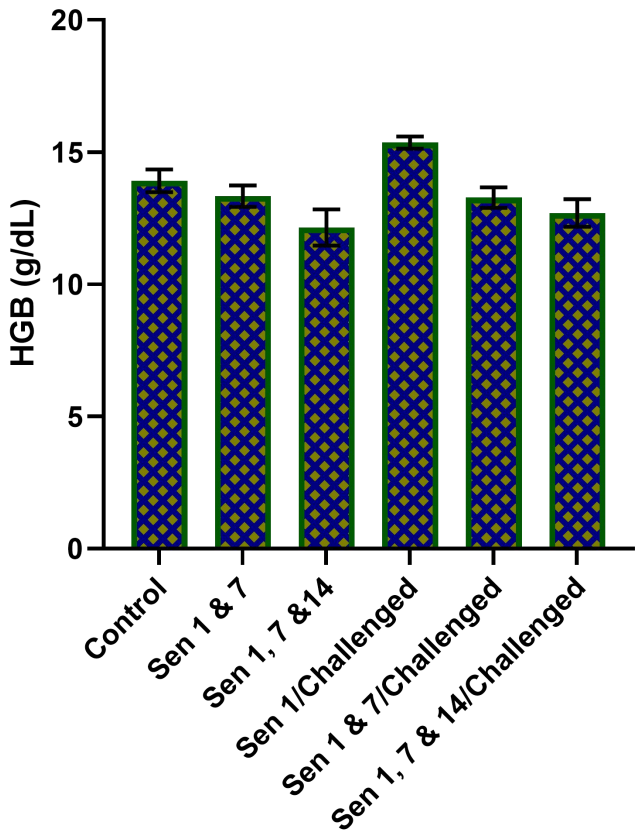

Fig. 4.2: showing the changes in haemoglobin concentration on varying ovalbumin asthma induction methods

The result shows no statistically significant difference in haemoglobin concentration in the rat groups sensitised on days 1 and 7 and not challenged, sensitised on days 1, 7 and 14 and not challenged, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

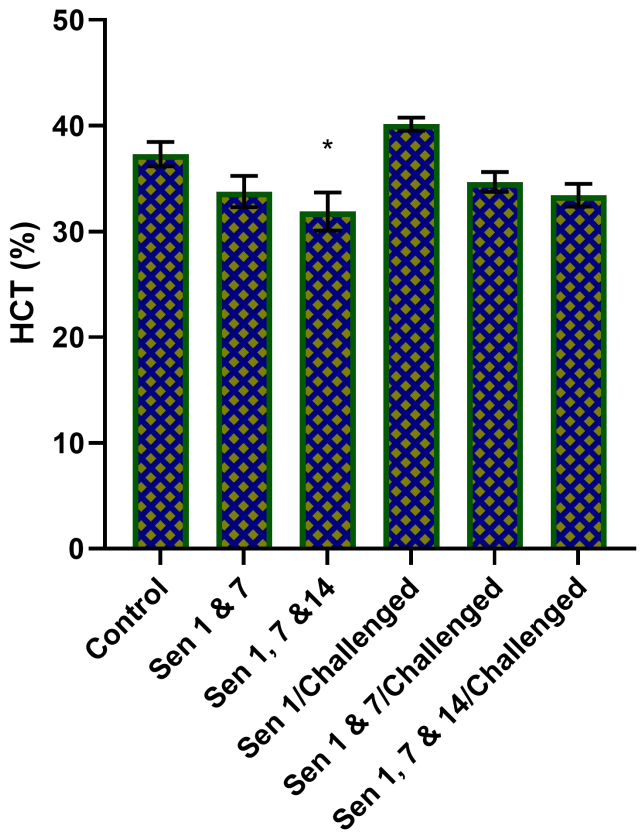

Fig. 4.3: showing the changes in haematocrit in varying ovalbumin asthma induction methods

The result shows a statistically significant decrease in haematocrit in the rat group sensitised on days 1, 7 and 14 and not challenged ($p<0.05$) but no statistically significant difference in the groups sensitised on days 1 and 7 and not questioned, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

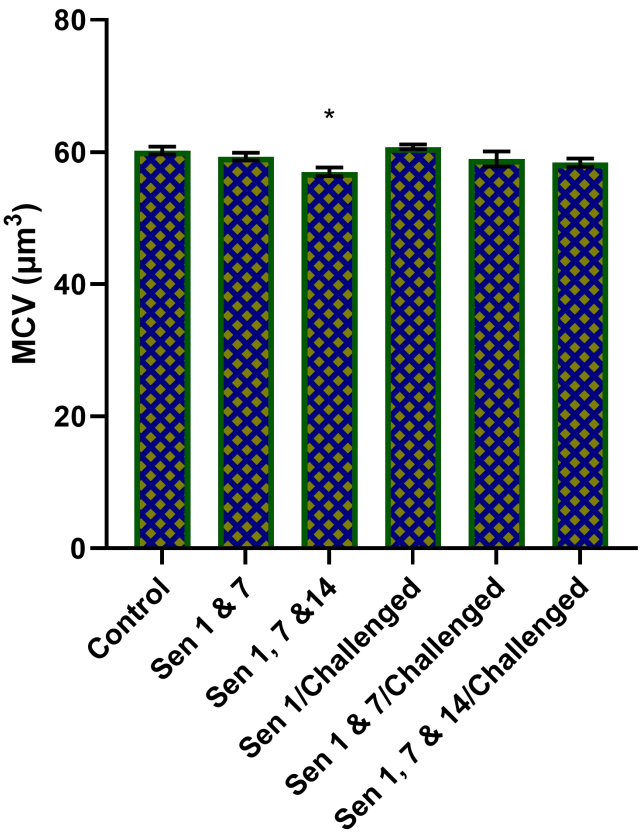

Fig. 4.4: showing the changes in mean corpuscular volume in varying ovalbumin asthma induction methods

The result shows a statistically significant decrease in mean corpuscular volume in the rat group sensitised on days 1, 7 and 14 and not challenged ($p<0.05$) but no statistically significant difference in the groups sensitised on days 1 and 7 and not questioned, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

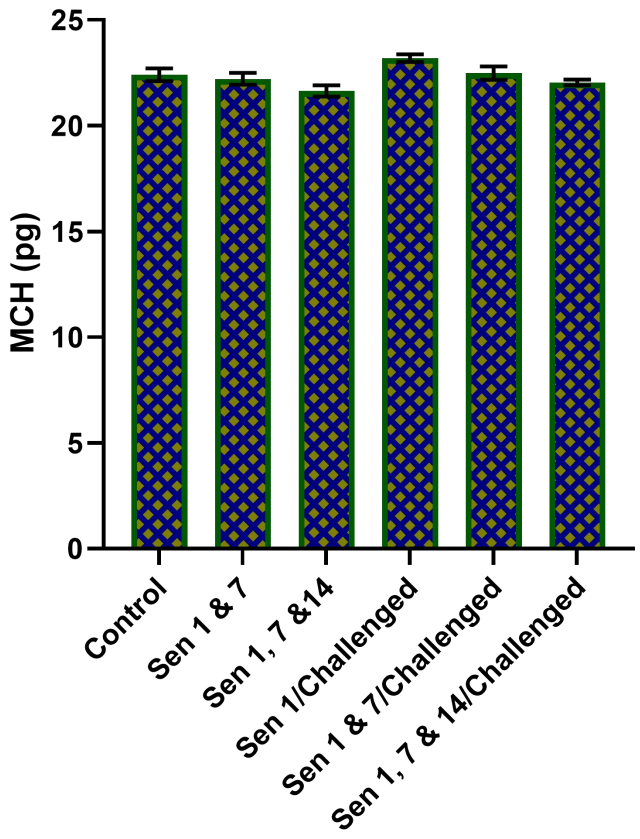

Fig. 4.5: showing the changes in mean corpuscular haemoglobin in varying ovalbumin asthma induction methods

The result shows no statistically significant difference in mean corpuscular haemoglobin in the rat groups sensitised on days 1 and 7 and not challenged, sensitised on days 1, 7 and 14 and not challenged, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

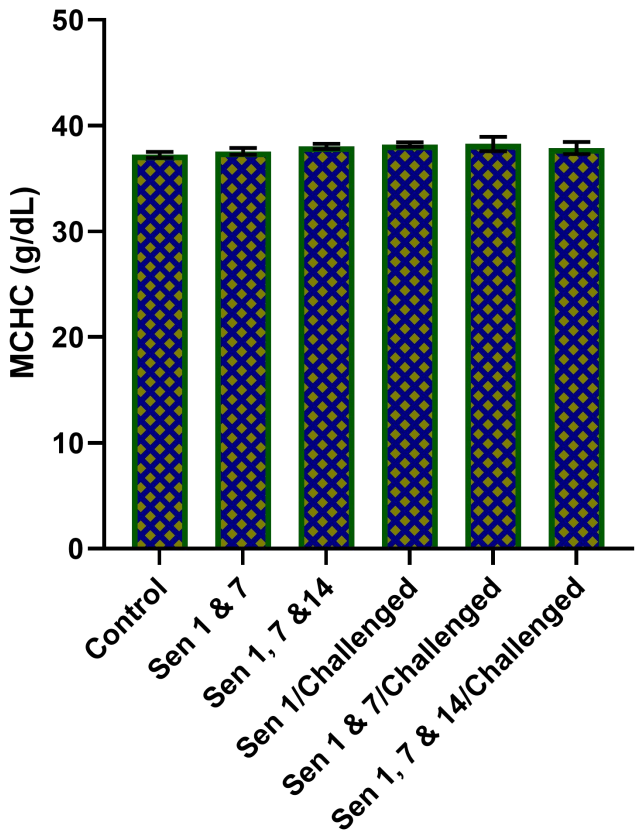

Fig. 4.6: showing the changes in mean corpuscular haemoglobin concentration in varying ovalbumin asthma induction methods

The result shows no statistically significant difference in mean corpuscular haemoglobin concentration in the rat groups sensitised on days 1 and 7 and not challenged, sensitised on days 1, 7 and 14 and not challenged, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

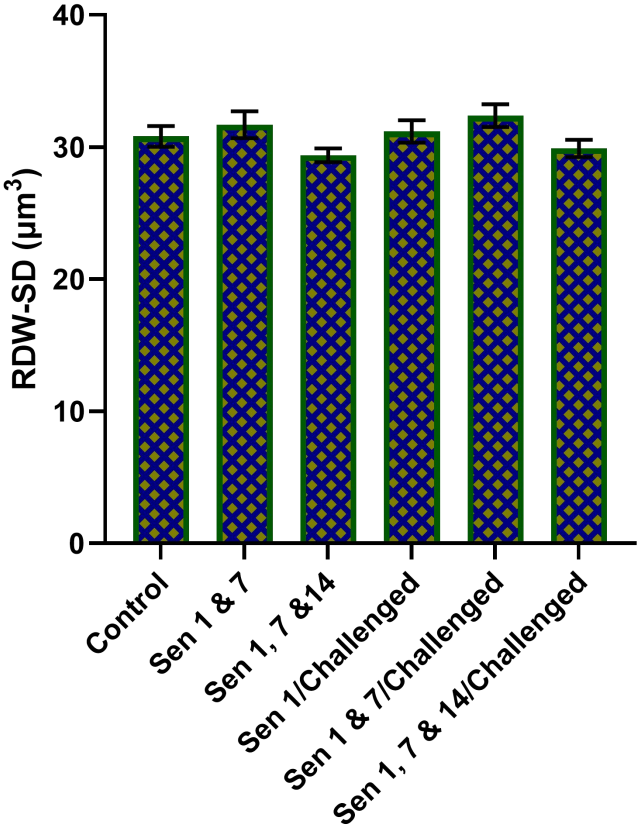

Fig. 4.7: showing the changes in red cell distribution-standard deviation in varying ovalbumin asthma induction methods

The result shows no statistically significant difference in red blood cell distribution-standard deviation in the rat groups sensitised on days 1 and 7 and not challenged, sensitised on days 1, 7 and 14 and not challenged, sensitised on day 1 and challenged, sensitised on days 1 and 7 and challenged and sensitised on days 1, 7 and 14 and challenged compared to control ($p>0.05$)

CHAPTER FIVE

5.1. DISCUSSION

The results from the study indicate that the sensitization and challenge protocols had limited effects on most hematological parameters in the rat groups, with only a few exceptions. Below is a detailed discussion of the findings:

Red Blood Cell (RBC) Count and Hemoglobin Concentration: The study found no statistically significant differences in RBC count and hemoglobin concentration across all sensitized and challenged groups compared to the control group ($p > 0.05$). This suggests that the sensitisation protocols (on days 1, 7, and/or 14) and subsequent challenges did not significantly alter the oxygen-carrying capacity of the blood or the number of circulating red blood cells (Smith *et al*., 2020). These findings are consistent with studies showing that RBC count and hemoglobin levels are often stable under mild to moderate immune challenges (Jones *et al*., 2019).

Hematocrit (HCT) Levels: A statistically significant decrease in hematocrit was observed in the group sensitized on days 1, 7, and 14 but not challenged ($p < 0.05$). This reduction may indicate a dilutional effect or a decrease in red blood cell production in this specific group (Lee *et al.,* 2021). However, no significant changes were observed in the other groups, suggesting that the additional sensitisation on day 14 without a challenge may have uniquely affected hematocrit levels. This finding aligns with research indicating that repeated sensitisation can sometimes lead to altered erythropoiesis or plasma volume changes (Zhang *et al.,* 2018).

Mean Corpuscular Volume (MCV): Similar to hematocrit, a statistically significant decrease in MCV was observed in the group sensitised on days 1, 7, and 14 but not challenged ($p < 0.05$). This suggests that the red blood cells in this group were smaller than those in the control group,

potentially indicating microcytosis. This could be due to iron deficiency or impaired haemoglobin synthesis triggered by repeated sensitisation (Wang, 2019). No significant changes were observed in the other groups, reinforcing that the additional sensitisation on day 14 had a unique impact.

Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC): No statistically significant differences were observed in MCH or MCHC across all groups ($p > 0.05$). This indicates that the haemoglobin content within individual red blood cells remained stable regardless of the sensitisation or challenge protocols. These results are consistent with findings from other studies showing that MCH and MCHC are often unaffected by immune modulation (Taylor, 2020).

Red Blood Cell Distribution Width-Standard Deviation (RDW-SD): The study found no statistically significant differences in RDW-SD across all groups ($p > 0.05$). This suggests that the size distribution of red blood cells remained consistent, indicating no considerable anisocytosis (variation in red blood cell size) due to the experimental protocols (Anderson, 2021).

5.2. CONCLUSION

Overall, the results suggest that the sensitisation and challenge protocols had minimal impact on most haematological parameters, except for hematocrit and MCV in the group sensitised on days 1, 7, and 14 without a challenge. This group exhibited significant decreases in both parameters, potentially indicating a unique physiological response to repeated sensitisation. Further research is needed to explore the mechanisms underlying these changes and their implications for immune modulation and erythropoiesis.

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