EFFECTS OF *Citrullus lanatus* JUICE ON RED BLOOD CELL INDICES OF ANAEMIC RATS.

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BMS2005115

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

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this project work on "**EFFECTS OF *Citrullus lanatus* JUICE ON RED BLOOD CELL INDICES OF ANAEMIC RATS**" was carried out by **Olowolagba Joy ASHIMAKOME**, with the Matriculation Number: **BMS2005115**; in partial fulfilment for the Award of Bachelor of Science (B.Sc) Degree in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this project to God Almighty for his love and infinite mercy, to him alone be all the glory forever.

ACKNOWLEDGEMENTS

I want to thank my parents for their love and support all through my study.

I also want to thank my project supervisor, Dr. Mrs. Akpitanyi Idutua for her rich advice and guidance in the course of writing this project.

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ABSTRACT

Anaemia is a prevalent global health concern, particularly in developing regions. Conventional treatments such as iron supplements often have side effects, prompting interest in natural alternatives. *Citrullus lanatus* (watermelon) is rich in bioactive compounds, including lycopene, citrulline, and essential minerals, which may influence hematopoiesis. This study evaluates the effects of *Citrullus lanatus* juice on red blood cell (RBC) indices in anaemic rats. Seventy seven male Sprague-Dawley rats were divided into five groups: control, anaemia-induced (untreated), anaemia + vitamin B complex/folic acid, and anaemia + watermelon juice. Anaemia was induced using phenylhydrazine, and treatments were administered for three weeks. Blood samples were collected weekly and analyzed for RBC indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW). Data were analyzed using ANOVA with a significance level of $p < 0.05$. *Citrullus lanatus* juice administration significantly increased MCV and MCH compared to the control ($p < 0.05$), suggesting enhanced erythropoiesis. RDW-SD showed a notable increase in the watermelon-treated group, indicating RBC production. However, no significant changes were observed in mean corpuscular hemoglobin concentration (MCHC) ($p > 0.05$). The results suggest that *Citrullus lanatus* juice positively influences RBC indices in anaemic rats, potentially enhancing erythropoiesis. This study supports the use of *Citrullus lanatus* as a dietary intervention for anaemia, warranting further research in human models.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

Anaemia is a global health challenge affecting millions of people particularly in developing countries. Approximately one-third of the world's 5.5 billion people are anaemic. Globally, about 40% of children aged 0–12 years, 35% of all women, 51% of pregnant women, and 18% of men are affected by anaemia. These figures likely underestimate the true prevalence, as the diagnosis of anaemia is based on the World Health Organization's (WHO) definition; Haemoglobin less than 11 g/dL for children aged 0–4 years and pregnant women, Haemoglobin less than 12 g/dL for children aged 5–12 years and non-pregnant women, Haemoglobin less than 13 g/dL for men. These thresholds apply to global populations, encompassing both developed and developing nations. The decrease in hemeoglobin concentrations, red blood cells or hematocrit levels, leads to reduced oxygen-carrying capacity of the blood (Camaschella, 2017). The causes of anaemia ranges from nutritional deficiencies or malnutrition, High pregnancy rates, to chronic diseases with iron-deficiency anaemia being the most prevalent type of anaemia worldwide (WHO, 2021). The management of anaemia often involves dietary interventions and supplements, showcasing the role of nutrition in improving hematological parameters. (Gardner *et al*., 2023; WHO, 2024).

Fruits and natural products have gained considerable attention in recent years for their potential therapeutic properties. *Citrullus lanatus* (Watermelon) is a tropical fruits with reported health benefits due to its rich phytochemical and nutrient profiles. Papaya is an excellent source of vitamin A, C and E, folate and antioxidants. *Citrullus lanatus* is known for high water content, vitamin C and bioactive compounds such as Lycopene and citrulline. This fruit is also rich in minerals, including potassium and magnesium, which are essential for the body's physiological functions, including hematopoiesis (Eze *et al*., 2018; Collins *et al*., 2007).

1.2 Statement of Problem

Anaemia remains a major challenge, affecting millions of people worldwide, particularly pregnant women, children and individuals with chronic illness (WHO, 2021). Conventional treatments such as iron supplements, such as iron supplements often have side effects in gastrointestinal discomfort and poor compliance. There is a growing need for alternative, natural remedies such as water melon (citrullus lanatus) that are affordable accessible and effective in managing anaemia.

1.3 Justification of Study

The use of natural remedies in diseases managements is gaining widespread attention due to their minimal side effects and cost effectiveness. *Citrullus lanatus* is a readily available fruit with known medicinal properties, including antioxidant and anti-inflammatory effects. If proven effective, it could serve as therapeutic interventions for anaemia reducing reliance on synthetic supplements. This study will contribute to scientific knowledge on the role of fruit based therapy in health and may provide the basis for future clinical research on humans.

1.4 Aim of study

To evaluate the effects of *Citrullus lanatus* juice on the Red blood cell (RBC) indices profile of anaemic Sprague-Dawley rats.

1.5 Objectives of Study

i. To evaluate the effects of *Citrullus lanatud* juice on red cell indices (MCV, MCH, MCHC, CHR, and RDW) in anaemic rats.

1.6 Research questions

i. How does the administration of *Citrullus lanatus* juice affect the red cell indices of anaemic rats?

ii. Can the administration of Carica Papaya serve as an effective alternative to conventional anemia treatment medications?

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Anaemia and Its Implications

Anaemia is a widespread public health challenge, particularly in developing countries, affecting individuals across all age groups. It is defined as a condition in which the quantity or quality of circulating red blood cells (RBCs) is reduced below normal levels. Anaemia is characterized by a decreased concentration of haemoglobin, which leads to insufficient oxygen transport to tissues. Red blood cells, or erythrocytes, are biconcave discs which are critical for gaseous exchange. Their major product is haemoglobin, the carrier for O_2 and CO_2 throughout the body. At the center of the heme is iron which is essential for gaseous transport. It is haemoglobin which produces the characteristic redness associated with erythrocytes. The anaemic condition is often associated with symptoms such as fatigue, weakened immunity, and an increased susceptibility to infections (Camaschella, 2015). More than half of the pregnant women in low income countries suffer from anaemia and iron deficiency is the most common cause of anaemia in pregnancy (WHO 2001). According to the World Health Organization (WHO) Vitamin and Mineral Nutrition Information System for 1993 to 2005, global anaemia prevalence was estimated to be 41.8% in pregnant women (McLean 2009). The high prevalence of anaemia has been associated with low socio economic status (odds ratio (OR) 1.419, 95% CI 1.05 to 1.90) (Noronha 2010).

In addition to impairing the production of RBCs, anaemia also affects white blood cells (WBCs), which play a crucial role in the immune response. The most common method of diagnosing anaemia is by measuring haemoglobin concentration in the blood. Haemoglobin levels are regulated by a homeostatic mechanism and may vary slightly among individuals within the normal range (Gardner *et al*., 2023). The specialized function of red blood cells is the transport of O2 from pulmonary capillaries to tissue capillaries, where it is exchanged for CO_2. Iron plays a central role in O_2 transport by haemoglobin (Orsango *et al.,* 2021). Iron deficiency anaemia are caused by failure of iron to be absorbed, transported, and taken up by cells. Severe destruction of red cells in hemolytic anaemias can be caused by infections, glycolytic defects. A group of diseases known as haemoglobinopathies involve mutations to globin genes; these include thalassaemias which can result to sickle cell anaemia, where a point mutation produces an abnormal globin protein which produces a characteristic sickle shape (Kendall, 2001). Hence, causes of anaemia include iron deficiency, often resulting from inadequate dietary intake, malabsorption, or chronic blood loss (e.g., menstruation or gastrointestinal bleeding) (WHO, 2023). Vitamin B12 deficiency, frequently caused by pernicious anaemia, vegetarian diets, or malabsorption conditions like Crohn’s disease (Green and Allen, 2023), primarily affects red blood cells.

2.1.1conventional Treatment for Anaemia

Conventional treatments of anaemia include Iron supplements such as ferrous sulfate, are only prescribed for iron deficiency anaemia. However, side effects such as constipation and gastrointestinal discomfort limit compliance (Killip *et al*., 2021). Dietary sources of iron including leafy vegetables, meat and fortified cereals are also recommended. Also, in chronic cases such as kidney failure, erythropoietin injections stimulate RBC production (Weiss *et al*., 2005), while for severe anaemia, blood transfusions provide an immediate increase in RBC count but carries risks of infections and immune reactions (Yip and dallman, 2022.). Red blood cell (RBC) transfusion constitutes one of the mainstays of therapy in the management of anaemic patients. With a prevalence of anaemia as high as 95% in intensive care unit (ICU) patients by day 3, critically ill intensive care unit (ICU) patients in particular, as well as medical and haematology–oncology patients, are among the largest groups of users of RBC products. While anaemia is common in these patients, its treatment and management, including appropriate thresholds for RBC transfusion, remain controversial (Wang and Klien, 2009). Given the limitations of these conventional methods of anaemia management, there is an increase of interest in natural remedies like fruits and vegetables.

2.2 Red Blood Cell Indices

Red blood cell (RBC) indices are vital diagnostic tools used to evaluate the characteristics of red blood cells, aiding in the diagnosis of various hematological disorders. These indicies, which together give information about the size, hemoglobin content, and concentration of hemoglobin inside RBCs, include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. Termed red cell indices, these values are useful in elucidating the etiology of anemias (Klinken, 2002). MCV defines the size of the red blood cells and is expressed as femtoliters. MCH quantifies the amount of hemoglobin per red blood cell. MCHC indicates the amount of hemoglobin per unit volume. In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell. RDW represents the coefficient of variation of the red blood cell volume distribution (size) and is expressed as a percentage (Zhang *et al.,* 2022).

Mean corpuscular volume (MCV) measures the size of red blood cells, expressed in femtoliters (fl; 10^{1}) or cubic microns (μm^3), with a normal range of 87 ± 7 fl. Mean corpuscular hemoglobin (MCH) quantifies the hemoglobin content per red blood cell, with normal values of 29 ± 2 picograms (pg) per cell. Mean corpuscular hemoglobin concentration (MCHC) reflects hemoglobin content per unit volume, correlating hemoglobin with cell volume, and is expressed as g/dl or percentage, with a normal range of 34 ± 2 g/dl. Red cell distribution width (RDW) indicates the variability in red blood cell size, expressed as a percentage, with a normal value of 13 ± 1.5% (Veda, 2013). For the initial classification of anemic diseases, the two traditional primary criteria are reticulocyte count (Hilman, 1969), and mean corpuscular volume (MCV) (Wintrobe, 1981).

Table 2.1: Definition of Anaemia and Abnormal Red Blood Cell Indices

Parameter	Abbreviation	Unit	Abnormalities	Reference
Haemoglobin	Hb	g/dL	Anaemia: <12 g/dL for women and <13 g/dL for men	Khan *et al*., 2023
Mean Corpuscular Volume	MCV	fL	Low MCV: <81 fL for women and <80 fL for men	WHO, 2024
Mean Corpuscular Haemoglobin	MCH	pg	Low MCH: <26 pg for women and <27 pg for men	WHO, 2024
Mean Corpuscular Haemoglobin Concentration	MCHC	g/dL	Low MCHC: <32 g/dL	WHO, 2024
Mean Reticulocyte Haemoglobin Content	CHR	pg	Low CHR: <28 pg	Thomas *et al*., 2005; Mast *et al*., 2002; Beutler and Waalen, 2006

Red Cell Distribution Width	RDW	%	High RDW: >14.5%	Al-Naijar *et al*., 2009

2.3 *Citrullus lanatus* (watermelon)

Citrullus lanatus (Watermelon) is a vine-like plant belonging to the Cucurbitaceae family, known for producing a juicy fruit that is widely grown for human consumption. As an annual plant, it follows well-established growth patterns that thrive in hot climates. The typical weight of the fruit ranges from 1-2 kg, though it can reach up to 20 kg. In desert areas, watermelon is highly valued as a source of water (Perkins-Veazie *et al*., 2001). China leads the world in watermelon production, followed by Turkey, India, Iran, Algeria, Brazil, the United States, and Korea (Lin *et al*., 2014; Naz *et al*., 2013). The scientific name, *Citrullus lanatus*, combines the Greek word "Citrullus," meaning citrus, and the Latin "Lanatus," referring to the fuzzy hairs on the plant's leaves and stems. Watermelon is composed of approximately 68% pulp, 30% rind, and 2% seeds (Pons, 2003). The fruit has a thick, smooth outer skin with light green or grey stripes over a deep green color. Its flesh is red, with dark brown or black elliptical seeds found in the central third. Seedless varieties, which either lack seeds or contain small, jelly-like white ones, have also been developed (Nunes, 2008). Watermelon is highly nutritious, hydrating, and low in calories (Okonmah *et al*., 2011). Although now grown worldwide, it was first harvested in Egypt more than 5,000 years ago.

Plate 2.1: ***CItrullus lanatus* plant and fruits**

Plate 2.2: **cross section of Citrullus lanatus fruit showing pulp and seeds (source: Steve Evans)**

2.3.1 Botanical classification

Kingdom- Plantae

Phylum- Embryophyta

Class- Dicotyledoneae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Citrullus*

Species: *C. lanatus*

Citrullus lanatus

2.3.2 Nutrient composition

Table 2.2: Nutritional composition of watermelon

Nutrient	Watermelon (Per 152 g)	Watermelon Seed (Per 100 g)	Watermelon Rind	Watermelon Seed Flour	Watermelon Juice (Per 100 g)
Calories	45.60 kcal	557 kcal	-	-	30 kcal
Energy	-	2,330 kJ	-	-	127 kJ
Moisture	-	5.05 g	10.61%	9.77%	90.1-92.42 g
Lipids	0.23 g	47.37 g	2.44%	0.64%	0.05-0.27 g
Protein	0.93 g	28.33 g	11.17%	2.23%	0.4-0.84 g
Ash	0.38 g	3.94 g	13.09%	2.15%	0.1-0.37 g
Dietary Fiber	0.61 g	-	17.28%	0.65%	0.4-0.7 g
Carbohydrates	11.48 g	15.31 g	56.02%	84.57%	7.55 g
Total Sugars	9.42 g	-	-	-	5.74-6.59 g
Lycopene	6,888.64 µg	-	-	-	3,040-5,590 µg
β-Carotene	-	-	96.44%	-	-
References	(Mateljan, 2020)	(USDA, 2020)	(Al-Sayed and Ahmed, 2011)	(Ubbor and Akobundo, 2009)	(USDA, 2020)

2.3.3 Mineral composition of *Citrullus lanatus*

Table 2.3: Mineral composition of *Citrullus lanatus*

Minerals	**Watermelon (Per 152 g)**	**Watermelon Juice (Per 152 g)**	**Watermelon Rind (Per 100 g)**	**Watermelon Seed (Per 100 g)**
Calcium	10.64 mg	4-14 mg	29.15 mg	54 mg
Iron	0.36 mg	0.08-0.55 mg	1.29 mg	7.28 mg
Fluoride	-	1-2.1 µg	-	-
Magnesium	15.20 mg	5-12 mg	1.48 mg	515 mg
Phosphorous	16.72 mg	5-19 mg	135.24 mg	755 mg
Potassium	170.24 mg	58-191 mg	1.37 mg	648 mg
Sodium	1.52 mg	0-5 mg	12.65 mg	99 mg
Zinc	0.15 mg	0.04-0.63 mg	1.29 mg	10.24 mg
Copper	0.06 mg	0.006-0.166 mg	0.45 mg	0.686 mg
Manganese	0.06 mg	0-0.08 mg	1.42 mg	1.614 mg
Boron	142.50 µg	-	-	-
Selenium	0.61 µg	0-0.7 µg	-	-
References	(Mateljan, 2020)	(USDA, 2020)	(Gladvin *et al.*,	(USDA, 2020)

			2017)	

2.3.4: Phytochemical properties of *Citrullus lanatus*

Table 2.4: Phytochemical Composition of *Citrullus lanatus* (watermelon)

Phytochemical	Percentage composition	Function/Benefits	References
Lycopene		Antioxidant, reduces risk of certain cancers, promotes heart health, and improves skin health.	Fahey *et al.,* 2001, Perkins-Veazie and Collins, 2007)
Alkaloids	0.57%	Analgesic, Antimalarial, CNS Stimulant, Neurostimulant	Omoboyowa *et al*., 2015
Citrulline		Improves blood flow, enhances exercise performance, and may lower blood pressure.	Sweeney and Cooper, 2013)
Beta-carotene		Antioxidant, supports immune function, and promotes eye health.	Kumar and Srivastava, 2018)

Flavonoids	0.01%	Antioxidant, anti-inflammatory, may reduce the risk of cardiovascular diseases.	Omoboyowa *et al*., 2015
Phenolic compounds	0.02%	Antioxidant, supports anti-inflammatory effects, may reduce cancer risk.	Omoboyowa *et al*., 2015
Tannins	0.02%	Antimicrobial, Anti-inflammatory, Astringent	Omoboyowa *et al*., 2015
Triterpenoids		Antioxidant, anti-inflammatory, may have anticancer and anti-obesity effects.	Kumar and Srivastava, 2018
Saponins	0.08%	Antimicrobial, Immunomodulatory, Hypocholesterolemic	Omoboyowa *et al*., 2015
Vitamins (A, C, B6)		Immune support, skin health, antioxidant, supports nervous system function.	Wall, 2006
Minerals		Supports muscle and nerve	Wall, 2006

(Potassium, Magnesium)		function, regulates blood pressure, and maintains electrolyte balance.	

2.3.5 Ethno-medicinal properties

Citrullus lanatus holds significant ethno-medicinal importance in traditional herbal practices. The fruit, when ripe or even slightly decayed, is used as a febrifuge (Saiwal, 2019). Its root is considered emetic and purgative in sufficient doses (Khalid, 2021). The seeds exhibit demulcent, pectoral, and tonic properties, and are used for treating urinary tract infections and bedwetting (Erhirhie and Ekene, 2013). The fruit has diuretic properties, aiding in the treatment of dropsy and renal stones. Additionally, it acts as a vermifuge and has been linked to lowering blood pressure due to its high water content (Sharma *et al.,* 2020).

In Northern Sudan, *Citrullus lanatus* is used for burns, swellings, rheumatism, gout, and as a laxative. It also treats diarrhea, gonorrhea, and other gastrointestinal issues. The plant's seeds yield tar for scabies treatment and a tanning agent for the skin (Sharma *et al.,* 2020). Seed oil demonstrates potent anthelmintic activity, even surpassing pumpkin seed oil (Ijaz *et al.,* 2022). Despite its widespread traditional use, many of its therapeutic properties await further scientific validation.

2.3.6 Pharmacological Properties of *Citrullus lanatus*

Watermelon's unique composition, including its vitamins, minerals, and phytochemicals, is reported to have significant therapeutic and pharmacological properties (Banurek and Mahendran, 2011; Jiang *et al*., 2020; Nkoana *et al*., 2021; Ubbor and Akobundo, 2009; Zhao *et al*., 2021). The seeds are rich in proteins, oils, and unsaturated fatty acids like stearic, palmitic, linoleic, and oleic acids. Watermelon is also a good source of natural sugars such as glucose, sucrose, and fructose, along with carotenoids like β-carotene (Khan *et al*., 2020). The fruit contains phytochemicals, including cucurbitacins and their glycoside derivatives, which have notable medicinal properties, including hepatoprotective, anti-inflammatory, anti-tumor, antimicrobial, and anthelmintic activities (Biswas *et al*., 2017; Nkoana *et al*., 2021). In Sudan, watermelon is used to treat a variety of conditions, including gastrointestinal issues, rheumatism, inflammation, and gout. In South Africa, both the leaves and fruit are employed in traditional and alternative medicine to address hypertension (Aderiye *et al*., 2020; Nkoana *et al*., 2021; Rashid *et al*., 2020). Additionally, roasted watermelon seeds are consumed as an appetite stimulant and to help alleviate constipation (Biswas *et al*., 2017). Recently, there has been growing interest in natural, plant-based food products as alternatives to pharmaceutical drugs for treating health conditions (Ahmad *et al*., 2020; Biswas *et al*., 2017; Nkoana *et al*., 2021). This review highlights the current understanding of watermelon's phytochemical composition and therapeutic effects, including its limited ethnomedicinal applications. It suggests that watermelon possesses a rich nutritional profile, numerous phytochemicals, and various therapeutic benefits.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Fruit Samples.

Fresh Fruit samples of *Citrullus lanatus* were purchased from Uselu Market, Ugbowo, Edo state Nigeria. The fruits were authenticated and the juices were then extracted from the fruits.

3.2 Equipment and Materials used

Materials used for this research includes: Hand-gloves, Sponges, Bucket, Weighing Scale, Syringes, Chloroform, Cotton Wool, Dissecting Tools, Beaker, Refrigerator, Measuring Cylinder, EDTA sample bottles, 77 Male Sprague Dawley Rats, Plastic cages, Wire Mesh, Watermelon Extract, Feed, Water, Plates, , Juice extractor, folic acid, Distilled water, Phenyl Hydrazine Hydrochloride (99%), pH meter.

3.3 Rats Study

Seventy seven (77) male Sprague-Dawley rats (*Rattus norvegicus*.) weighing between 150g-200g were used for this research. The animals were purchased and kept in the animal house of the Department of Anatomy, School Of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. They were kept in a clean cages at room temperature and had free access to water and rat feed. They were acclimatized to the laboratory conditions for two weeks before anaemia was induced and confirmed after three days. Administration began at the animal house of the Department of Anatomy for three weeks. This study was conducted for six weeks, including acclimatization period. Ethical approval was obtained from the University Animal Care and Use Committee (ACUC), ensuring compliance with ethical guidelines for animal research.

3.4 Methodology

3.4.1 Extraction of *Citrullus lanatus* juice

Extraction of juice from the fruits were done weekly in the Laboratory using a fruit Juice extractor and stored in the refrigerator.

3.4.2 Experimental Design and work plan

Phase one: Rats study

Rats Group

Male Sprague Dawley rats were assigned into 5 groups.

Group 1 (5 rats): Control

Group 2 (18 rats): Anaemia + distilled water.

Group 3 (18 rats): Anaemia + vitamin B complex plus folic acid

Group 4 (18 rats): Anaemia + *Citrullus lanatus* juice

Work Plan

1/10/24: Acclimatization

7/10/24: End of acclimatization

7/10/24-9/10/24: Induction of anaemia

To induce Anaemia, each groups were administered 20mg/Kg body weight intra peritoneal phenyl hydrazine hydrochloride injection. Rats which has its PCV level less than 35% were considered anaemic.

10/10/24: confirmation of anaemia,

To treat Anaemia, each group were administered 2ml/g body weight orally of their respective juices.

17/10/24: First sample collection and analysis

24/10/24: Second sample collection and analysis

21/10/24: Final sample collection and analysis

Phase two: Sickle cell study

7/10/24: Introduction to sickle cell and sample collection

8/10/24 – 10/10/24: Sample collection and analysis

14/10/24 – 17/10/24: Sample collection and analysis

21/10/24 – 24/10/24: Sample collection and analysis

28/10/24 – 1/11/24: Sample collection and analysis

The animals in each group were eventually sacrificed, the blood samples were collected in Ethylene diamine tetraacetic acid (EDTA) bottles, heparin, bottles and plain bottles

List of analysis

1. Full blood count (UBTH)
2. Stained slides of sample (UBTH)

3.5 Statistical Analysis

Data were analyzed using GraphPad Prism version 8.1 and expressed as mean ± SFM. A two-way analysis of variance (ANOVA) was performed to assess group differences, followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant. Findings were presented using graphs and tables where utilized.

CHAPTER FOUR

RESULTS

4.1 Red Cell Indices of Anaemic Rats Treated With Distilled Water, Vitamin B/Folic Acid Combination and Watermelon after First Week of Administration

The graphs show how different hematological parameters in Sprague-Dawley rats with anemia were affected by distilled water, a mixture of vitamin B and folic acid, and watermelon juice for a week. All treatment groups showed statistically significant increases in MCV, MCH, and RDW-SD when compared to the control ($p < 0.05$), with the exception of the anemia-induced group, which did not demonstrate any significant differences in RDW-SD ($p > 0.05$). MCHC, on the other hand, did not reveal any statistically significant differences between the groups ($p > 0.05$). Furthermore, there was no significant difference between the vitamin B/folic acid and watermelon-treated groups and the control group ($p > 0.05$), while RDW-CV significantly dropped in the anemia-induced group and increased in the distilled water group ($p < 0.05$).

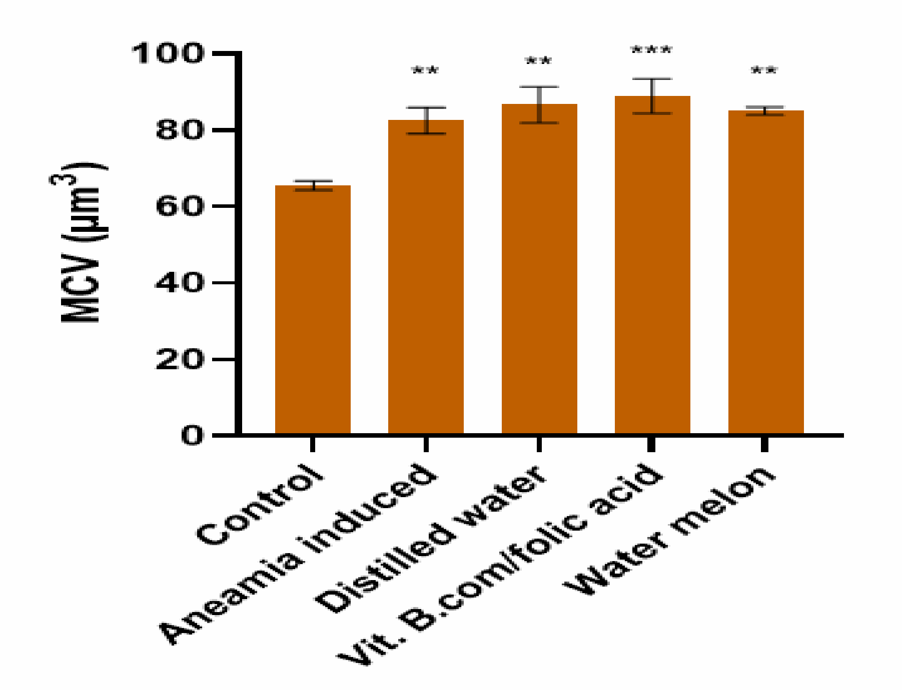

Figure 4.1: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 1 week on MCV in Sprague-Dawley induced with anaemia.

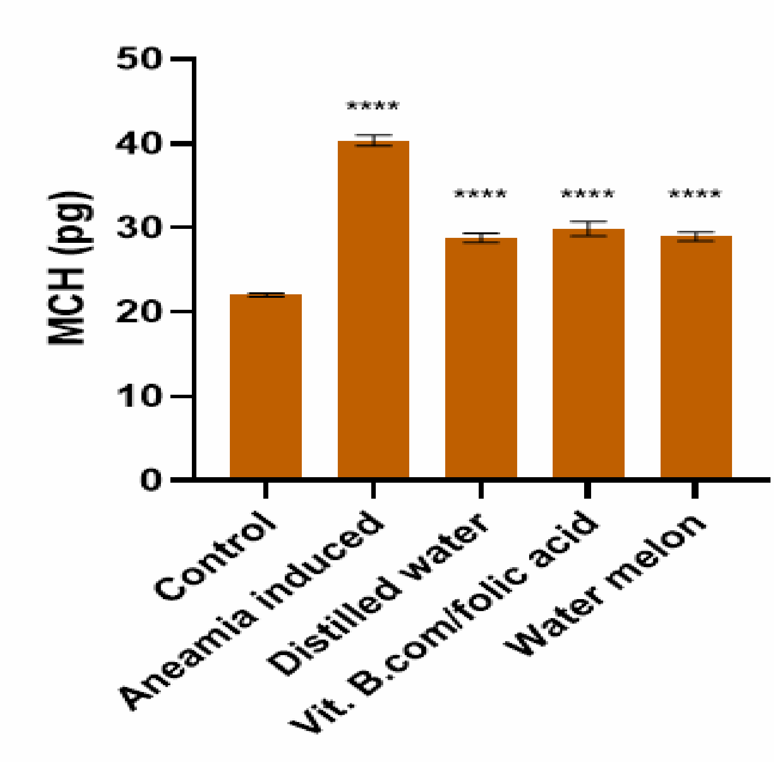

Figure 4.2: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 1 week on MCH in Sprague-Dawley induced with anaemia.

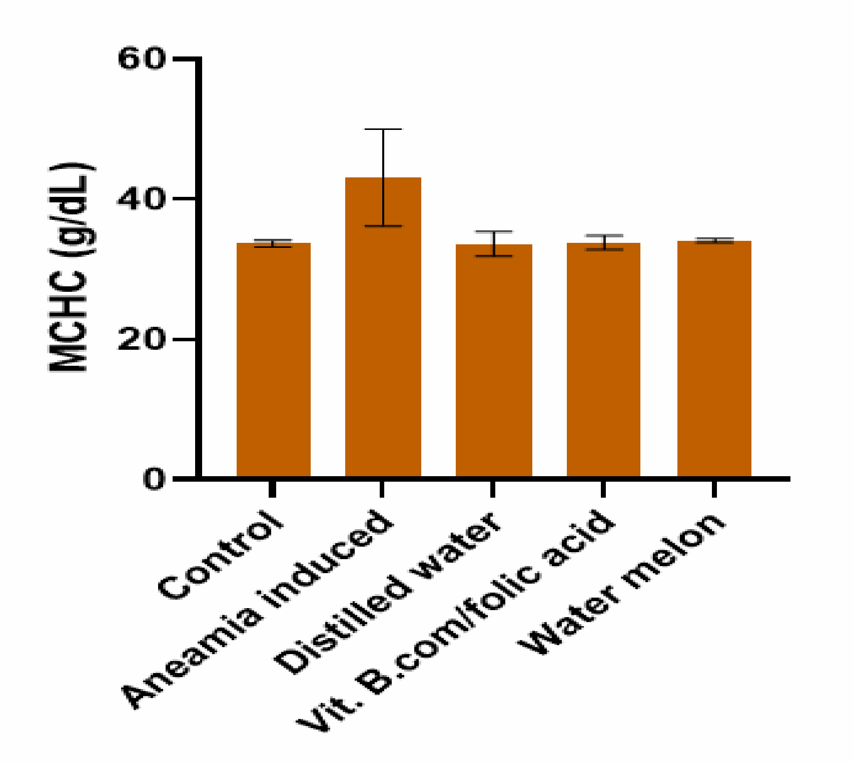

Figure 4.3: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 1 week on MCHC in Sprague-Dawley induced with anaemia.

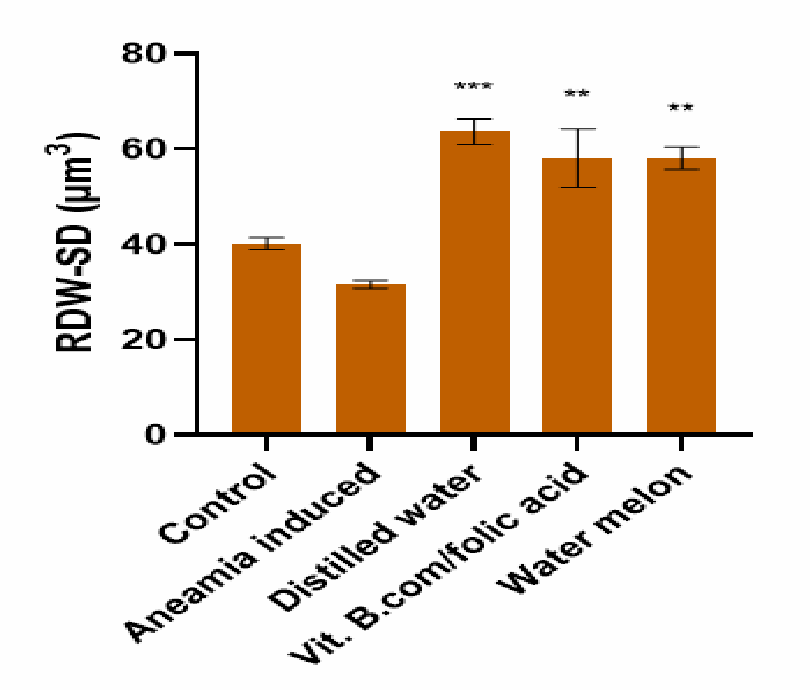

Figure 4.4: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 1 week on RDW-SD in Sprague-Dawley induced with anaemia.

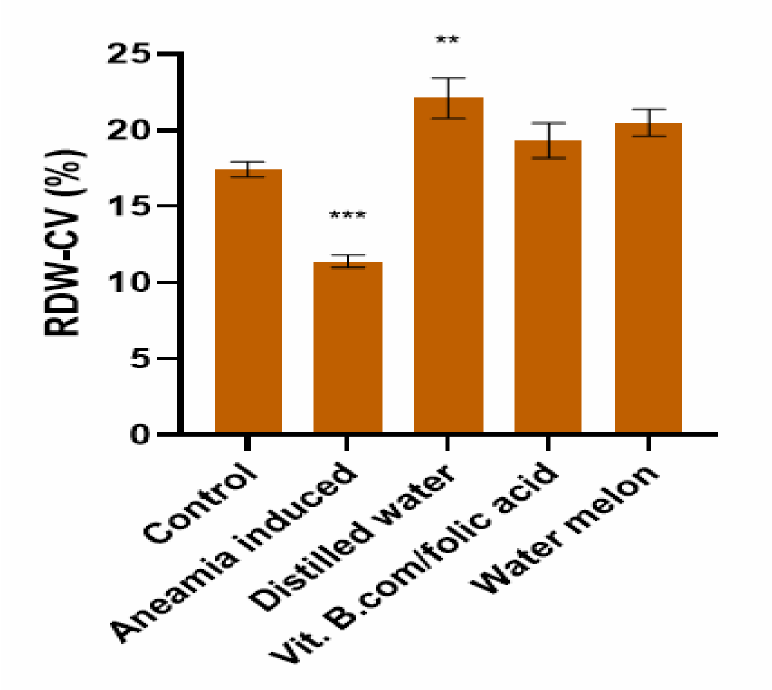

Figure 4.5: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 1 week on RDW-CV in Sprague-Dawley induced with anaemia

4.2 Red Cell Indices of Anaemic Rats Treated With Distilled Water, Vitamin B/Folic Acid Combination and Watermelon after Second Week of Administration

The graphs show that MCV and MCH increased statistically significantly in all treatment groups when compared to the control ($p < 0.05$). Although there was no statistically significant difference in MCHC across the groups ($p > 0.05$), RDW-SD rose significantly in the groups treated with distilled water, vitamin B/folic acid, and watermelon ($p < 0.05$), but stayed the same in the group that was induced by anaemia ($p > 0.05$). Furthermore, in comparison to the control, RDW-CV significantly decreased in the anaemia-induced group while increasing in the groups treated with distilled water, vitamin B/folic acid, and watermelon ($p < 0.05$).

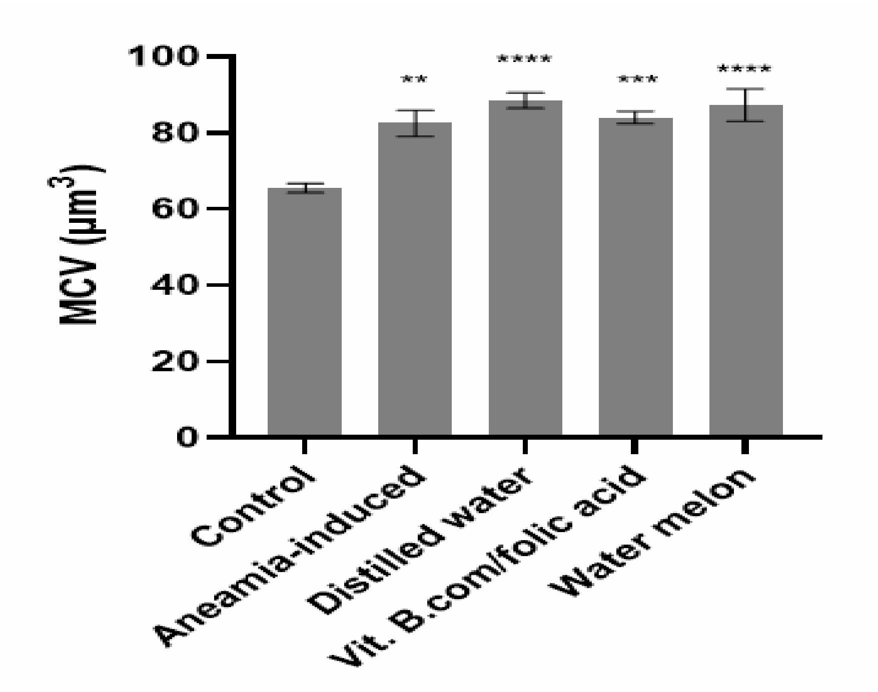

Figure 4.6; Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 2 weeks on MCV in Sprague-Dawley induced with anaemia

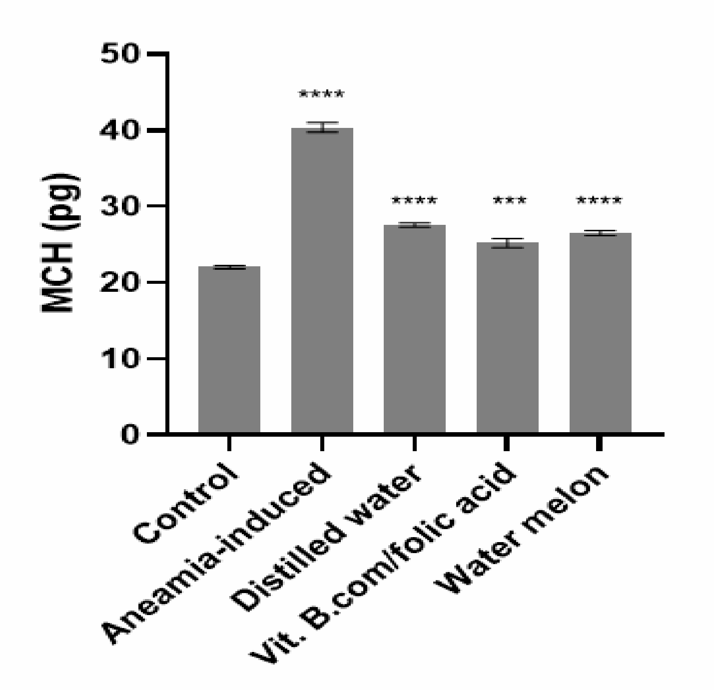

Figure 4.7: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 2 weeks on MCH in Sprague-Dawley induced with anaemia.

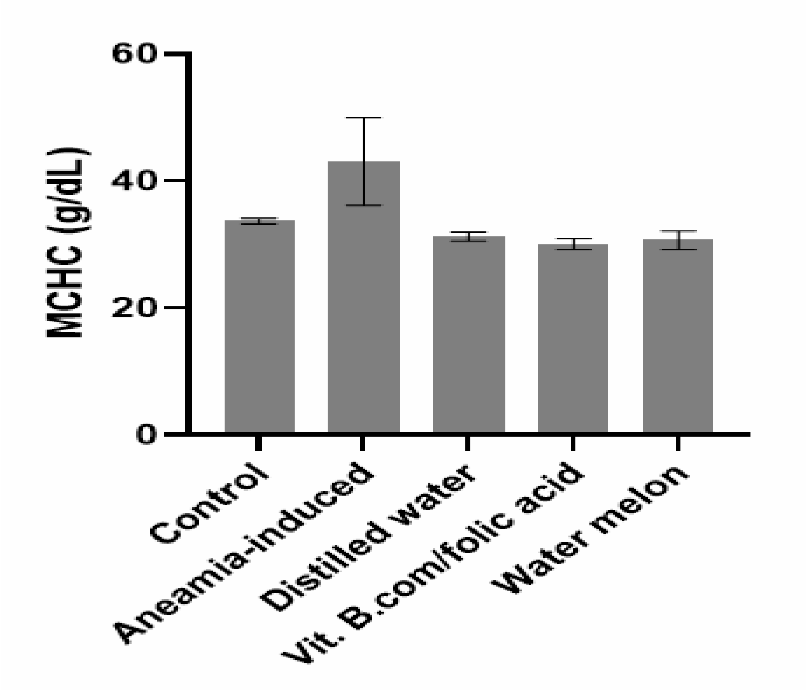

Figure 4.8: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 2 weeks on MCHC in Sprague-Dawley induced with anaemia.

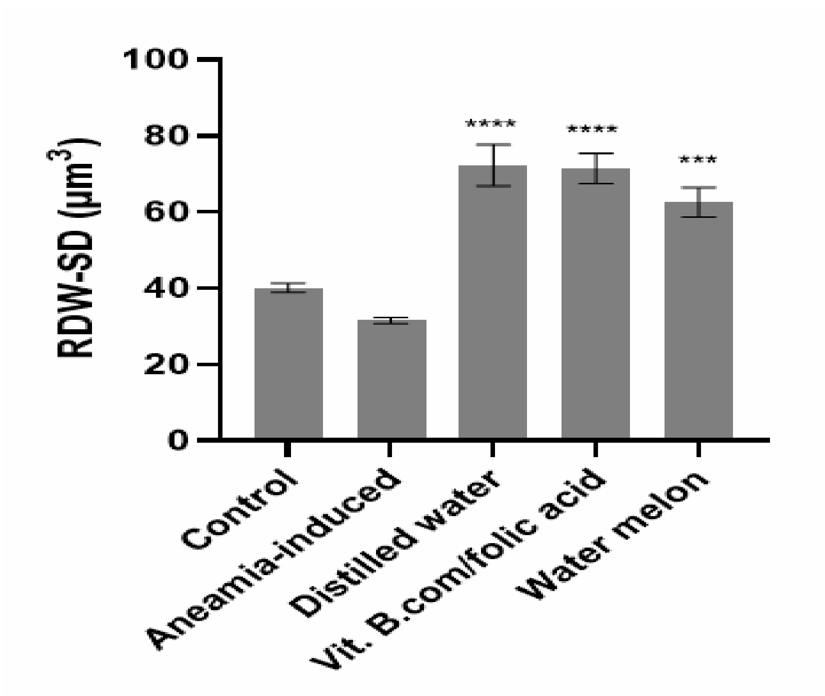

Figure 4.9: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 2 weeks on RDW-SD in Sprague-Dawley induced with anaemia

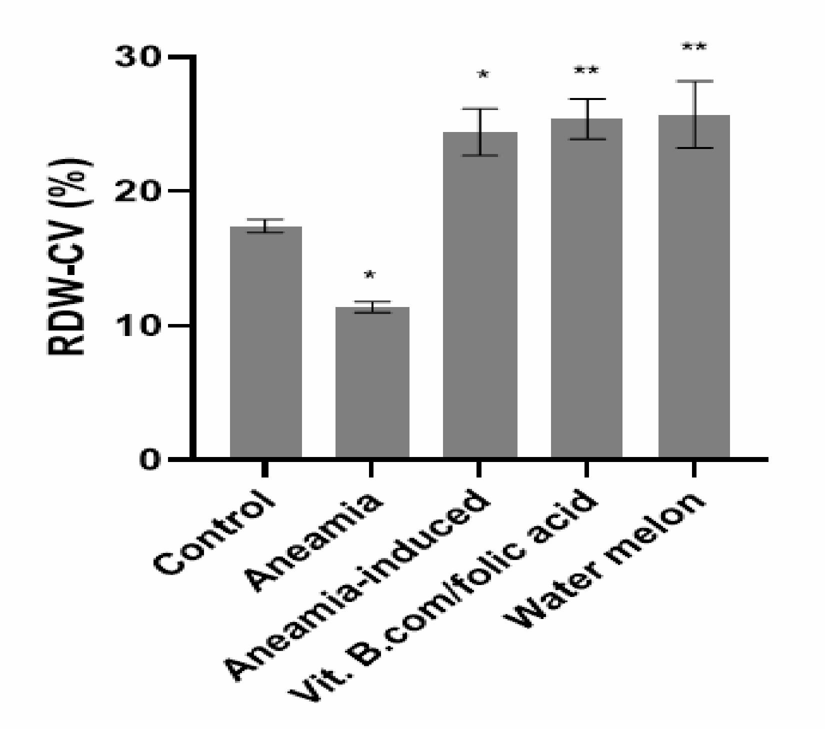

Figure 4.10: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 2 weeks on RDW-CV in Sprague-Dawley induced with anaemia.

4.3 Red Cell Indices of Anaemic Rats Treated With Distilled Water, Vitamin B/Folic Acid Combination and Watermelon after Third Week of Administration

The graphs show that, as compared to the control, the MCV increased statistically significantly in the anemia-induced and vitamin B/folic acid-treated groups ($p < 0.05$), but not significantly in the distilled water and watermelon-treated groups ($p > 0.05$). Across all groups, MCHC did not reveal any statistically significant differences ($p > 0.05$). In comparison to the control group, RDW-SD significantly decreased in the anemia-induced group but increased in the groups treated with watermelon, distilled water, and vitamin B/folic acid ($p < 0.05$). In a similar vein, RDW-CV rose in the vitamin B/folic acid and watermelon-treated groups ($p < 0.05$), declined considerably in the anemia-induced group, and did not significantly vary in the distilled water group from the control group ($p > 0.05$).

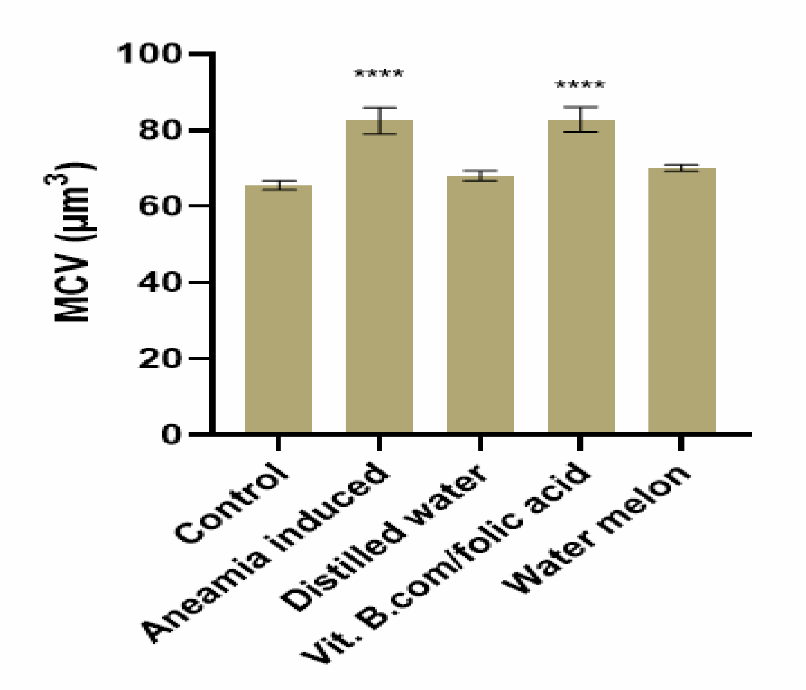

Figure 4.11: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 3 weeks on MCV in Sprague-Dawley induced with anaemia

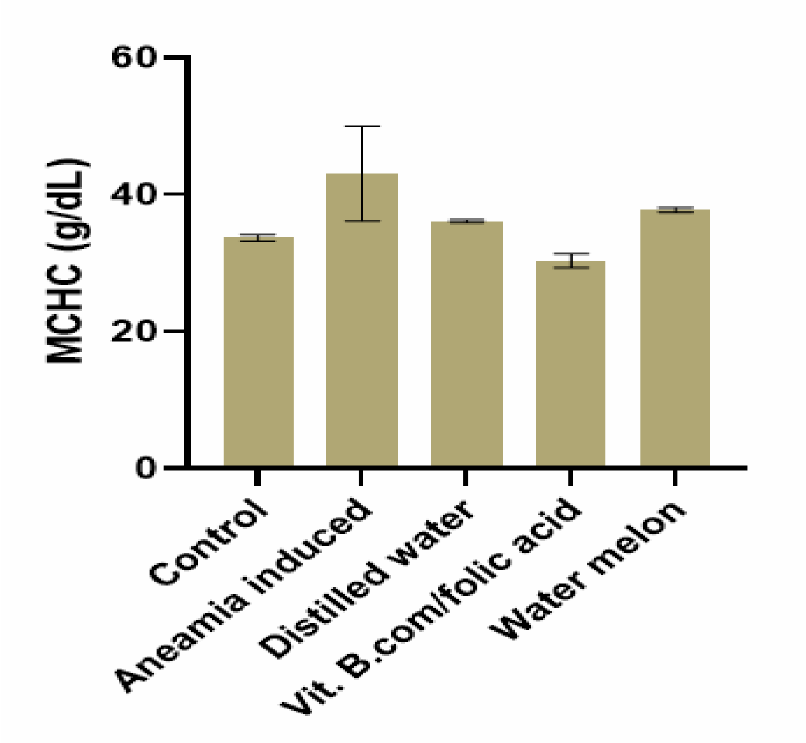

Figure 4.12: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 3 weeks on MCHC in Sprague-Dawley induced with anaemia.

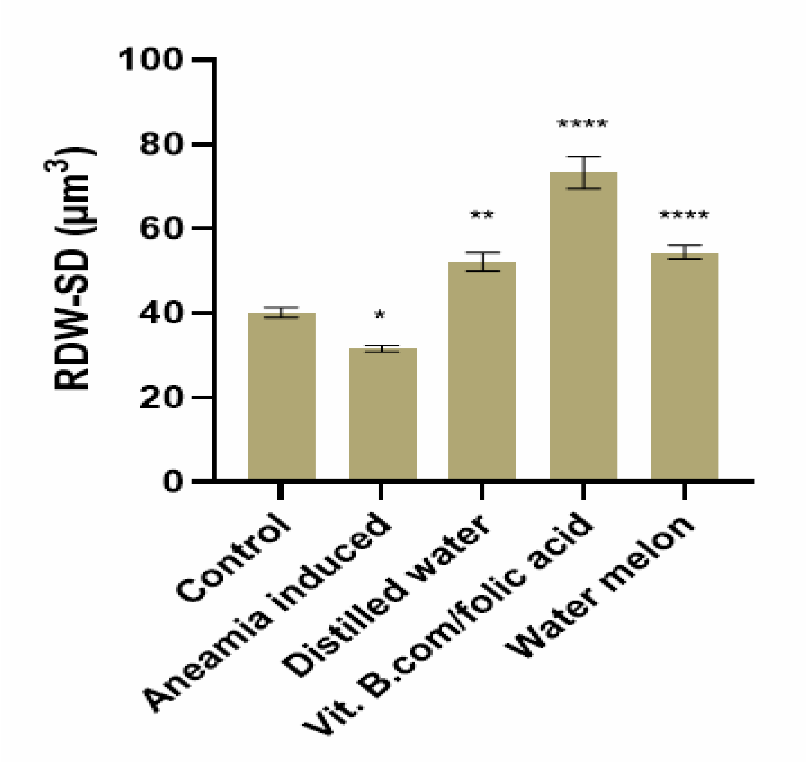

Figure 4.13: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 3 weeks on RDW-SD in Sprague-Dawley induced with anaemia

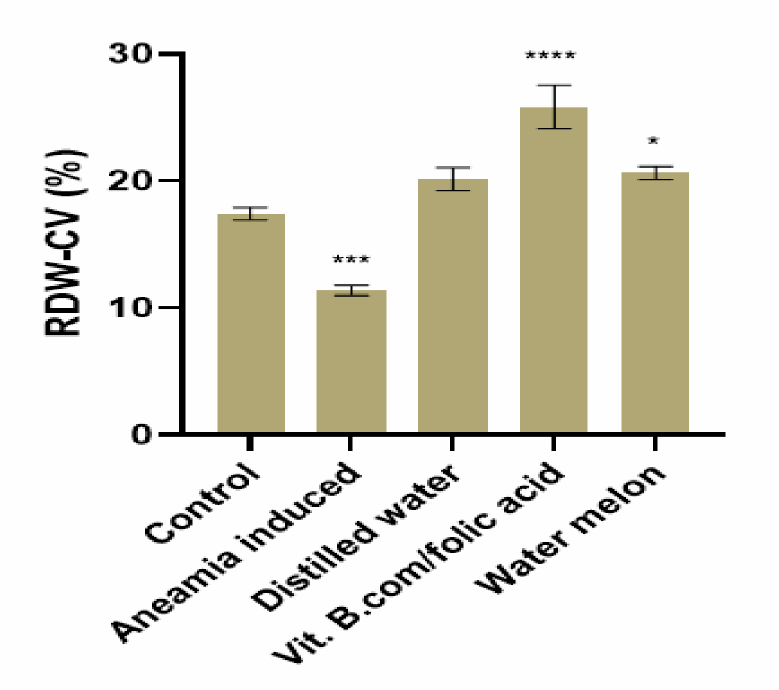

Figure 4.14: Chart showing the effect of distilled water, vitamin B/folic acid combination, and watermelon juice treatment for 3 weeks on RDW-CV in Sprague-Dawley induced with anaemia.

CHAPTER FIVE

DISCUSSION

The study's results and analysis show that during the course of three weeks, the administration of *Citrullus lanatus* juice improved red blood cell (RBC) indices, specifically mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The *Citrullus lanatus*-treated group's statistically significant increase in MCV and MCH over the control group raises the possibility that *Citrullus lanatus* juice could promote erythropoiesis and red blood cell formation.

5.1 Mean Corpuscular Volume (MCV)

All treatment groups showed a statistically significant increase in MCV when compared to the control ($p < 0.05$). The rise in MCV in all treatment groups points to better erythropoiesis, which can be brought on by *Citrullus lanatus's* bioactive and nutritional properties, such as lycopene, citrulline, and iron. It has been demonstrated that lycopene possesses antioxidant and hematopoietic properties (Rao *et al*., 2006) and also upregulates erythropoiesis (Wolffenbuttel *et al*., 2019). It does this by protecting the erythrocyte precursors from oxidative stress, which may hinder RBC development and function. An important part of hemoglobin synthesis, iron helps produce red blood cells and could be the cause of the observed rise in MCV (Naz *et al*., 2013). (Collins *et al.,* 2007; Zang *et al*., 2022).

5.2 Mean Corpuscular Hemoglobin (MCH)

MCH is a measure of the average amount of hemoglobin (a protein in red blood cells that carries oxygen) in each red blood cell. The MCH values in all treatment groups increased significantly, according to the results ($p < 0.05$). A significant increase in MCH values suggests enhanced hemoglobin synthesis in *Citrullus lanatus*-treated groups. An increase in MCH is usually as a result of larger, more hemoglobin-containing RBCs are produced. The most common cause of elevated MCH levels is macrocytic. The underlying cause of this is a Vitamin B12 deficiency or may involve the fruit's iron, and antioxidant properties anemia (Green and Allen, 2023). *Citrullus lanatus* iron content, which promotes iron absorption and utilization, may be responsible for this improvement (Naz *et al*., 2013; Ford, 2013).

5.3 Mean Corpuscular Hemoglobin Concentration (MCHC)

Mean Corpuscular Hemoglobin Concentration (MCHC) is a measure of the concentration of hemoglobin in a given volume of packed red blood cells. Throughout the three weeks of administration, no statistically significant changes in MCHC were seen across all groups ($p > 0.05$), in contrast to MCV and MCH. This suggests that *Citrullus lanatus* increased the absolute hemoglobin content per cell, but did not affect the relative concentration of hemoglobin in each cell.

5.4 Red Cell Distribution Width - Standard Deviation (RDW-SD)

When compared to the control group, the results show a substantial rise in RDW-SD in the *Citrullus lanatus*-treated group ($p < 0.05$), suggesting a difference in RBC size. The rise in RDW-SD is consistent with studies that show *Citrullus lanatus* juice increases red cell vatriation and stimulates RBC synthesis, both of which are crucial for managing anemia. This could be due to its high antioxidant and anti-inflammatory effects as Lycopene and other antioxidants in watermelon may reduce oxidative stress on RBCs, improving cell lifespan and quality (Smeet *et al*., 2022). This could contribute to improved RBC heterogeneity, as seen in higher RDW. The juice promoted the synthesis of red blood cells because erythropoiesis occurs after anemia is induced (Zhang *et al*., 2022).

5.5 Red Cell Distribution Width - Coefficient of Variation (RDW-CV)

During the course of the three weeks of administration, RDW-CV demonstrated a statistically significant drop in the anemia-induced group and an increase in the *Citrullus lanatus*-treated group. This implies that juice might aid in restoring RBC variability, which is essential for managing anemia. The results are similar with existing r research that highlights *Citrullus lanatus* nutritional and medicinal benefits. Iron, vitamin C, and citrulline are essential for the synthesis and upkeep of red blood cells (Collins *et al.,* 2007). Regular consumption of this juice may enhance hematological parameters because of its high level of bioactive substances, such as lycopene, flavonoids, and phenolic compounds, according to studies by Biswas *et al.,* (2017) and Perkins-Veazie *et al*. (2001).

FINDINGS

According to the results, watermelon juice improves RBC indices, especially via raising MCV and MCH. This suggests that during anaemia, it might improve hemoglobin concentration and increase the synthesis of red blood cells. The lack of significant changes in MCHC, however, indicates that although erythropoiesis is induced, the total hemoglobin content per cell stays constant.

CONCLUSION

According to the results obtained, *Citrullus lanatus* juice can have a beneficial effect on anaemic RBC indices, especially MCV and MCH. While more research is required to demonstrate its efficacy in human subjects, watermelon's nutritional profile suggests that include it in dietary regimens for the management of anemia may be therapeutic.

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