EXPRESSION OF ERFE GENE IN ALUMINUM CHLORIDE-INDUCED ANAEMIA BEARING WISTAR RATS TREATED WITH AQUEOUS LEAVES EXTRACT OF *Icacina trichantha*

BY:

AUDU WINNIFRED OMOYE
BMS2005026

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCE,
UNIVERSITY OF BENIN, BENIN CITY,
EDO STATE.

SEPTEMBER, 2025.

EXPRESSION OF ERFE GENE IN ALUMINUM CHLORIDE-INDUCED ANAEMIA BEARING WISTAR RATS TREATED WITH AQUEOUS LEAVES EXTRACT OF *Icacina trichantha*

BY:

AUDU WINNIFRED OMOYE
BMS2005026

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE (BMLS) IN MEDICAL LABORATORY SCIENCE.

SEPTEMBER, 2025

CERTIFICATION

This is to certify that this project is an authentic work carried out AUDU WINNIFRED OMOYE with matriculation number BMS2005026 under the supervision of DR. (MRS) P. A. OBAZELU in partial fulfillment of the requirement for the award of Bachelor's in Medical Laboratory Science Degree (BMLS) of the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin City, Benin City, Edo state.

DR. (MRS) Z. OMORUYI **DATE**

Head of Department

DR. (MRS) P. A. OBAZELU **DATE**

Supervisor

EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project to God almighty for making this project work a huge success.

ACKNOWLEDGEMENTS

I give thanks to God almighty for his grace upon my life, for granting me the strength, resources and wisdom needed for this work and seeing me through this project work.

My profound gratitude goes to my supervisor DR. (MRS.) P. A. OBAZELU for her concern, guidance, constructive and supportive idea which has aided this project work.

My very special thanks to the Head of Department, Medical Laboratory Science, DR. (MRS.) ZAINAB OMORUYI and also to the entire staff of the department for investing so much in my academic development. My appreciation goes to the entire scientist at the university of Benin Teaching Hospital.

I am deeply grateful for my parents, Mr. Audu Fidelis for sponsorship and endless support to my education and Mrs. Audu Victoria for her constant love and words of encouragement and to my lovely siblings for their love and support, encouragement and prayers.

I'm also grateful for my amazing friend, Osuji Rejoice for her impeccable love and support. May God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Anaemia is influenced by erythroferrone (ERFE), a regulator of hepcidin during stress erythropoiesis. Aluminium chloride induces anaemia through oxidative stress and impaired iron utilization. *Icacina trichantha*, a medicinal plant with reported haematopoietic and

antioxidant effects, may modulate ERFE expression in anaemic conditions. The aim of this study is to determine the effect of aqueous leaf extract of *Icacina trichantha* on ERFE gene expression in aluminium chloride-induced anaemia in albino Wistar rats. A total of sixty (60) adult male albino Wistar rats were divided into six (6) groups; A, B, C, D, E and F representing control, aluminium chloride group, ferrous sulphate group, aluminium chloride + 100 mg/kg *Icacina trichantha* extract, aluminium chloride + 200 mg/kg *Icacina trichantha* extract and aluminium chloride + 400 mg/kg *Icacina trichantha* extract respectively. Haematological parameters, blood cell morphology and mRNA ERFE expression were determined using haematology autoanalyzer, manual method and polymerase chain reaction respectively. Data obtained were analyzed by the Statistical Package for Social Science (SPSS) software. The comparison of red blood cell parameters showed that red blood cell count (RBC) of groups C (7.65±0.21), D (8.18±0.06), E (7.84±0.31) and F (8.05±0.29) showed no significant difference when compared with groups A (8.09±0.12) and B (7.74±0.21) ($p>0.05$). Haemoglobin Concentration (g/dL) of groups C (15.06±0.36), D (15.5±0.22), E (15±0.59) and F (15.1±0.61) was not significantly different from groups A (15.33±0.24) and B (14.84±0.32) ($p>0.05$). Haematocrit (%) of groups C (44.46±0.99), D (45.1±0.73), E (44.32±1.46) and F (43.86±1.52) was not significantly different from groups A (44.88±0.78) and B (44.52±0.96) ($p>0.05$). Mean cell volume (MCV) of group F (54.64±0.96) was significantly lower than group C (58.22±0.49) ($p<0.05$), while no significant differences were observed in other groups. Mean cell haemoglobin (MCH) of group F (18.72±0.23) was also significantly lower when compared to group C (19.66±0.07) ($p<0.05$). Mean cell haemoglobin concentration (MCHC), RDW-SD, and RDW-CV showed no significant differences among the groups ($p>0.05$). Normochromatic and normocytic cells were moderately present in most groups (A, C, D, E), while Group B showed reduced levels with increased crenated cells. Group F demonstrated higher normocytic cell presence alongside mild crenation. Polychromatic cells were absent across all groups. Groups B showed significantly higher expression of RUNX1 when compared to groups A ($p<0.05$). Groups B showed significantly lower expression of ERFE when compared to groups A ($p<0.05$). Group C, D, E and F had significantly higher expression of ERFE when compared to group B ($P<0.05$). In conclusion, treatment with ferrous sulphate and varying doses of *Icacina trichantha* extract caused changes in red blood cell parameters and also improved ERFE expression, with the highest extract dose showing the greatest effect.

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

Medicinal plants have been a major part of traditional healing systems for centuries, long before the introduction of synthetic drugs (Obazelu and Omoregie, 2024; Jamshidi *et al*., 2017). Across different cultures (African, Chinese, Indian, and even Native American), plants were used to treat a different health condition based on knowledge passed down through generations (Pan *et al*., 2014). In recent decades, scientific interest in these plants has increased significantly due to the discovery that many of their therapeutic effects are attributed to the presence of phytochemicals which are naturally occurring compounds with pharmacological activity (Ogbuagu *et al*., 2022). These phytochemicals include flavonoids, alkaloids, saponins, tannins, terpenes, glycosides, and phenolic compounds, and they all have various biological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Kaushik *et al*., 2021). Additionally, medicinal plants are often rich in essential minerals like iron, calcium, magnesium, potassium, and zinc which are elements that are important and necessary for numerous physiological functions including blood formation, enzyme activation, immune support, and oxygen transport (Ceccanti *et al*., 2021). The combination of these bioactive and nutritional constituents makes medicinal plants especially valuable in managing diseases related to oxidative stress, inflammation, and nutrient deficiency (Rapa *et al*., 2019). As the global demand for natural and cost-effective treatments with minimal side effects grows, there is a strong need to look towards and scientifically validate the health benefits of traditional plants used in local communities (Benyene *et al*., 2016).

One such plant that has gained attention for its medicinal potential is *Icacina trichantha*, a drought-resistant shrub indigenous to West and Central Africa. Known locally

by various names, it is primarily recognized for its large underground tubers, which are starchy and can serve as emergency food during times of scarcity (Che *et al*., 2016). However, beyond its nutritional role, *Icacina trichantha* has a good reputation in traditional African medicine. Different parts of the plant, especially the tubers and leaves, are used in the treatment of a variety of conditions including food poisoning, malaria, rheumatism, hypertension, asthma, and even as an aphrodisiac (Okieimen *et al*., 2018; Otun *et al*., 2015). The plant is often prepared by crushing or soaking the leaves or tubers in water or local gin to extract its medicinal compounds. Phytochemical studies have shown that *I. trichantha* contains an abundance of flavonoids, terpenoid, glycosides, tannins, and phenols which are compounds known to possess strong antioxidant and haematoprotective properties (Otun *et al*., 2015). These antioxidants help to scavenge free radicals and protect cells, especially in organs like the bone marrow where new blood cells are formed (Obazelu and Williams, 2024; Engwa *et al*., 2022). In addition, mineral analysis reveals the presence of iron, magnesium, calcium, and potassium, which are important for red blood cell production and maintenance of overall haematological health (Alawode, 2024). These properties make *Icacina trichantha* a strong contender for investigating its potential in reversing anaemia, particularly that which is caused by toxic chemical exposure (Alawode, 2024).

Anaemia is a public health issue affecting over a billion people worldwide. It occurs when the blood lacks sufficient healthy red blood cells or haemoglobin, leading to reduced oxygen delivery to tissues. This can result in fatigue, weakness, poor concentration, and, in severe cases, organ damage (Noreen *et al*., 2020). Anaemia has a wide range of causes, including nutritional deficiencies (especially iron, vitamin B12, and folate), chronic diseases (like kidney failure or cancer), genetic disorders (such as sickle cell disease), and infections (like malaria or HIV) (Hussein *et al*., 2023). In recent times, attention is also being drawn to environmental and chemical factors as contributors to anaemia. One such chemical is

aluminum chloride, a compound commonly found in industrial and household products, including water treatment agents, antiperspirants, and certain pharmaceuticals (Sanajou *et al*., 2021). Although it has many practical uses, aluminum chloride can be toxic when it accumulates in the body. Studies have shown that aluminum ions can generate reactive oxygen species (ROS), which cause oxidative stress and damage to cellular structures. This includes the bone marrow, where blood cells are produced (Kumar and Gill, 2014). When the hematopoietic stem cells in the bone marrow are exposed to oxidative stress, their ability to divide and differentiate into mature red blood cells is impaired, leading to a drop in red cell production and the onset of anaemia (Richardson *et al*., 2015).

One of the regulators of red blood cell production under stress is a hormone known as erythroferrone (ERFE). Erythroferrone is a protein hormone secreted by erythroblasts, the immature red blood cell precursors in the bone marrow, especially during conditions that demand an increased production of red blood cells, such as anaemia or after significant blood loss (Srole and Ganz, 2021). Its primary function is to regulate iron homeostasis by acting as a suppressor of hepcidin, a liver-derived peptide that is the central regulator of systemic iron metabolism (Babar and Saboor, 2024). Hepcidin normally inhibits intestinal iron absorption and traps iron within storage sites by degrading ferroportin, the only known cellular iron exporter (Nemeth and Ganz, 2021). During anaemia or hypoxia, ERFE is released in greater amounts to counteract hepcidin, thereby increasing the availability of circulating iron necessary for efficient erythropoiesis. This mechanism ensures that iron is mobilized from stores in the liver and macrophages and made available for haemoglobin synthesis in developing red blood cells (Coffey and Ganz, 2018).

However, in pathological conditions such as aluminum chloride-induced anaemia, the normal expression and function of ERFE can be impaired. Exposure to aluminum chloride leads to oxidative damage of erythroid progenitor cells, reducing their capacity to synthesize ERFE.

As a result, hepcidin levels remain inappropriately high despite the anaemic state, restricting iron availability and further compromising red blood cell production (Babar and Saboor, 2024). This disrupts the natural compensatory mechanism meant to correct anaemia, creating a state of functional iron deficiency where the body has adequate iron stores but cannot effectively utilize them due to persistent hepcidin activity (Ganz, 2019). This makes ERFE not only a critical hormone in iron balance but also a sensitive biomarker for evaluating the severity and progression of anaemia, especially in toxin-induced models. Investigating ERFE gene expression provides important information into the molecular pathways affected by toxicity and also offers a measurable way to assess the efficacy of potential therapeutic agents (Tomasz *et al*., 2021).

1.2. Statement of Problem

Aluminum chloride, a commonly used industrial and pharmaceutical compound, has been shown to exert toxic effects on the hematopoietic system by generating reactive oxygen species that cause oxidative stress and damage to bone marrow progenitor cells. This oxidative damage interferes with normal erythropoiesis and can affect the regulation of important molecular signals such as erythroferrone (ERFE), a hormone that plays a role in mobilizing iron for red blood cell production. ERFE functions by suppressing hepcidin, the main regulator of iron metabolism, thereby ensuring adequate iron availability during periods of increased erythropoietic demand. However, under toxic conditions such as exposure to aluminum chloride, the expression of the ERFE gene may be reduced, leading to iron-restricted erythropoiesis and worsening of the anaemic condition. Despite the recognized importance of ERFE in erythropoietic regulation, there is limited research on how its expression is affected in aluminum chloride-induced anaemia. In addition, while *Icacina trichantha* is a medicinal plant traditionally used in African communities for the treatment of various ailments, its potential role in protecting against anaemia through modulation of ERFE

gene expression has not been adequately studied. Although the plant is known to contain antioxidant and haematoprotective phytochemicals, scientific evidence supporting its effect on molecular regulators of erythropoiesis remains scarce.

1.3. Justification of Study

Anaemia is a common health problem, especially in developing countries where exposure to toxins like aluminum chloride contributes to its development by damaging the bone marrow and disrupting red blood cell production. One important regulator that can be affected is erythroferrone (ERFE), a hormone that controls iron availability for making new blood cells, but there is limited research on how ERFE expression changes in aluminum chloride-induced anaemia. *Icacina trichantha* contains antioxidants and minerals that may protect against oxidative damage and support blood formation, yet its effect on molecular factors like ERFE remains unclear. This study is important because it will investigate how ERFE gene expression is altered in aluminum chloride-induced anaemia and whether treatment with *Icacina trichantha* leaf extract can help restore normal erythropoiesis, potentially offering an affordable natural therapy for toxin-related anaemia.

1.4. Aim of Study

The aim of this study is to determine the expression of erythroferrone (ERFE) gene in aluminium chloride-induced anaemia bearing wistar rats treated with aqueous leaves extract of *Icacina trichantha.*

1.5. Specific Objectives

1. To determine the expression of ERFE gene in aluminium chloride-induced anaemia bearing wistar rats treated with aqueous leaves extract of *Icacina trichantha*.
2. To determine the effect of *Icacina trichantha* aqueous leaf extract on some haematological parameters in aluminium chloride-induced anaemia bearing wistar rats.

3. To observe the effect of *Icacina trichantha* aqueous leaf extracts on the morphology of blood cells in aluminium chloride-induced anaemia bearing wistar rats.
4. To determine the ameliorative capacity of *Icacina trichantha* aqueous leaf extract on aluminium chloride-induced anaemia bearing wistar rats.

1.6. Research Questions

1. Does the aqueous leaf extract of *Icacina trichantha* have any effect on ERFE gene expression in aluminium chloride-induced anaemia in Wistar rats?
2. Does the aqueous leaf extract of *Icacina trichantha* have any effect on haematological parameters in aluminium chloride-induced anaemia in Wistar rats?
3. Does the aqueous leaf extract of *Icacina trichantha* have any effect on the morphology of blood cells in aluminium chloride-induced anaemia in Wistar rats?
4. Does the aqueous leaf extract of *Icacina trichantha* possess ameliorative capacity against aluminium chloride-induced anaemia in Wistar rats?

1.7. Research Hypothesis

1.7.1. Null Hypothesis (H_o)

1. Aqueous leaf extract of *Icacina trichantha* does not have any effect on ERFE gene expression in aluminium chloride-induced anaemia in Wistar rats.
2. Aqueous leaf extract of *Icacina trichantha* does not have any effect on haematological parameters in aluminium chloride-induced anaemia in Wistar rats.
3. Aqueous leaf extract of *Icacina trichantha* does not have any effect on the morphology of blood cells in aluminium chloride-induced anaemia in Wistar rats.
4. Aqueous leaf extract of *Icacina trichantha* does not possess ameliorative capacity against aluminium chloride-induced anaemia in Wistar rats.

1.7.2. Alternate Hypothesis (H_A)

1. Aqueous leaf extract of *Icacina trichantha* has an effect on ERFE gene expression in aluminium chloride-induced anaemia in Wistar rats.
2. Aqueous leaf extract of *Icacina trichantha* has an effect on haematological parameters in aluminium chloride-induced anaemia in Wistar rats.
3. Aqueous leaf extract of *Icacina trichantha* has an effect on the morphology of blood cells in aluminium chloride-induced anaemia in Wistar rats.
4. Aqueous leaf extract of *Icacina trichantha* possesses ameliorative capacity against aluminium chloride-induced anaemia in Wistar rats.

1.8. Scope of Study

This study was designed to cover the effect of *Icacina trichantha* and Aluminium chloride on some molecular markers of Iron metabolism and erythropoiesis and also some haematological parameters.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of *Icacina trichantha*

Icacina trichantha Oliv., a member of the family *Icacinaceae*, is an indigenous shrub native to the lowland tropical zones of West and Central Africa, where it is naturally distributed across a broad ecological range extending from Guinea and Sierra Leone in the west to Cameroon, Gabon, and the Democratic Republic of Congo in the central part of the continent (Ekeke *et al*., 2021). The species is believed to have originated in the Guineo-Congolian and Sudanian savanna regions, which serve as centers of plant endemism and domestication in sub-Saharan Africa. Its distribution is closely linked to traditional subsistence systems, as it has long been utilized by indigenous communities for both its medicinal and nutritional value (EI and Agianaku, 2015). It flourishes in secondary forests, savanna grasslands, and open woodlands, particularly on sandy or lateritic soils that are well-drained and low in fertility conditions under which many other food crops fail to thrive (Olde, 2021). In Nigeria, where the plant is most extensively documented, *I. trichantha* grows predominantly in the southern, south-eastern, and southwestern regions, especially in Enugu, Anambra, Ebonyi, Osun, Oyo, and Ogun states (Ekeke *et al*., 2021). It is known as “Urumbia” or “Eriagbo” among the Igbo people, where it is often associated with traditional healing and purging practices, while the Yoruba communities refer to it as “Gbegbe”, a name rooted in its cleansing and detoxifying properties. In these regions, it is not only harvested from the wild but is occasionally preserved in home gardens or protected in the wild for later use, especially during periods of food scarcity (Adedeji, 2023). The shrub typically reaches a height of 1.5 to 2 meters and is

most recognizable by its large underground tuberous roots, which can weigh up to several kilograms and serve as emergency food during famine or prolonged droughts. These tubers are rich in starch but contain bitter and potentially toxic secondary metabolites, necessitating traditional processing techniques such as soaking, fermentation, and drying before consumption (Enebeli *et al*., 2021). The geographic spread of I. *trichantha* has been supported not only by its ecological resilience but also by the deep cultural knowledge and ethnobotanical practices of local populations who have passed down its uses through oral traditions and practical applications. The plant's fruits (small drupes with a sweet outer pulp) also contribute to its propagation, as animals and humans aid in the dispersal of its seeds (Chauhan *et al*., 2018). Although not widely cultivated on a commercial scale, its presence in local pharmacopoeias and as a famine-resilient “wild crop” has prompted growing scientific interest in its domestication, conservation, and pharmacological evaluation (Sharma *et al*., 2021).

Figure 2.1. Leaves of *Icacina trichantha* (Alawode, 2024).

Table 2.1. Some species of *Icacina* according to International Board for Plant Genetic Resources (IBPGR, 1992).

S/N	Species
1	*Icacina trichantha*
2	*Icacina oliviformis*
3	*Icacina mannii*
4	*Icacina senegalensis*
5	*Icacina guessfeldtii*
6	*Icacina claessensii*
7	*Icacina schweinfurthiana*

Table 2.2. Taxonomy of *Icacina trichantha* (Alawode, 2024).

Domain	*Eukaryota*
Kingdom	*Plantae*
Class	*Angiosperms*
Order	*Icacinales*
Family	*Icacinaceae*
Genus	*Icacina*
Species	*trichantha*

2.1.1. Uses of Some Plant Parts of *Icacina trichantha*

- Seeds: The seeds of *Icacina trichantha* are highly valued in various West African communities for their medicinal significance. Traditionally, they are employed as natural remedies for fever reduction and are consumed as general health tonics to promote overall well-being (Otun *et al*., 2015). In many local traditions, the seeds are crushed and mixed with water or combined with extracts from other herbal plants to form a drinkable solution used in the treatment of respiratory ailments, such as bronchitis, catarrh, and pneumonia. Additionally, the seeds are believed to possess aphrodisiac properties, and in some communities, they are administered specifically to enhance male sexual performance and vitality, especially in older men or those experiencing fatigue or reduced libido (Orimisan *et al*., 2023).
- **Fruits**: The fruits of *Icacina trichantha* are not only edible but also serve important nutritional and medicinal purposes. The soft, sweet pulp of the drupe is often consumed raw, particularly by rural dwellers, as a source of quick energy and relief from digestive problems, notably constipation and dysentery (Idu *et al*., 2017). In some regions, the fruit is fermented with local beverages, such as palm wine, to amplify its medicinal effects and create a tonic believed to improve gastrointestinal function. Interestingly, the outer shell or skin of the fruit is also used medicinally. It is often soaked in palm wine or herbal brews and consumed as a febrifuge, particularly during malaria episodes, to help lower body temperature and reduce chills (Atawodi *et al*., 2014).

- **Leaves**: The leaves of *Icacina trichantha* have a wide range of applications in traditional medicine, particularly for their anthelmintic, anti-inflammatory, and antimicrobial properties. They are primarily known as natural vermifuges, employed to expel intestinal worms and parasites, especially in children and in communities with limited access to pharmaceutical deworming agents. The fresh leaf sap is traditionally instilled into the ear canal to relieve earaches and treat ear infections, likely due to its presumed anti-inflammatory action. Additionally, crushed fresh leaves are prepared as poultices or topical applications, which are applied to the skin to soothe irritation, insect bites, cuts, wounds, and inflammatory skin lesions (Olayemi *et al*., 2022).
- **Bark**: The bark of *Icacina trichantha* is traditionally harvested and used as a potent purgative and laxative, supporting the body's detoxification processes and cleansing the gastrointestinal tract. It is commonly used in the treatment of venereal diseases, such as syphilis and gonorrhoea, as well as in the management of other systemic infections (Obode *et al*., 2020). A decoction of the bark, often prepared by boiling the dried or fresh bark in water, is consumed to reduce fever and alleviate symptoms of febrile illnesses, including malaria, jaundice, and typhoid-like syndromes. In some cultures, the bark is processed into a bitter tonic that is regularly consumed to support liver function, enhance bile secretion, and purify the blood. These uses highlight the bark's integral role in traditional detoxification regimens and infectious disease management (Olayemi *et al*., 2022).
- **Root**: The root of *Icacina trichantha* is widely used in traditional medicine due to its diverse therapeutic benefits. It functions primarily as a vermifuge and general health tonic, commonly administered to eliminate intestinal parasites and restore physical strength. Decoctions and infusions made from the root are often consumed to treat

malaria, manage high fevers, and relieve symptoms of pneumonia and other respiratory infections. The root is also considered effective in promoting healthy digestion and relieving gastrointestinal disturbances, such as stomach cramps, diarrhea, and bloating. Beyond its curative properties, the root is also ingested for immune system enhancement and as a vitality booster, especially in individuals recovering from illness or prolonged fatigue. This wide-ranging use underscores the root's reputation as a rejuvenating herbal medicine in many indigenous African healthcare systems (Ekpo *et al*., 2008).

2.1.2. Minerals in the Leaves of *Icacina trichantha*

Proximate analysis reveals that the leaves are exceptionally rich in crude fibre, with a content of 43.25%, indicating their potential usefulness in promoting digestive health and preventing constipation. They also possess a high crude protein content of 26.97%, which is notably significant for a leafy plant, suggesting their role as a valuable protein source in local food systems. Moisture content is relatively low at 0.19%, indicating good shelf stability when dried. The ash content stands at 7.20%, reflecting the total mineral content present in the leaves. Carbohydrate content is 22.37%, contributing to the energy value of the leaves, while crude fat is minimal at 0.02%, characteristic of most green leafy vegetables (Alawode, 2024). Mineral analysis further highlights the nutritional richness of the leaves. Potassium (K) is the most abundant mineral at 1111.00 mg/100g, making the leaves beneficial for cardiovascular health and muscle function. The calcium (Ca) content is 290.00 mg/100g, essential for bone development and maintenance. Magnesium (Mg), another key macro-mineral, is present at 338.00 mg/100g, supporting enzymatic activities and neuromuscular function. Notably, the leaves also contain appreciable levels of iron (Fe) at 21.14 mg/100g, which is vital for hemoglobin synthesis and the prevention of anaemia. Trace elements such as zinc (Zn) at

6.83 mg/100g and copper (Cu) at 0.76 mg/100g are present in concentrations supportive of immune function and antioxidant defense (Alawode, 2024).

2.1.3. Phytochemical Constituents of the Leaves of *Icacina trichantha*

Phytochemical screening of *Icacina trichantha* leaf extracts revealed the presence of several important secondary metabolites, confirming its medicinal relevance. The screening was carried out using three solvents: n-hexane (HEX/IT), ethyl acetate (EA/IT), and ethanol (ET/IT). Tannins, known for their astringent and antimicrobial properties, were detected in trace amounts in all extracts. Phenolic compounds, which possess antioxidant activity, were also present in all extracts, with the ethanol extract showing a stronger presence. Terpenoids, which contribute to anti-inflammatory and anticancer effects, were observed in all extracts in moderate amounts. Glycosides, associated with cardioprotective and antimicrobial actions, were strongly present in each extract. Steroids, often linked to anti-inflammatory effects, were found in small quantities across the three extracts. Flavonoids, well-known for their antioxidant and anti-inflammatory roles, were also present in trace amounts in all samples. Interestingly, saponins were absent in all extracts, suggesting they are not a major component of *Icacina trichantha* leaves. Among the solvents, the ethanol extract showed the most robust phytochemical presence, particularly in phenols and glycosides (Otun *et al*., 2015).

2.1.4. Antioxidant Activity of *Icacina trichantha*

Several *in vitro* investigations have explored the antioxidant properties of different parts of *Icacina trichantha*, including the leaves, wood, and roots. These studies consistently reported moderate free radical scavenging activity, primarily evaluated using the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. This method assesses how effectively plant extracts neutralize free radicals and reduce oxidative stress (Udeh and Nwaehujor, 2011). Among the parts tested, the leaf extracts demonstrated particularly notable antioxidant activity, which closely correlated with their total phenolic content. Phenolic compounds are known for their

ability to stabilize free radicals by donating hydrogen atoms or electrons, thereby mitigating potential cellular damage (Oke and Hamburger, 2002). Further fractionation studies confirmed that the hexane extract of *Icacina trichantha* leaves also exhibited significant antioxidant activity. This was further validated by several independent reports, which observed similar effects and suggested that non-polar bioactive constituents in the hexane fraction significantly contribute to the plant's antioxidant capacity (Otun *et al*., 2015).

2.1.5. Antimicrobial Activity of *Icacina trichantha*

The antimicrobial activity of *Icacina trichantha* leaf was first demonstrated *in vitro* against *Pseudomonas aeruginosa* and *Escherichia coli*, two clinically important bacterial pathogens (Timothy and Idu, 2011). Later studies revealed that both ethanol and water extracts of the leaves could inhibit a broader range of microorganisms, including *Staphylococcus aureus*, a common cause of skin infections; *Candida albicans*, a fungal pathogen responsible for opportunistic infections; and *Klebsiella pneumoniae*, often linked to respiratory and urinary tract infections (Shagal and Kubmarawa, 2013). Additionally, non-polar solvent extracts like hexane and ethyl acetate showed antimicrobial effects against *E. coli*, *P. aeruginosa*, and *Klebsiella oxytoca*, indicating that different groups of bioactive compounds contribute to the plant’s antimicrobial activity. The wide spectrum of activity observed among the various extracts suggests the presence of multiple phytochemicals capable of combating diverse pathogens (Sofidiya *et al*., 2006). These findings validate the traditional use of *I. trichantha* in treating infections and emphasize its potential as a natural source for new antimicrobial agents, especially important given the increasing problem of antibiotic resistance (Otun *et al*., 2015).

2.1.5. Antidiabetic activity of *Icacina trichantha*

Icacina trichantha has demonstrated promising antidiabetic properties, particularly through experimental models involving alloxan-induced diabetic mice. Alloxan is a known β-cell cytotoxin that selectively destroys the insulin-producing β-cells of the islets of Langerhans in the pancreas, leading to a significant reduction in endogenous insulin secretion and impaired glucose utilization by peripheral tissues. This results in elevated blood glucose levels, decreased protein synthesis, and increased concentrations of cholesterol and triglycerides, thereby mimicking the metabolic profile observed in human diabetes mellitus (Ezeigbo, 2010). In an experimental study, methanolic extracts of *I. trichantha* leaves (ITLE) were administered orally at graded doses of 200 mg/kg, 300 mg/kg, and 450 mg/kg body weight to diabetic mice. The results revealed a dose-dependent reduction in fasting blood glucose levels over a 12-hour period, with the most significant glucose-lowering effects observed at the 6- and 12-hour time points. At a dose of 450 mg/kg, ITLE significantly reduced blood glucose from 8.87 mmol/L at baseline to 3.32 mmol/L at 12 hours. The 300 mg/kg dose also produced notable reductions, demonstrating the extract's efficacy in managing hyperglycaemia. These effects were comparable to those observed with glibenclamide, a standard antidiabetic drug used as a positive control. The study suggests that *I. trichantha* leaves possess bioactive phytochemicals capable of enhancing glycemic control, potentially through mechanisms such as stimulation of residual β-cell activity, enhancement of peripheral glucose uptake, or antioxidant-mediated protection of pancreatic tissues (Ezeigbo, 2010).

2.2. Iron Physiology

Iron ranks as the second most abundant metal on Earth, accounting for roughly 5% of the Earth's crust. It holds immense importance in human biology as a vital micronutrient essential for life. As a transition metal located in the d-block of the periodic table, iron can shift

between different oxidation states. This property enables it to participate in electron transfer processes and bind with various biological molecules (Pantopoulos *et al*., 2012). The two principal forms of iron are the ferrous state (Fe^{2+}) and the ferric state (Fe^{3+}). In the human body, iron functions as a cofactor for both haem and non-haem proteins. Haemoproteins include hemoglobin and myoglobin, which are involved in oxygen transport and storage, along with enzymes such as catalase and peroxidase that play roles in oxygen metabolism. Cytochromes also require iron to facilitate electron transport and mitochondrial respiration. Non-haem iron proteins are equally important, contributing to DNA synthesis, cell growth and differentiation, gene regulation, drug metabolism, and the production of steroid hormones (Pantopoulos *et al*., 2012).

Iron is crucial for blood formation and plays a central role in hemoglobin structure, cytochrome activity, and enzymatic reactions. Within the human body, iron exists in both functional and storage forms, and it can be efficiently recycled from aging red blood cells. The small intestine is the primary site of iron absorption, although only about 10% of ingested iron is actually absorbed. A regular dietary intake is required to support growth in children and to replenish iron lost through the shedding of skin and intestinal cells (Gulec *et al*., 2014). The need for iron is even greater during infancy and adolescence due to increased growth demands. The efficiency of iron absorption depends largely on its chemical form. Haem iron, found in animal sources, is more readily absorbed compared to non-haem iron. Non-haem iron requires reduction to the ferrous state by gastric secretions before it can be released from food and absorbed. Certain dietary substances such as tea tannins, calcium, and phytates found in grains and legumes hinder the absorption of non-haem iron. In contrast, vitamin C enhances iron absorption (Lane and Richardson, 2014).

Once absorbed, iron is bound to transferrin, a transport protein synthesized in the liver. The production of transferrin is influenced by the body's iron status. In conditions of iron

deficiency, transferrin synthesis increases. In chronic illnesses, its synthesis tends to decrease. Iron is stored in the body in the form of ferritin or hemosiderin. During red blood cell production, these iron reserves are mobilized. Ferritin, which is soluble and readily accessible, is stored in hepatocytes, splenic macrophages, bone marrow, serum, and red blood cells. The concentration of ferritin in the blood reflects the total amount of stored iron (Wang and Pantopoulous, 2011). Iron is essential for synthesizing haem-containing enzymes and other iron-dependent enzymes that support electron transfer and oxidation-reduction reactions. It also contributes to the production of oxygen-carrying proteins such as hemoglobin and myoglobin (Bilska *et al*., 2017). Approximately 15% of total body iron is found in myoglobin within muscle tissue and in enzymes responsible for oxidative metabolism and other cellular activities. Nearly two-thirds of body iron is present in hemoglobin within circulating red blood cells. About 25% is kept in storage forms that can be mobilized when needed (Frazer and Anderson, 2005).

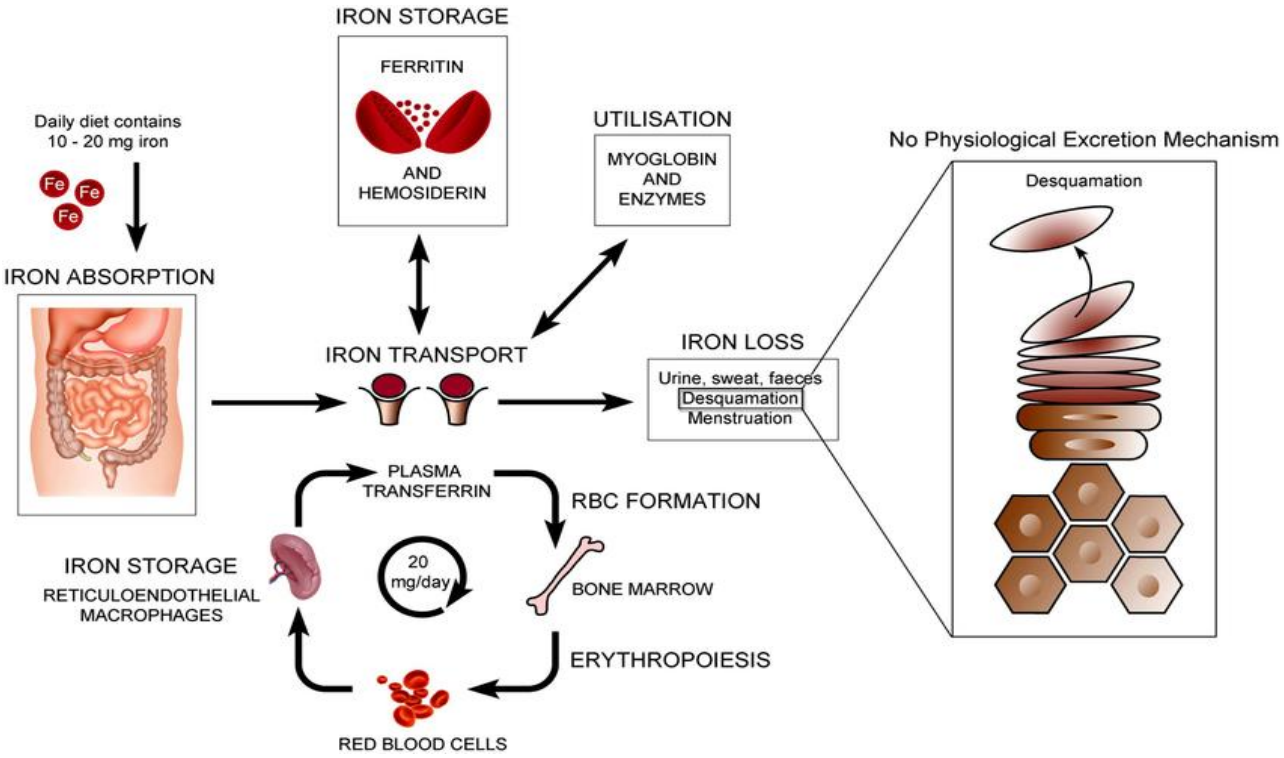


Figure 2.2. Normal Physiology of Iron (Wright *et al*., 2014)**.**

2.2.1. Serum Iron

In males and females, serum iron concentrations typically range from 14–32 µmol/L and 10–28 µmol/L respectively. This iron is primarily bound to the globulin component of serum proteins. Serum iron testing is used to assess the amount of iron circulating in the bloodstream, although values can fluctuate throughout the day. In most individuals, the highest levels are usually recorded in the morning (Tacke *et al*., 2016). However, because serum iron levels alone do not provide a reliable indication of iron deficiency or iron overload, the test is often conducted alongside other parameters to evaluate overall iron status. Several conditions can lead to reduced serum iron levels, including blood loss, nutritional iron deficiency, and chronic inflammatory disorders. Elevated serum iron levels may occur following the use of oral contraceptives, which stimulate transferrin synthesis (Tekle *et al*., 2022). Increases are also seen in cases of iron overload, such as hemochromatosis, after the intake of iron supplements, and during liver inflammation such as hepatitis, where stored iron is released into circulation (Tang *et al*., 2020). To measure serum iron accurately, the iron must first be separated from transferrin by lowering the pH of the blood sample. It is then chemically reduced from its ferric (Fe^{3+}) to ferrous (Fe^{2+}) form. Once in the ferrous state, it reacts with a chromogen to produce a colored complex. The concentration of serum iron is then determined by measuring the absorbance of this iron–chromogen complex at a specific wavelength (Strzelak *et al*., 2017).

2.2.2. Iron Metabolism

2.2.2.1. Absorption of Iron

The process of iron absorption begins with the conversion of ferric iron (Fe^{3+}) to its ferrous form (Fe^{2+}), a reaction catalyzed by the enzyme duodenal cytochrome b reductase. This step

is critical because the metal transporter DMT1 (divalent metal transporter 1), located on the apical membrane of duodenal enterocytes, permits the passage of only divalent metals. These include iron, copper, lead, and manganese (Gulcin and Alwasel, 2025). While DMT1 plays a major role in iron uptake, it is not the only transport mechanism. Another key molecule involved is the heme carrier protein, which is responsible for transporting heme-bound iron across the apical membrane into the enterocyte. Within enterocytes and macrophages, the extraction of iron from heme involves a complex metabolic sequence. A central enzyme in this pathway is heme oxygenase 1, which helps release iron from the heme molecule (Yanatori and Kishi, 2019). After iron is in the ferrous form, it is transported out of the enterocyte and into the bloodstream by ferroportin. This transporter is found on the basolateral surface of enterocytes and also on macrophage membranes. When the body's iron levels are elevated, the liver increases its production of hepcidin. Hepcidin binds to the external domain of ferroportin, which leads to the internalization, ubiquitination, and degradation of the transporter. This mechanism serves to reduce the movement of iron into the blood (Nemeth and Ganz, 2021). Like DMT1, ferroportin is selective for ferrous iron. However, for iron to bind to transferrin in the circulation, it must be in the ferric form. The oxidation of Fe^{2+} to Fe^{3+} is carried out by hephaestin, an enzyme located near ferroportin on enterocyte membranes. In macrophages, a similar role is played by ceruloplasmin, a hephaestin-like protein. Thus, ferrous iron exported from enterocytes is oxidized by hephaestin, while ferrous iron released from macrophages is oxidized by ceruloplasmin to allow for proper binding to transferrin (Pietrangelo, 2017).

2.2.2.2. Transport of Iron

The plasma iron pool is quite small, containing only about 3–4 mg, yet it must be recycled multiple times each day to satisfy the substantial iron requirements of erythropoiesis and other tissues, which total around 20–25 mg daily. The iron transport protein transferrin plays

a central role in managing iron distribution. By binding to its widely expressed receptor, transferrin receptor 1 (TFR1), transferrin delivers iron to cells via the well-characterized endosomal uptake pathway (Hentze *et al*., 2010). This process is vital not only for red blood cell production but also for muscle function and the activities of B and T lymphocytes, as demonstrated by the discovery that a homozygous mutation in TFR1 causes a combined immunodeficiency with only mild anaemia (Jabara *et al*., 2016). Beyond its role in iron import, TFR1 is critical in the intestinal epithelium for maintaining homeostasis. In liver cells (hepatocytes), TFR1 is not required for basic iron uptake but is necessary during iron overload to help regulate hepcidin levels precisely (Fillebeen *et al*., 2019). Additionally, transferrin acts as a key regulator of iron balance by interacting with a second receptor, transferrin receptor 2 (TFR2). TFR2 binds transferrin with less affinity than TFR1 and is mainly expressed in hepatocytes and erythroblasts. When plasma iron is abundant, transferrin fully loaded with iron binds to TFR2, which triggers increased production of hepcidin in hepatocytes and reduces the sensitivity of erythroid cells to erythropoietin through TFR2's interaction with erythropoietin receptors (Forejtnikova *et al*., 2010). The opposite response occurs during iron deficiency. The dual role of transferrin as both an iron transporter and a regulator is thought to depend on the differing iron-binding capacities of its N-terminal and C-terminal lobes and is mediated through the distinct interactions of monoferric transferrin with TFR1 and TFR2 (Parrow *et al*., 2019).

2.2.2.3. Transferrin Saturation

Transferrin saturation describes the proportion of iron-binding sites on transferrin that are occupied by iron at a given time. Transferrin is a protein responsible for binding iron and transporting it through the bloodstream to various organs such as the liver and bone marrow (Camaschella, 2015). This measurement is a key indicator of the body's iron status and is frequently used to help diagnose conditions like iron deficiency anaemia and other disorders

related to iron imbalance. Transferrin saturation is expressed as a percentage, calculated by dividing the serum iron concentration by the total iron-binding capacity (TIBC) and then multiplying by 100. Normal values generally range from 20% to 50%. Values below 20% suggest iron deficiency, whereas levels above 50% may point to iron overload (Camaschella, 2015).

2.2.2.4. Iron Storage

Ferritin (Ft) is a protein made up of 24 subunits, which include heavy (H) and light (L) chains (Yeo *et al*., 2019). These subunits assemble to form a complex capable of storing up to 4,500 iron atoms, making ferritin the primary iron storage protein within cells (Brissot *et al*., 2012). The subunits create a cage-like structure that securely binds and stores ferric (Fe^{3+}) ions in a stable form, preventing the formation of harmful reactive oxygen species. Ferritin-bound iron serves as the main storage form of iron in macrophages and liver hepatocytes. Additionally, other cell types such as erythroblasts can absorb ferritin-bound iron and use it to support their development (Nairz *et al*., 2017). The release of iron from ferritin is controlled through ferritinophagy, a process in which nuclear receptor coactivator 4 (NCOA4) specifically binds to the ferritin light chain and directs the complex to the autolysosome for degradation. This process liberates iron, making it accessible for various biosynthetic functions (Fuhrmann *et al*., 2020).

2.2.3. Regulation of Iron Metabolism

2.2.3.1. Cellular Mechanism

This regulatory system operates through the interaction between iron regulatory proteins (IRPs) and iron-responsive elements (IREs) located in the untranslated regions of target mRNAs. During iron deficiency, IRP1 and IRP2 promote increased iron uptake by stabilizing transferrin receptor 1 (TFR1) mRNA, while simultaneously reducing iron storage and export by inhibiting the translation of ferritin and ferroportin (Zhang *et al*., 2014). In cells with

sufficient iron, iron-sulphur (Fe/S) clusters convert IRP1 into cytosolic aconitase, and IRP2 is degraded via an iron-dependent proteasomal pathway. This conversion between IRP1 and aconitase links iron regulation to the tricarboxylic acid cycle and cellular metabolism, demonstrating that iron availability is self-regulated through Fe/S clusters. IRP2 primarily binds to IREs under normal oxygen conditions, whereas IRP1 functions mainly in hypoxic tissues like the duodenum and kidneys (Costain *et al*., 2019).

2.2.3.2. Systemic Mechanism

Iron balance in the body is primarily controlled by hepcidin, a small peptide hormone made up of 25 amino acids, which is produced by the liver and can be measured in both blood and urine. Hepcidin serves as a central regulator of systemic iron metabolism by managing iron absorption in the intestines as well as its release from macrophages and enterocytes. It helps maintain equilibrium between dietary iron intake, storage, and utilization (Nemeth and Ganz, 2023). The production of hepcidin is finely tuned by various physiological signals such as anaemia, low oxygen levels (hypoxia), inflammation, and oxidative stress (Wojciechowska *et al*., 2021). During anaemia or hypoxia, the body increases red blood cell production, causing a decrease in hepcidin levels. This decline permits greater iron absorption from the gut and more iron release from storage sites within macrophages and liver cells to support new red blood cell formation (Pagani *et al*., 2019). This regulatory mechanism involves erythroferrone (ERFE), a hormone secreted by erythroblasts in the bone marrow in response to erythropoietin stimulation. ERFE suppresses hepcidin production in the liver, thereby increasing iron availability for hemoglobin synthesis and red blood cell production during times of heightened erythropoiesis (Coffey and Ganz, 2018). Hepcidin controls iron export by interacting with ferroportin, the only known protein that exports iron from cells. When hepcidin levels are low, ferroportin remains on the cell surface, allowing iron to enter the bloodstream. In contrast, high hepcidin levels cause ferroportin to be internalized and

degraded, which reduces the release of iron from intestinal cells and storage sites (Reichert *et al*., 2017).

2.2.4. Iron and Erythropoiesis

Iron plays an essential role in erythropoiesis, the process by which new red blood cells (erythrocytes) are produced in the bone marrow (Camaschella *et al*., 2016). As a critical component of haemoglobin, iron enables red blood cells to transport oxygen efficiently throughout the body. During erythropoiesis, iron is incorporated into protoporphyrin IX to form heme, which binds oxygen in mature erythrocytes. The demand for iron increases significantly during active erythropoiesis, and its supply must be tightly regulated to meet the needs of developing erythroblasts (Chiabrando *et al*., 2014). Iron is delivered to erythroid precursors primarily through transferrin-bound iron, which binds to transferrin receptors on the cell surface and is internalized via endocytosis (Gammella *et al*., 2017). In conditions such as anaemia or hypoxia, the body responds by increasing erythropoietin (EPO) secretion from the kidneys, stimulating erythroid proliferation and differentiation. This increase in erythropoietic activity, in turn, suppresses hepcidin, a key iron-regulatory hormone, allowing more iron to be absorbed from the intestine and mobilized from stores (Tsiftsoglou, 2021). Recently, erythroferrone (ERFE), a hormone secreted by erythroblasts in response to EPO, has been identified as a crucial mediator in this feedback loop. ERFE acts by downregulating hepcidin, thereby promoting iron availability to support effective erythropoiesis (Babar and Saboor, 2024).

2.3. Erythroferrone (ERFE)

Erythroferrone is a relatively recent discovery in the field of iron homeostasis and erythropoiesis. It is a glycoprotein hormone primarily produced by erythroblasts in the bone marrow in response to erythropoietin, a hormone that stimulates red blood cell production during conditions such as anaemia, blood loss, or hypoxia (Coffey and Ganz, 2018). The

main role of erythroferrone is to regulate systemic iron availability by suppressing the hepatic expression of hepcidin, which is the master regulator of iron metabolism. Under normal conditions, hepcidin inhibits iron absorption in the intestine and iron release from macrophages and hepatocytes by degrading ferroportin, the only known cellular iron exporter (Ramirez *et al*., 2020). When erythroferrone is secreted, it acts on the liver to reduce hepcidin synthesis, thereby enhancing dietary iron absorption and mobilization of iron stores to meet the increased demand for erythropoiesis. This mechanism ensures a coordinated response between red blood cell production and iron supply (Ganz, 2019).

2.3.1. Gene and Protein Structure of Erythroferrone

The ERFE gene is located on chromosome 2 (2p14) in humans and encodes a protein that belongs to the C1q/TNF-related protein (CTRP) family, which is characterized by a globular C1q domain involved in protein-protein interactions. Structurally, the ERFE protein is composed of several distinct domains, including an N-terminal signal peptide that directs its secretion, a collagen-like domain responsible for oligomerization, and the C-terminal globular domain essential for its biological function (Srole and Ganz, 2021). ERFE functions as a secreted glycoprotein that binds to liver cells to suppress the synthesis of hepcidin, thereby promoting increased iron availability to support red blood cell production (Kautz *et al*., 2015).

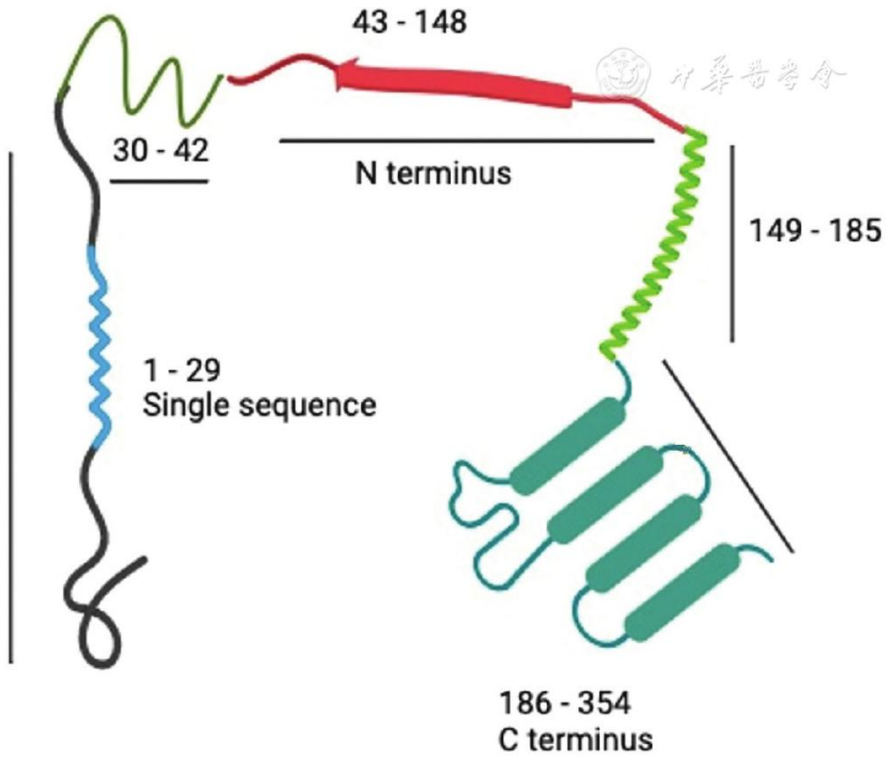


Figure 2.3. Structure of Erythroferrone (Srole and Ganz, 2021).

2.3.2. Role of ERFE in Iron Metabolism

Erythroferrone (ERFE) is an essential hormone involved in the regulation of iron metabolism, particularly during periods of increased red blood cell production (El-Gamal *et al*., 2020). ERFE is produced by erythroblasts in the bone marrow in response to stimulation by erythropoietin. One of its primary functions is to suppress the synthesis of hepcidin, a liver-derived hormone that serves as the master regulator of systemic iron homeostasis. ERFE reduces hepcidin production by interfering with the signaling pathways in hepatocytes that normally promote hepcidin expression, such as the bone morphogenetic protein (BMP) pathway (Papanikolaou and Pantopoulos, 2017). By inhibiting hepcidin synthesis, ERFE indirectly influences ferroportin, the only known cellular iron exporter located on the surface of enterocytes in the intestine and macrophages involved in iron recycling (Mleczko-Sanecka and Silvestri, 2021). When hepcidin levels decrease, ferroportin remains on the cell surface and continues to export iron into the bloodstream, increasing systemic iron availability. This increase in circulating iron is crucial to meet the heightened iron demands of active erythropoiesis, as iron is required for hemoglobin synthesis and the formation of new red blood cells (Drakesmith *et al*., 2015). ERFE thereby serves as a vital link that coordinates iron supply with erythropoietic demand, ensuring that iron homeostasis is maintained even when the body requires increased red blood cell production (Anderson and Frazer, 2017).

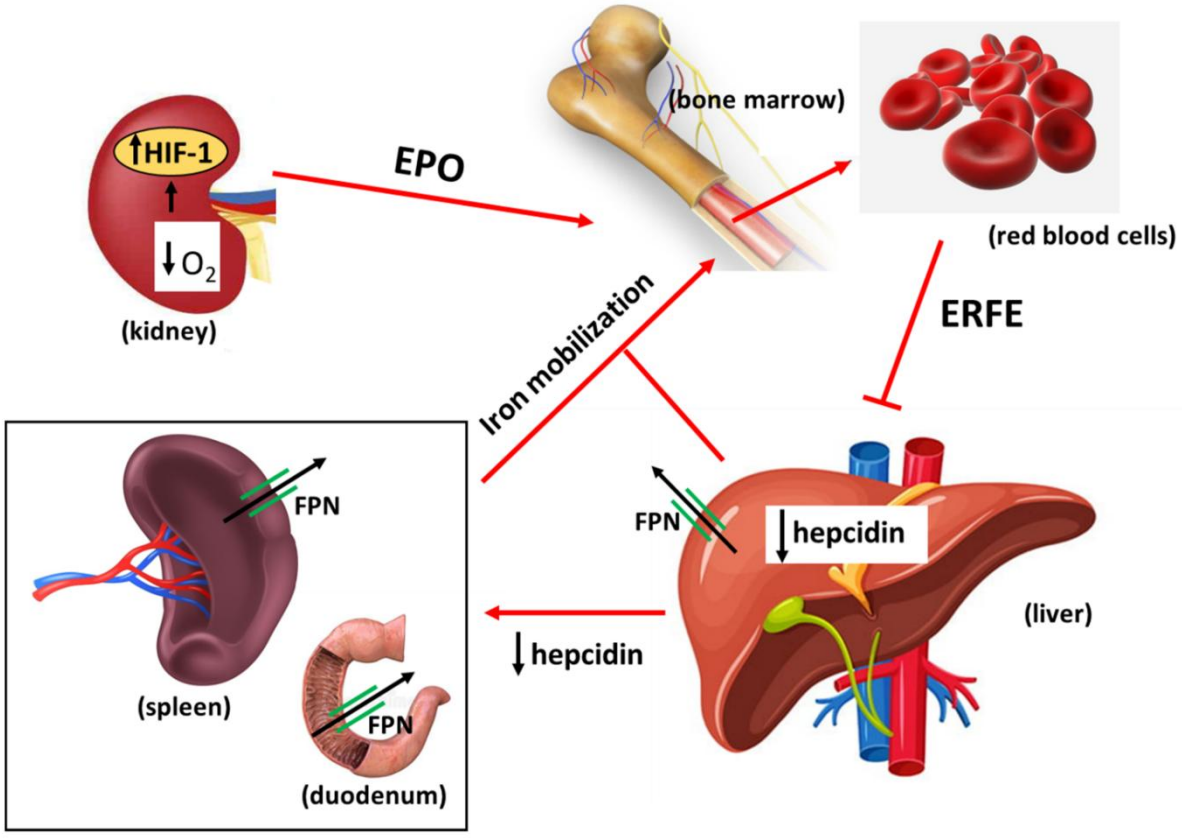


Figure 2.4. Erythroferrone and Iron Metabolism (Correnti *et al*., 2022).

2.3.3. Erythroferrone in Physiological Conditions

Erythroferrone (ERFE) plays a vital role in maintaining iron balance under normal physiological conditions, particularly in response to the body's varying needs for red blood cell production (Babar and Saboor, 2024). Under steady-state conditions, ERFE levels are typically low, reflecting the baseline erythropoietic activity required to replace aging red blood cells. However, when the body experiences increased demand for erythropoiesis, such as after blood loss, during hypoxia, or in response to erythropoietin stimulation, ERFE production by erythroblasts in the bone marrow rises significantly (Xu *et al*., 2024). This increase in ERFE acts as a signaling molecule to suppress hepcidin synthesis in the liver, thereby promoting iron mobilization from storage sites and enhancing intestinal iron absorption (Dahar *et al*., 2017). By fine-tuning hepcidin levels, ERFE ensures that sufficient iron is available to support enhanced haemoglobin synthesis and the accelerated production of red blood cells. This adaptive response is important for maintaining adequate oxygen delivery to tissues during stress or increased metabolic demand (Srole and Ganz, 2021). Additionally, ERFE's regulatory function helps prevent iron restriction that could limit erythropoiesis, while avoiding excessive iron accumulation under normal conditions. ERFE serves as an important physiological mediator that balances iron availability with erythropoietic needs, contributing to overall systemic iron homeostasis (Tang and Wang, 2023).

2.3.4. Erythroferrone in Pathological States

2.3.4.1. Erythroferrone in Anaemia and Increased Erythropoietic Activity

In pathological states characterized by heightened erythropoietic demand, such as anaemia caused by blood loss or haemolysis, ERFE levels rise significantly. This elevation occurs as erythroblasts in the bone marrow respond to increased erythropoietin signaling. The higher ERFE production suppresses hepcidin synthesis in the liver, leading to enhanced iron absorption from the intestine and mobilization of iron from stores (Georgatzakou *et al*., 2016). This mechanism helps to meet the urgent need for iron to support rapid red blood cell production. In conditions such as β-thalassemia and other chronic anaemias with ineffective erythropoiesis, ERFE is often excessively produced, which can lead to prolonged suppression of hepcidin. While this initially helps alleviate iron-restricted erythropoiesis, persistent hepcidin suppression may contribute to systemic iron overload, increasing the risk of tissue damage (Dias *et al*., 2020).

2.3.4.2. Erythroferrone in Chronic Kidney Disease

In chronic kidney disease (CKD), anaemia is a common complication due to reduced erythropoietin production and altered iron metabolism (Hanna *et al*., 2021). Studies have shown that ERFE levels may be dysregulated in CKD patients. Despite the impaired erythropoietic response, elevated ERFE has been observed, possibly reflecting a compensatory attempt to suppress hepcidin and increase iron availability (Wojtaszek *et al*., 2020). However, the inflammatory environment typical of CKD often causes elevated hepcidin production, which can counteract ERFE's effects, leading to functional iron deficiency. This imbalance complicates anaemia management and underscores the importance of understanding ERFE's role in CKD-related anaemia (Malyszko *et al*., 2019).

2.3.4.3. Erythroferrone in Inflammatory Diseases

During chronic inflammatory states such as rheumatoid arthritis or infections, the body's iron regulation is significantly altered by inflammatory cytokines that induce hepcidin production (Schmidt, 2015). Elevated hepcidin reduces iron availability by promoting ferroportin degradation, leading to anaemia of inflammation (Langer and Ginzburg, 2017). In these conditions, ERFE production may increase as a physiological attempt to suppress hepcidin and restore iron availability. However, persistent inflammation often maintains high hepcidin levels, overwhelming the inhibitory effect of ERFE. This results in impaired iron mobilization despite increased ERFE, contributing to anaemia and reduced erythropoietic efficiency (Coffey and Ganz, 2018).

2.3.4.4. Erythroferrone in Iron Overload Disorders

In hereditary haemochromatosis and other iron overload disorders, the regulation of ERFE and hepcidin is disrupted. Typically, hepcidin production is inadequate relative to body iron stores, causing excessive iron absorption and accumulation. ERFE levels in such conditions may be abnormally high due to ineffective erythropoiesis or expanded erythroid precursors (Piperno *et al*., 2020). Elevated ERFE further suppresses hepcidin, worsening iron overload. This pathological feedback loop contributes to progressive iron deposition in organs, increasing the risk of liver disease, cardiomyopathy, and endocrinopathies (Yun and Vincelette, 2015).

2.4. Haematological Parameters

Blood, an essential circulatory tissue, comprises cells floating in plasma, primarily functioning to maintain homeostasis (Isaac *et al*., 2013). Haematological parameters, including erythrocytes and their indices, leukocytes, and platelets, are quantifiable components originating from haematopoietic stem cells. At birth, the bone marrow is entirely filled, but as one ages, it is gradually replaced by fatty tissue, affecting blood parameters.

Assessing haematological parameters is crucial as they are vital markers for evaluating immunological state, informing therapeutic decisions, and tracking disease progression and treatment efficacy to provide optimal patient care (Azuonwu *et al*., 2017).

2.4.1 Red blood cells

Red blood cells, also called erythrocytes, are the most numerous and essential cellular elements in the bloodstream (Humphry and Armstrong, 2022). They are uniquely adapted to their role in gas exchange through their biconcave disc shape, which increases their surface area-to-volume ratio, thereby promoting efficient diffusion of oxygen into tissues and the removal of carbon dioxide from them (Fischbach, 2003). This shape, combined with their high flexibility, allows erythrocytes to easily navigate through narrow capillaries without rupturing. The average adult has about 5 litres of blood, and approximately 2 litres consist of red blood cells, reflecting their critical role in maintaining tissue oxygenation (Fischbach, 2003). Each litre of blood contains around 5 trillion red blood cells, although this number may fluctuate based on an individual's health status, age, gender, altitude of residence, and physiological demands such as pregnancy or physical activity. Erythrocytes are produced continuously in the red bone marrow through a tightly regulated process known as erythropoiesis. Once matured and released into circulation, they function for approximately 120 days before being cleared from the bloodstream by the spleen and liver. Their removal is balanced by the constant production of new red cells to maintain homeostasis (Cheesbrough, 2006).

2.4.2 Packed cell volume (PCV)

Packed cell volume (PCV), also referred to as haematocrit (HCT) or erythrocyte volume fraction (EVF), is the proportion of whole blood that is made up of red blood cells (RBCs), expressed as a percentage. It is a fundamental hematological parameter that provides insight into the oxygen-carrying capacity of blood (Farooq *et al*., 2023). PCV is directly related to

the number and size of red blood cells and serves as an indicator of various physiological and pathological states (Etim *et al*., 2014). A decreased PCV may suggest anaemia, blood loss, or fluid overload, while elevated PCV may be seen in conditions such as polycythaemia vera, dehydration, or chronic hypoxia (Cheesbrough, 2006). Since red blood cells are the primary vehicles for oxygen and nutrient transport, PCV is critical in assessing the adequacy of tissue oxygenation. Monitoring PCV is essential in clinical practice, particularly in cases of blood transfusion, hydration status evaluation, and diagnosis of hematological disorders. Normal range of packed cell volume for males is 40-52 (%), females is between 36-48 (%) (Hoffbrand *et al*., 2016).

2.4.3. Haemoglobin

Haemoglobin is an iron-containing, oxygen-transporting metalloprotein found abundantly within red blood cells (Wang *et al*., 2016). Structurally composed of four globin chains, each bound to a heme group containing iron, haemoglobin plays a crucial role in the physiological process of gas exchange. It is responsible for imparting the characteristic red colour to red blood cells due to the iron component in the heme moiety (Fischbach, 2003). The primary function of haemoglobin is to facilitate the efficient transport of oxygen from the lungs to peripheral tissues, where it is released to support cellular metabolism. In return, haemoglobin binds carbon dioxide and transports it back to the lungs for exhalation. Its ability to reversibly bind both oxygen and carbon dioxide is essential for maintaining homeostasis and ensuring adequate tissue oxygenation throughout the body (Hoffbrand *et al*., 2016). Normal range of Haemoglobin in males is 135-175g/L and females 115-155g/L (Hoffbrand *et al*., 2016).

2.4.4. Red Cell Indices

2.4.4.1. Mean Cell Haemoglobin Concentration (MCHC)

The mean cell haemoglobin concentration gives the concentration of Haemoglobin in g/l of packed red cells. It is calculated from the haemoglobin (Hb) and PCV as follows:

$$\text{MCHC (g/l)} = \frac{\text{(Haemoglobin in g/100m by l of blood) x100 g/d}}{\text{Volume of packed cells/100ml of blood.}}$$

Normal range for MCHC is between 315-360g/l (Cheesbrough, 2006).

Low MCHC values are associated with iron deficiency anaemia, thalassaemia trait, while increase MCHC is associated marked spherocytosis which is rare (Cheesbrough, 2006).

2.4.4.2. Mean Cell Volume (MCV)

The mean red cell volume (MCV) provides information on red cell size. It is measured in femtolitres (fl) and is determined from PCV and obtained RBC count. It can be calculated as

$$\text{MCV} = \frac{\text{(Volume of packed cells/100 ml of blood) fl}}{\text{Red blood cell count in millions/ml}} \text{ (fl)}$$

Normal range is between 80-98fl low MCV values is found in microcytic anaemia's such as iron deficiency anaemia, anaemia of chronic disease and thalassaemia while increased MCV value is found in macrocytic anaemia, marked reticulocytosis, and chronic alcoholism (Cheesbrough, 2006).

2.4.4.3. Mean Cell Haemoglobin (MCH)

The MCH gives the amount of haemoglobin in pictograms (pg) in an average red cell. It is calculated from the obtained haemoglobin and RBC count.

Normal range is 27-34 pg (Hoffbrand *et al*., 2016).

$$\text{MCH} = \frac{\text{(Haemoglobin in g/100ml of blood)pg/cell}}{\text{Red blood cell counts in millions/ml.}}$$

2.4.4.4. Red Cell Distribution Width (RDW)

Red cell distribution width (RDW) is a measure used to determine how much variation there is in the size of red blood cells in the bloodstream (Salvagno *et al*., 2015). Normally, red blood cells are fairly uniform in size, but when there is a noticeable difference, it may point to certain health issues. A high RDW suggests a wide range of red cell sizes, which is often seen in conditions like iron deficiency anaemia, vitamin B_{12} or folate deficiency, and in some cases where more than one type of anaemia is present at the same time (Hoffbrand *et al*., 2016). RDW is usually interpreted alongside other red blood cell indices, such as the mean corpuscular volume (MCV), to help healthcare providers better understand the cause of the abnormality. Normal range is between 11.5-14.5 coefficients of variation (CV) of red cell size (Fischbach, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Reagents

Reagents used in this study were of analytical grade.

3.2. Study Area

In this study, animal (rats) model was used. A total of sixty (60) of the albino wistar strain were purchased from the animal holdings of the Department of Anatomy, University of Benin, Benin city, Nigeria. The rats were housed at the animal housing wing of the Department of Anatomy, University of Benin.

3.3. Identification of *Icacina Trichantha* Leaves

Icacina trichantha leaves were harvested from Ekosodin community in Ovia North East Local Government Area of Edo state, Nigeria on the 2nd of April 2025. The leaves were

identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin city, with voucher number UBH-1185.

3.3.1. Processing of *Icacina Trichantha* leaves

The procedure began by removing any unhealthy leaves from the sample. Subsequently, the leaves underwent a thorough washing process followed by drainage. To facilitate proper grinding, the leaves were air-dried under shade for 2 weeks. Further drying was then carried out using a hot air oven at 50ºc for 24hours. This ensured that the leaves were adequately dried and prepared for grinding. The grinding process itself was conducted using a high-speed grinding machine, specifically an industrial 1000A high-speed grinder.

3.4. Preparation of Plants Extract

1500grams of the pulverized plant was mixed with 15litres of distilled water and soaked with constant stirring for 24hours. The extract was filtered using Whatman's (Nitro cellulose 45; 0.45µm pore size) filter paper and the filtrates were concentrated using a water bath at 37ºc. Thereafter, it was put in an airtight container and refrigerated until use (Obazelu and Ogiza, 2024).

3.5. Animal Care

Animals were housed in a cross-ventilated room in the animal holdings of the Department of Anatomy, University of Benin, Benin City. Animals were exposed to 12 hours dark and light cycles with access to feed and water *ad libitum*. The rats were acclimatized for a period of two (2) weeks before commencement of the experiment (Obazelu and Gaius-Igboanugwo, 2024).

3.5.1. Inclusion Criteria

- Apparently healthy Wistar rats weighing between 150-200g
- Male rats

3.5.2. Exclusion Criteria

- Rats with excessive breathing
- Rats with reduced appetite
- Sick rats
- Rats weighing less than 150g (<150g)

3.6. Ethical Consideration

Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: HA/737/25/D/06180723 issued on 18th, June, 2025).

3.7. Preparation of Aluminium Chloride and Ferrous Sulphate Drug Solution

3.7.1. Aluminium Chloride Solution

Aluminium chloride solution was prepared by mixing 0.1gram of aluminium chloride powder (Manufactured by Guangdong Guanghua Sci-Tech co, LTD, Batch number: T/CSTM 00071-2019) with 100mililitre of distilled water. Subsequently, 0.1mililitre of aluminium chloride solution was administered to each animal in the various test groups, with an average weight of 150grams

3.7.2. Ferrous Sulphate Drug Solution

Ferrous sulphate drug solution was made by mixing 1000mg of the powdered drug in 50ml of distilled water. 0.3ml of this drug solution was administered orally to each animal in group C of an average weight of 150g, every 48 hours for 28 days (Obazelu and Evwaire, 2024).

3.8. Research Design

Grouping of Animals: Sixty (60) mature Wistar rats weighing 150-200g were randomly selected and divided into six groups (n = 10 per group). The groups were Group A, Group B, Group C, Group D, Group E and Group F.

Group A: This was the control group. Animals in this group received only standardized feed (Manufactured by KARMA AGRIC FEEDS AND FOOD LIMITED, Oyo State) and clean water *ad libitum.*

Group B: This group received only aluminium chloride intraperitoneally.

Group C: Animals in this group were administered aluminium chloride solution and treated with the standard drug solution (ferrous sulphate) intraperitoneally.

Group D: Animals in this group were administered aluminium chloride solution intraperitoneally and treated with low dose of *Icacina trichantha* leaves extract orally.

Group E: Animals in this group were administered aluminium chloride solution intraperitoneally and treated with a higher dose of *Icacina trichantha* leaves extract orally.

Group F: Animals in this group were administered aluminium chloride solution intraperitoneally and treated with the highest dose of *Icacina trichantha* leaves extract orally.

3.8.1. Dosage of Ferrous Sulphate Administered

40mg/kg.bw

40mg - 1000g

? - 150g (Mean weight of rats)

$= \frac{40 \times 150}{1000} = 6\text{mg}$

1000mg of ferrous sulphate powder = 50mls

6mg = x

$X = \frac{6 \times 50}{1000} = 0.3\text{ml of 6mg/ml 48 hourly for 4 weeks}$

3.8.2. Extract Dosing

The dosage given to each group is calculated by;

Weight of the animal = g/kg

Dose of extract = mg/kg

Stock of extract = mg/ml

$$\text{Volume to administer} = \frac{\text{Weight} \times \text{Dose of extract}}{\text{Stock}}$$

40g of the extract was weighed

40g is equivalent to 40000mg

40g of the extract is dissolved in 400ml of distilled water

$$\text{Concentration of extract} = \frac{40000\text{mg}}{400\text{ml}} = 100\text{mg/ml}$$

Group A was the control group which received only feed and water *ad libitum*

Group B was administered aluminium chloride intraperitoneally

Group C was administered 40mg/kg ferrous sulphate drug solution

Group D was administered 100 mg/kg of *Icacina trichantha* orally

Group E was administered 200 mg/kg of *Icacina trichantha* orally

Group F was administered 400 mg/kg of *Icacina trichantha* orally

Calculating dose of extract for each group using;

$$\text{Volume to administer} = \frac{\text{Weight} \times \text{Dose of extract}}{\text{Stock}}$$

Group D

Average weight of 10 rats = 150kg

Dose = 100mg/kg

Stock = 100mg/ml

150g to kg = 0.150kg

$$\text{Volume to administer} = \frac{\text{Weight} \times \text{Dose of extract}}{\text{Stock}}$$

$$= \frac{0.150 \times 100}{100} = 0.15\text{ml}$$

Group E

Average weight of 10 rats = 150kg

Dose = 200 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

$$\text{Volume to administer} = \frac{\text{Weight} \times \text{Dose of extract}}{\text{Stock}}$$

$$= \frac{0.150 \times 200}{100} = 0.3\text{ml}$$

Group F

Average weight of 10 rats = 150kg

Dose = 400 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

$$\text{Volume to administer} = \frac{\text{Weight} \times \text{Dose of extract}}{\text{Stock}}$$

$$= \frac{0.150 \times 400}{100} = 0.6\text{ml}$$

3.8.3. Administered Doses of *Icacina trichantha* Leaves Extract

Group A (control) received only standardized feed and clean water *ad libitum*. Group B (aluminium chloride treated group) were administered 0.2ml of aluminium chloride solution intraperitoneally every 48 hours for 28 days. Group C (ferrous sulphate drug solution treated group) were administered 0.2ml of aluminium chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 6mg/ml of ferrous sulphate 48 hourly for 28 days. Group D were administered with 0.2ml of aluminium chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of *Icacina trichantha* leaves extract orally using a gavage tube every 24 hours for 28 days. Group E were administered with 0.2ml of aluminium chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of *Icacina trichantha*

leaves extract orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.2ml of aluminium chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of *Icacina trichantha* leaves extract orally using a gavage tube every 24 hours for 28 days (Obazelu and Osazee, 2024).

3.9. Physical Examination of Animals

Animals were weighed to check for any increase or decrease in body weight throughout the experiment.

3.9.1. Measurement of Body Weight

The body weights of the animals were measured two times during the course of the experiment. This was done at day 0 and 28 i.e., initial and final body weight. A weighing scale was used to measure the individual weight of each animal. This was done by removing the animals from the cage and placing them on the scale. The weights were read and recorded while the animals were resting on the scale.

3.10. Sacrifice of Animals and Collection of Samples

At the end of the experimental period, the animals were grossly observed for general physical characteristics. A midline incision was made through the ventral wall of the rats after anaesthetizing (using chloroform) and cervical dislocation. Five millilitres (5ml) of blood were collected from each rat using a sterile syringe and placed in an Ethylene Diamine Tetra-acetic Acid (EDTA) container for full blood count analysis and peripheral blood film. Bone marrow samples were also obtained from the rats by opening the femur longitudinally and exposing the marrow cavity. A sterile forceps was used to obtain the bone marrow from the cavity and placed in an Eppendorf container containing Trizol for molecular analysis.

3.11. Laboratory Analysis

3.11.1. Haematological Profile

The full blood count parameters were analysed immediately after sample collection using the automated three parts ERMA Haematology Auto analyser PCE-210N (Diamond Diagnostic; Holliston, USA). Calibration and standardization of the equipment, processing and analysis of the samples were done strictly according to the manufacturer's instructions (Obazelu and Wasa, 2023).

3.11.1.1. Detection Principle of Haematology Autoanalyzer

The instrument counts and sizes the cells. It detects and measures changes in electrical resistance when a particle (such as a cell) passes through a gem aperture sensor. Sample was diluted in a conductive liquid. Each time a blood cell will pass through the aperture a resistant signal will be generated because blood cells are bad conductors. When cell goes through the aperture, the resistance increases with increase in cell volume. According to the Ohm formulary: U=RI (U =Voltage I =Current R =Resistance). If I is invariable, U is increased as cell volume increases. Treat by magnifying circuit, the voltage signal is amplified; background noise is removed, and receives the signal to analysis. WBC and RBC/PLT are analysed by two different circuits. The MPU analyses and calculates the cells, then gives the histograms. The count of PLT adopts an advanced liquid, electron and soft system, which can settle the repetitive count of the cells. If RBC enters the analysis area, they will have similar pulses with PLT.

3.11.1.2. Procedure

The whole blood was properly mixed and inserted into the probe. Then 20 µL of the blood was aspirated into the instrument. The analysis was immediately done and the results displayed on the screen after about 1-2 minutes, which was printed by the printer.

3.11.2. Peripheral Blood Film (Obazelu and Olorunda, 2024)

3.11.2.1. Preparation of Leishman Stain

Stock Solution of Eosin Y

- 1 gram of Eosin Y powder was added to a clean, dry glass staining dish.
- 100 ml of distilled water was added to the dish and the mixture was stirred until the powder was completely dissolved.
- Solution was labelled as "Eosin Y stock solution."

Stock solution of Methylene blue:

- 1 gram of Methylene blue powder was added to another clean, dry glass staining dish.
- 100 ml of distilled water was added to the dish and the mixture was stirred until the powder is completely dissolved.
- It was then labelled as "Methylene blue stock solution."

Working solution of Leishman stain:

- 1 ml of the Eosin Y stock solution was added to a clean, dry staining jar.
- 1 ml of the Methylene blue stock solution was also added to the same staining jar.
- 98 ml of ethanol (95%) was added to the staining jar.
- The contents of the staining jar were mixed thoroughly using a glass stirring rod and then allowed to ripen for three (3) days after which it was labelled as "Leishman stain working solution."

3.11.2.2. Procedure for Leishman Staining

- A drop of blood sample from the sacrificed animals was placed on a clean grease free glass slide.
- Another clean grease free slide was placed at a 45-degree angle against the blood drop while allowing it to spread along the contact lines after which a smooth motion was

applied to create a thin and even blood film. The film was allowed to air dry completely.

- The film was flooded with the prepared working solution of Leishman stain for 2 minutes.
- After 2 minutes the slide was buffered with twice the volume of stain using a Sorensen's buffer solution for 8 minutes.
- After 8 minutes, slide was gently rinsed and allowed to dry after which a drop of immersion oil was place on it.
- The prepared slide was placed on the microscope stage, and the peripheral blood film was examined using ×100 objective lens.
- Different cellular components such as red blood cells, white blood cells, and platelets were observed for morphology and abnormalities.

3.11.3. Erythroferrone (ERFE) mRNA Assay

Isolation of Total RNA

Total RNA was isolated from whole drosophila samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

cDNA conversion

One (1 μg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Olumegbon *et al*., 2020).

PCR Amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of ERFE gene was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa). PCR amplification was performed in a total of 25µl volume reaction mixture containing cDNA, primer (forward and reverse SEE BELOW) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95ºC for 5 min, followed by 30 cycles of amplification (denaturation at 95ºC for 30 seconds, annealing for 30 seconds and extension at 72ºC for 60 seconds) and ending with final extension at 72ºC for 10 min. The amplicons were resolved on 1.0% agarose gel. The Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al*., 2020).

PRIMER SEQUENCES

ERFE

Forward: AAGAAAGGGACCACCACAAC

Reverse: GAAGAGCTCACTGCTATTCTCC

GAPDH

Forward: CTCCCTGGAGAAGAGCTATGA

Reverse: AGGAAGGAAGGCTGGAAGA

3.12. Statistical Analysis

Data obtained from this research was presented and analyzed using statistical package for social sciences (SPSS) version 21.0 (IBM Inc. USA). Analysis of variance (ANOVA) was used to compare treatment groups of continuous variables. Tukey HSD *post hoc* was applied where a significant difference was observed in the ANOVA. Bar charts was used to represent

the mRNA gene expression patterns. A p value of ≤0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Table 4.1 shows the comparison of Mean±SD of red blood cell count, Haemoglobin concentration, haematocrit and red cell indices of six groups namely; groups A, B, C, D, E

and F, representing control, $AlCl_3$ group, $AlCl_3$ + ferrous sulphate group, $AlCl_3$ + 100mg/kg *Icacina trichantha*, $AlCl_3$ + 200mg/kg *Icacina trichantha* and $AlCl_3$ + 400mg/kg *Icacina trichantha* respectively.

Red blood cell count (RBC) (µL) of groups C (7.65±0.21), D (8.18±0.06), E (7.84±0.31) and F (8.05±0.29) was not significantly different from groups A (8.09±0.12) and B (7.74±0.21) ($p>0.05$). Haemoglobin Concentration (g/dL) of groups C (15.06±0.36), D (15.5±0.22), E (15±0.59) and F (15.1±0.61) was not significantly different from groups A (15.33±0.24) and B (14.84±0.32) ($p>0.05$). Haematocrit (%) of groups C (44.46±0.99), D (45.1±0.73), E (44.32±1.46) and F (43.86±1.52) was not significantly different from groups A (44.88±0.78) and B (44.52±0.96) ($p>0.05$). Mean Cell Volume (fL) of groups C (58.22±0.49), D (55.22±0.91) and E (56.62±0.59) was not significantly different from groups A (55.55±0.73) and B (57.7±1.26) ($p>0.05$). MCV of group F (54.64±0.96) was significantly lower than that of group C (58.22±0.49) ($p<0.05$). Mean Cell Haemoglobin (pg) of groups C (19.66±0.07), D (18.9±0.23), E (19.06±0.18) and F (18.72±0.23) was not significantly different from groups A (18.9±0.07) and B (19.16±0.24) ($p>0.05$). MCV of group F (18.72±0.23) was significantly lower than that of group C (19.66±0.07) ($p<0.05$). MCHC (g/dL) of groups C (33.8±0.19), D (34.32±0.31), E (33.78±0.36) and F (34.36±0.46) was not significantly different from groups A (34.1±0.37) and B (33.3±0.39) ($p>0.05$). RDW-SD (fL) of groups C (37.18±1.09), D (35.9±1.24), E (35.04±1.1) and F (33.74±0.81) was not significantly different from groups A (36.35±1.24) and B (37.18±1.09) ($p>0.05$). RDW-CV (%) of groups C (16.26±0.42), D (16.3±0.37), E (15.68±0.56) and F (15.48±0.23) was not significantly different from groups A (16.48±0.45) and B (16.36±0.34) ($p>0.05$).

Table 4.1. Mean Comparison of Red Blood Cell Count, Haemoglobin Concentration and Red Cell Indices among the Studied Groups

Parameters	Group A (Control) (n=10)	Group B ($AlCl_3$ only) (n=10)	Group C ($AlCl_3$ + Ferrous Sulphate) (n=10)	Group D ($AlCl_3$ + 100mg/kg) (n=10)	Group E ($AlCl_3$ + 200mg/kg) (n=10)	Group F ($AlCl_3$ + 400mg/kg) (n=10)	F value	p value
RBC ($\times 10^9$/L)	8.09±0.12	7.74±0.21	7.65±0.21	8.18±0.06	7.84±0.31	8.05±0.29	0.8828	0.5083
Haemoglobin (g/dL)	15.33±0.24	14.84±0.32	15.06±0.36	15.5±0.22	15±0.59	15.1±0.61	0.3059	0.9043
HCT (%)	44.88±0.78	44.52±0.96	44.46±0.99	45.1±0.73	44.32±1.46	43.86±1.52	0.1445	0.9797
MCV (fL)	55.55±0.73	57.7±1.26	58.22±0.49	55.22±0.91	56.62±0.59	54.64±0.96^c	2.729	0.0446
MCH (pg)	18.9±0.07	19.16±0.24	19.66±0.07	18.9±0.23	19.06±0.18	18.72±0.23^c	3.049	0.0296
MCHC (g/dL)	34.1±0.37	33.3±0.39	33.8±0.19	34.32±0.31	33.78±0.36	34.36±0.46	1.289	0.3029
RDW-SD (fL)	36.35±1.24	37.18±1.09	37.18±1.09	35.9±1.24	35.04±1.1	33.74±0.81	1.513	0.2246
RDW-CV (%)	16.48±0.45	16.36±0.34	16.26±0.42	16.3±0.37	15.68±0.56	15.48±0.23	1.007	0.4361

Key: $p \leq 0.05$- Significant; $p \geq 0.05$- Not significant, $AlCl_3$=Aluminium Chloride. c represents significance with $AlCl_3$ + Ferrous sulphate.

RBC: Red Blood Cell

HCT: Haematocrit

MCV: Mean Cell Volume

MCH: Mean Cell Haemoglobin

MCHC: Mean Cell Haemoglobin Concentration

RDWCV: Red cell distribution width – Coefficient of Variation

RDWSD: Red cell distribution width – Standard Deviation

Table 4.2 shows the blood morphology of the studied groups namely; groups A, B, C, D, E and F, representing control, $AlCl_3$ group, $AlCl_3$ + ferrous sulphate group, $AlCl_3$ + 100mg/kg *Icacina trichantha*, $AlCl_3$ + 200mg/kg *Icacina trichantha* and $AlCl_3$ + 400mg/kg *Icacina trichantha* respectively. Red blood cell (RBC) morphology assessment across the studied groups is presented in Table 4.2. Normochromatic and normocytic cells were moderately present (++ each) in Groups A, C, D, and E. Group B showed only mild presence (+) of both normochromatic and normocytic cells, accompanied by a high presence (++) of crenated cells. Group F exhibited moderate presence of normochromatic cells (++) and a high presence (+++) of normocytic cells, with mild presence (+) of crenated cells. Polychromatic cells were absent (–) in all groups. Crenated cells were absent (–) in Group A, highly present (++) in Group B, and mildly present (+) in Groups C, D, E, and F.

Table 4.2. Red Blood Cell Morphology of the Studied Groups

Groups	Normochromatic Cells	Normocytic Cells	Polychromatic Cells	Crenated Cells
Group A	++	++	-	-
Group B	+	+	-	++
Group C	++	++	-	+
Group D	++	++	-	+
Group E	++	++	-	+
Group F	++	+++	-	+

Key: - = absent, + = mildly present, ++ = moderately present and +++ = highly present.

Figure 4.1 shows the body weight of groups A, B, C, D, E and F, representing control, $AlCl_3$ group, $AlCl_3$ + ferrous sulphate group, $AlCl_3$ + 100mg/kg *Icacina trichantha*, $AlCl_3$ + 200mg/kg *Icacina trichantha* and $AlCl_3$ + 400mg/kg *Icacina trichantha* respectively. The weight gain of the control group increased significantly at day 28 when compared to day 0 ($p<0.05$). Other groups showed consistent weight increase at day 28 when compared to day 0 although these weight increase did not reach statistical significance ($p>0.05$).

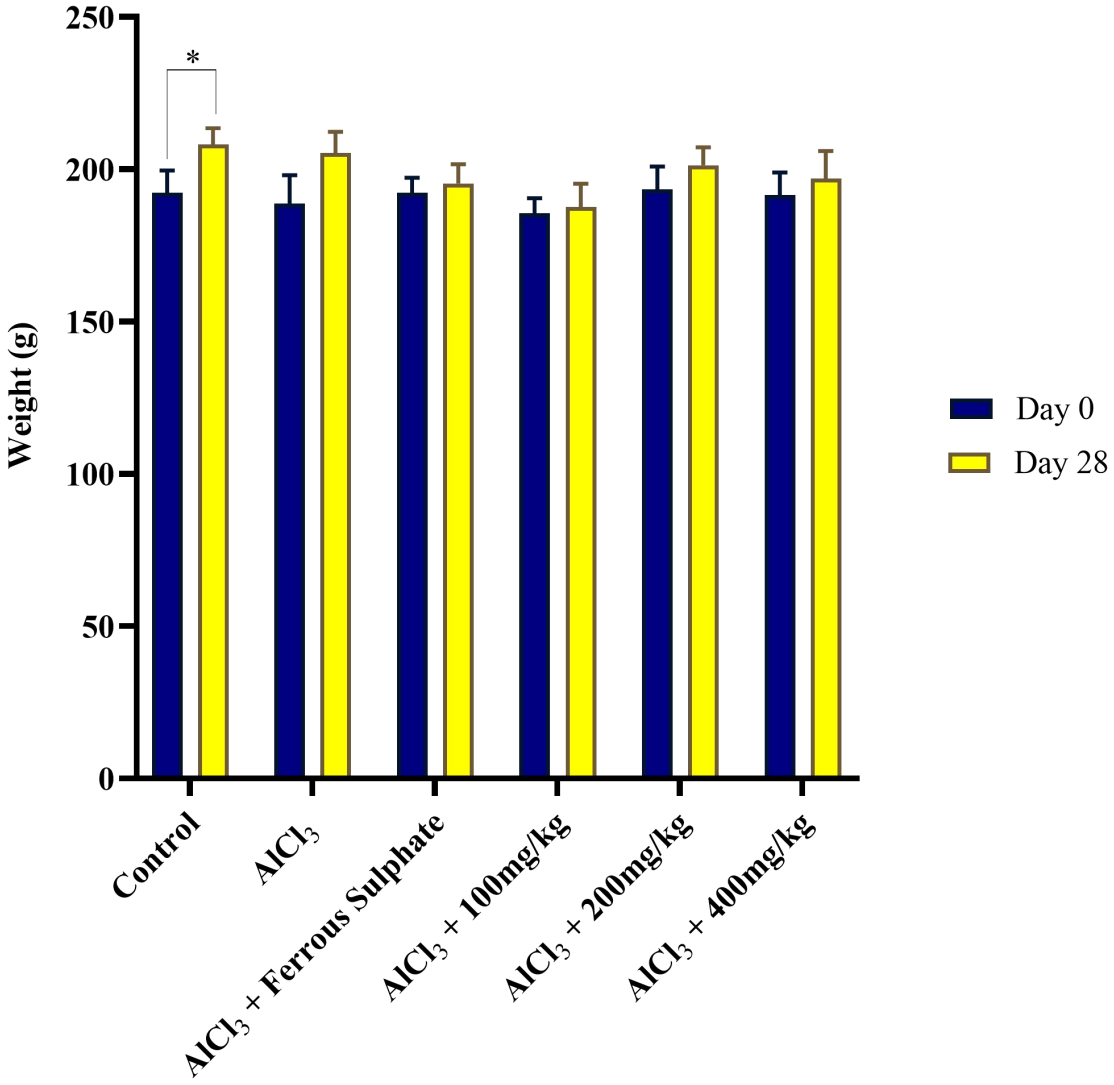


Figure 4.1. Body weights of the studied groups measured at Day 0 and Day 28.

Figure 4.2 shows the expression of genes as represented by gel electrophoresis picture and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GADPH}) of mRNA expression of Erythroferrone (ERFE) of groups A, B, C, D, E and F, representing control, $AlCl_3$ group, $AlCl_3$ + ferrous sulphate group, $AlCl_3$ + 100mg/kg *Icacina trichantha*, $AlCl_3$ + 200mg/kg *Icacina trichantha* and $AlCl_3$ + 400mg/kg *Icacina trichantha* respectively., represented on different bars on the bar chart. Groups B showed significantly lower expression of ERFE when compared to groups A ($p<0.05$). Group C, D, E and F had significantly higher expression of ERFE when compared to group B ($P<0.05$).

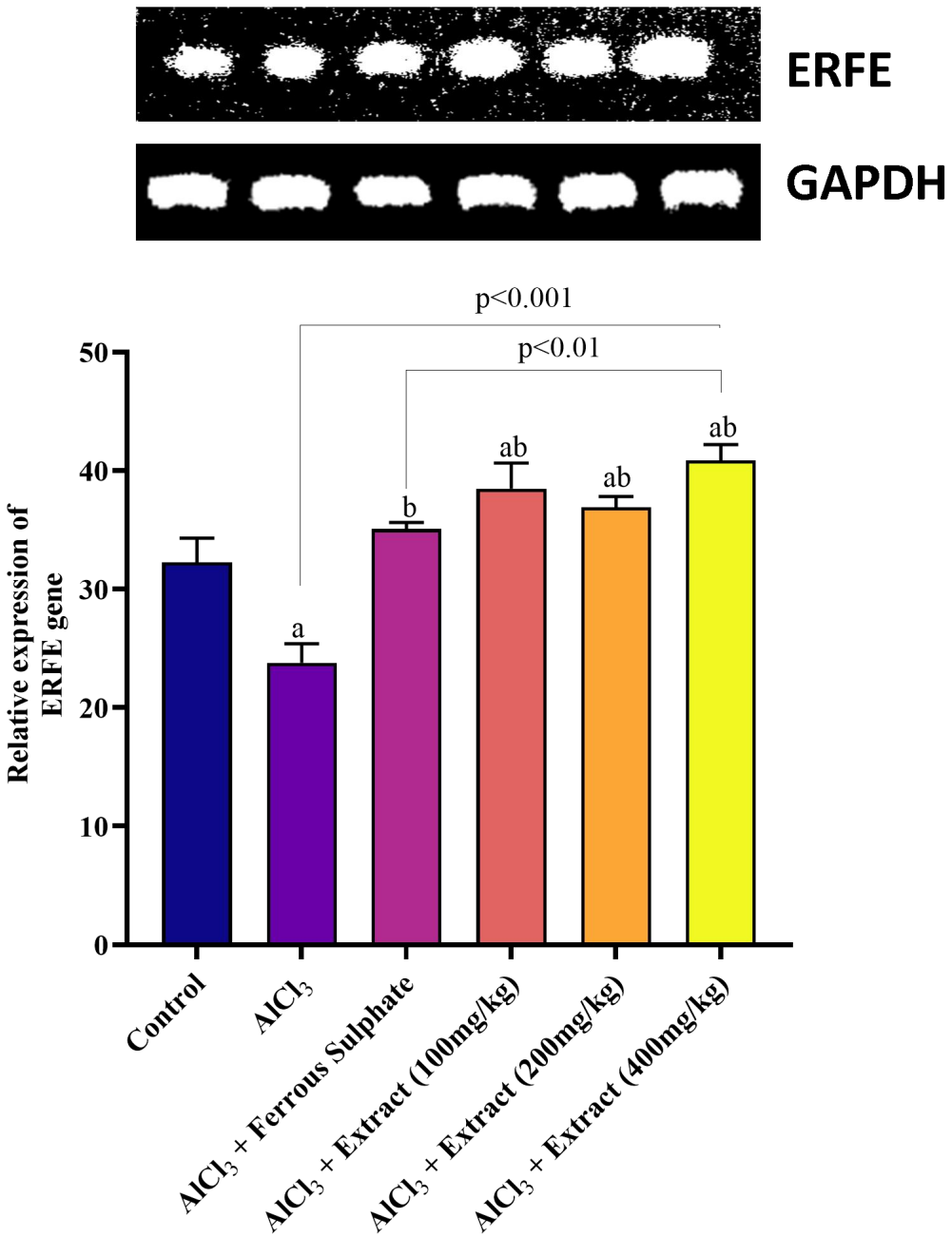


Figure 4.2. mRNA Expression of Erythroferrone (ERFE) of the Studied Groups.

Error bar shows Mean±SEM. $p<0.05$ was significant. a represent significance with control group, b represents significance with the group administered aluminium chloride ($AlCl_3$).

CHAPTER FIVE

5.1. DISCUSSION

Anaemia is a common health problem that reduces the blood's ability to carry oxygen, often leaving the body weak and fatigued (Bhadra and Deb, 2020). Aluminium chloride ($AlCl_3$), widely used in industry, can damage blood production by causing oxidative stress and disturbing iron balance, which may lead to anaemia (Ameh and Alafi, 2018). Erythroferrone (ERFE) is a hormone made by developing red blood cells that helps control iron availability by suppressing hepcidin. Changes in ERFE levels can affect how the body responds to anaemia (Srole and Ganz, 2021). *Icacina trichantha* is a medicinal plant traditionally used in Africa to boost blood health, with reported antioxidant and restorative effects. Studying how its aqueous leaf extract affects ERFE gene expression in aluminium chloride-induced anaemia could help explain its possible role in restoring healthy blood production (Che *et al*., 2016).

Red blood cells are essential for transporting oxygen from the lungs to tissues, and their quantity and indices are important indicators of the blood's ability to meet the body's oxygen demands (Nemkov *et al*., 2018). In this study, the administration of aluminium chloride and subsequent treatment with aqueous leaves extract of *Icacina trichantha* produced no significant alterations in the red blood cell count, haemoglobin concentration, haematocrit, or most red cell indices when compared to the control groups. This suggests that the experimental conditions did not elicit severe haematotoxic effects on erythropoiesis. The lack of significant changes in these parameters could be due to several factors, including the aluminium chloride dose and duration of exposure (Al-Hazmi *et al*., 2021). It is also possible that the anaemia induced was mild and transient, allowing the bone marrow to maintain adequate erythrocyte output during the treatment period (Bouchnita *et al*., 2016).

Our findings are in contrast with those of Kadhum (2017) and Azziz and Zabut (2011) who documented significant alterations in red blood cell counts and haemoglobin following exposure to aluminium chloride. The differences could be explained by variations in dosage, frequency of administration, and the physiological resilience of the test animals. The only notable deviation in our study was a reduction in mean cell volume (MCV) and mean cell haemoglobin (MCH) in the group treated with the highest dose of the extract when compared to the group administered ferrous sulphate. Such changes may indicate subtle alterations in red cell size and haemoglobin content, possibly reflecting early or subclinical microcytic anaemia (Buttarello, 2016). While these differences were statistically significant between these two groups, they did not translate into a consistent pattern across all treatment groups, further supporting the idea that any anaemic effect was minimal. The relatively stable red cell indices observed here may also point towards a mitigating role of *Icacina trichantha* extract. The plant is known to contain flavonoids, tannins, and alkaloids with antioxidant properties, which could protect erythrocyte membranes from oxidative damage and support normal haemopoietic function (Alawode, 2024). This aligns with reports by Aboderin *et al*. (2017), who found that certain medicinal plant extracts helped maintain haematological stability in toxin-exposed animals (Adebayor *et al*., 2010).

The red blood cell morphology assessment showed that exposure to aluminium chloride reduced the presence of normal normochromatic and normocytic cells, while increasing the occurrence of crenated cells. This suggests possible erythrocyte membrane damage or dehydration, which is consistent with the known oxidative and membrane-disruptive effects of aluminium (Exley, 2013). No polychromatic cells were observed in any group, indicating that there was no marked release of immature red blood cells during the study period. Treatment with *Icacina trichantha* extract at all tested doses, as well as ferrous sulphate, appeared to preserve red blood cell morphology, with a higher presence of normal cells and

fewer crenated cells compared to aluminium chloride alone. This supports the idea that both iron supplementation and antioxidant-rich plant extracts can help maintain erythrocyte integrity under toxic stress. Similar findings were reported by Aboderin *et al*. (2017), who observed improved red cell morphology in toxin-exposed animals treated with medicinal plants.

Body weight is an indicator in experimental studies that shows the general health status and physiological responses of the test animals (Burkholder *et al*., 2012). In this study, all groups, including those exposed to aluminium chloride and those treated with ferrous sulphate or varying doses of *Icacina trichantha* extract, showed a steady increase in body weight over the experimental period. The control group exhibited a statistically significant weight gain by day 28 compared to day 0, suggesting normal growth under standard conditions. Although weight gain was also observed in the aluminium chloride-exposed and treatment groups, these changes were not statistically significant, which may indicate that aluminium exposure slightly tempered growth potential despite supplementation or treatment (Igbokwe *et al*., 2019).

Erythroferrone (ERFE) is a critical erythroid-derived hormone that modulates iron homeostasis by suppressing hepcidin, a key hepatic peptide hormone that limits iron absorption and release from macrophages (Kautz *et al*., 2015). ERFE production is induced by erythropoietin (EPO) signaling in erythroblasts during increased erythropoietic activity, facilitating iron mobilization needed for haemoglobin synthesis (Ganz, 2019). In this study, aluminium chloride exposure significantly downregulated ERFE mRNA expression compared to controls, indicating disrupted erythropoietic signaling and impaired iron regulation. Aluminium toxicity is known to generate oxidative stress and interfere with bone marrow erythroid progenitors, reducing erythropoietin responsiveness and thus limiting ERFE production (Igbokwe *et al*., 2019). The diminished ERFE likely led to inadequate

suppression of hepcidin, resulting in iron sequestration and restricted availability for erythropoiesis, contributing to the anaemia observed (Ganz, 2019). This mechanistic disruption aligns with prior findings demonstrating aluminium's adverse effects on erythropoiesis through interference with iron metabolism and haematopoietic stem cell function (Igbokwe *et al*., 2019; Vota *et al*., 2012; Peto, 2010).

Treatment with ferrous sulphate and various doses aqueous leaves extract of *Icacina trichantha* significantly upregulated ERFE expression relative to the aluminium chloride-only group. The increase in ERFE following ferrous sulphate administration is consistent with the physiological response where enhanced iron availability stimulates erythropoiesis and ERFE secretion to coordinate iron homeostasis (Papanikolaou and Pantopoulos, 2017). Iron supplementation can potentiate erythroid precursor proliferation by providing substrate for haemoglobin synthesis and by indirectly modulating erythropoietin sensitivity (Ganz, 2019).

Icacina trichantha's ability to elevate ERFE expression likely stems from different factors. Its rich content of flavonoids, phenolics, and antioxidants can mitigate aluminium-induced oxidative stress in the bone marrow microenvironment, protecting erythroid progenitors and preserving their function (Otun *et al*., 2015). Oxidative stress impairs erythropoiesis by damaging progenitor cells and disrupting EPO receptor signaling (Zhao *et al*., 2016). By neutralizing reactive oxygen species, *Icacina trichantha* may enhance the erythropoietin-ERFE-hepcidin axis, restoring normal erythropoietic signaling. Studies have shown that antioxidant phytochemicals can upregulate ERFE expression thereby enhancing erythroblast viability and EPO responsiveness, which facilitates hepcidin suppression and iron mobilization (Imam *et al*., 2017).

The highest dose of *Icacina trichantha* extract (400 mg/kg) showed the greatest restoration of ERFE expression, surpassing the lower doses and closely approximating or exceeding the levels seen with ferrous sulphate treatment. This dose-dependent effect suggests that higher

concentrations of the extract provide more potent antioxidant and erythropoietic support, likely due to greater availability of active phytochemicals. The enhanced ERFE expression at the highest dose implies more effective suppression of hepcidin and improved iron availability, facilitating stronger recovery from aluminium-induced anaemia. Dose-dependent responses of this nature have been reported in other studies involving plant extracts, where increased bioactive compound exposure correlates with amplified therapeutic effects on haematological parameters (Obazelu and Abadaike-Elvis, 2024; Obazelu and Faluyi, 2023).

Molecularly, erythropoietin binds to its receptor on erythroid progenitors, triggering intracellular JAK2/STAT5 signaling pathways that induce ERFE gene transcription. ERFE then acts on hepatocytes to inhibit hepcidin synthesis, lifting the blockade on ferroportin-mediated iron export (Tomc and Debeljak, 2021). Aluminium chloride may disrupt this pathway by impairing erythropoietin receptor expression or downstream signaling, as well as by increasing oxidative stress, which inhibits JAK2/STAT5 activation (Vota *et al*., 2012). The restorative effects of *Icacina trichantha* likely involve reactivation of these pathways through antioxidant and possibly direct erythropoietic stimulation, resulting in increased ERFE and improved iron availability.

5.2. CONCLUSION

Data from this study revealed that red blood cell (RBC) counts, haemoglobin concentration and haematocrit showed no significant differences between all groups. Exposure to aluminium chloride resulted in a reduction of red blood cell morphology quality and a significant decrease in erythroferrone (ERFE) gene expression, indicating impaired erythropoiesis and iron regulation. However, treatment with ferrous sulphate and varying doses of *Icacina trichantha* extract helped restore red blood cell parameters and improved ERFE expression, with the highest extract dose showing the greatest effect.

REFERENCES

Adebayo, A. H., Abolaji, A. O., Opata, T. K. and Adegbenro, I. K. (2010). Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats. *African Journal of Biotechnology*. 9(14): 2145-2150.

Adedeji, J. A. (2023). Ecosystem Services of Yoruba Homegardens–Eco-cultural Indigenous Knowledge System for Wellbeing. In Ecological Urbanism of Yoruba Cities in Nigeria: An Ecosystem Services Approach. Cham: Springer International Publishing. Pp. 89-125.

Alawode, T. T. (2024). Phytochemical, proximate and mineral analysis of leaves and tubers of Icacina trichantha. *Journal of the Chemical Society of Nigeria*. 49(4): 606-614.

Alawode, T. T. (2024). Phytochemical, proximate and mineral analysis of leaves and tubers of Icacina trichantha. *Journal of the Chemical Society of Nigeria*. 49(4): 606–614.

Al-Hazmi, M. A., Rawi, S. M. and Hamza, R. Z. (2021). Biochemical, histological, and neuro-physiological effects of long-term aluminum chloride exposure in rats. *Metabolic Brain Disease*. 36(3); 429-436.

Al-Hazmi, M. A., Rawi, S. M. and Hamza, R. Z. (2021). Biochemical, histological, and neuro-physiological effects of long-term aluminum chloride exposure in rats. *Metabolic Brain Disease*. 36(3): 429-436.

Ameh, S. S. and Alafi, O. F. (2018). Effect of ethanol extract of Moringa oleifera leaves in protecting anaemia induced in rats by aluminium chloride. *IOSR Journal of Biotechnology and Biochemistry*. 4(6): 34-52.

Atawodi, S. E., Olowoniyi, O. D., Adejo, G. O., Liman, M. L. and Dubey, N. K. (2014). Review of the antioxidant potential of African medicinal and food plants. *Plants as a Source of Natural Antioxidants*. 34(1): 1-5.

Azuonwu, O., Nnenna, I. and Uwuma, O. E. (2017). Evaluation of haematological profile of geriatric subjects in port Harcourt metropolis of Niger Delta of Nigeria. *Journal of Clinical Laboratory Medicine*. 2(1): 3-7.

Babar, S. and Saboor, M. (2024). Erythroferrone in focus: emerging perspectives in iron metabolism and hematopathologies. *Blood Science*. 6(04): 218-224.

Beyene, B., Beyene, B. and Deribe, H. (2016). Review on application and management of medicinal plants for the livelihood of the local community. *Journal of Resources Development and Management*. 22(1): 33-39.

Bhadra, P. and Deb, A. (2020). A review on nutritional anemia. *Indian Journal of Natural Sciences*. 10(59): 18466-18474.

Bilska-Wilkosz, A., Iciek, M., Górny, M. and Kowalczyk-Pachel, D. (2017). The role of haemoproteins: hemoglobin, myoglobin and neuroglobin in endogenous thiosulfate production processes. *International Journal of Molecular Sciences*. 18(6): 1315.

Bouchnita, A., Eymard, N., Moyo, T. K., Koury, M. J. and Volpert, V. (2016). Bone marrow infiltration by multiple myeloma causes anemia by reversible disruption of erythropoiesis. *American Journal of Hematology*. 91(4): 371-378.

Brissot, P., Ropert, M., Le Lan, C and Loréal, O. (2012). Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 1820(3): 403-410.

Burkholder, T., Foltz, C., Karlsson, E., Linton, C. G. and Smith, J. M. (2012). Health evaluation of experimental laboratory mice. *Current Protocols in Mouse Biology*. 2(2): 145-165.

Buttarello, M. (2016). Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *International Journal of Laboratory Hematology*. 38: 123-132.

Camaschella, C. (2015). Iron-deficiency anemia. *New England Journal of Medicine*. 372(19): 1832-1843.

Camaschella, C., Pagani, A., Nai, A. and Silvestri, L. (2016). The mutual control of iron and erythropoiesis. *International Journal of Laboratory Hematology*. 38(1): 20-26.

Ceccanti, C., Brizzi, A., Landi, M., Incrocci, L., Pardossi, A. and Guidi, L. (2021). Evaluation of major minerals and trace elements in wild and domesticated edible herbs traditionally used in the Mediterranean area. *Biological Trace Element Research*. 199(9): 3553-3561.

Chauhan, H. K., Bisht, A. K., Bhatt, I. D., Bhatt, A., Gallacher, D., and Santo, A. (2018). Population Achanges of Trillium govanianum (Melanthiaceae) amid altered indigenous harvesting practices in the Indian Himalayas. *Journal of Ethnopharmacology*. 213(1): 302-310.

Che, C. T., Zhao, M., Guo, B. and Onakpa, M. M. (2016). Icacina trichantha, a tropical medicinal plant. *Natural Product Communications*. 11(7): 1934578X1601100740.

Cheesbrough, M. (2006). Investigation of bleeding disorders. District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press Publication. Pp. 340-347.

Chiabrando, D., Mercurio, S. and Tolosano, E. (2014). Heme and erythropoiesis: more than a structural role. *Haematologica*. 99(6): 973.

Coffey, R. and Ganz, T. (2018). Erythroferrone: an erythroid regulator of hepcidin and iron metabolism. *Hemasphere*. 2(2): 35-40.

Correnti, M., Gammella, E., Cairo, G. and Recalcati, S. (2022). Iron mining for erythropoiesis. *International Journal of Molecular Sciences*. 23(10): 5341-5347.

Costain, G., Ghosh, M. C., Maio, N., Carnevale, A., Si, Y. C., Rouault, T. A. and Yoon, G. (2019). Absence of iron-responsive element-binding protein 2 causes a novel neurodegenerative syndrome. *Brain*. 142(5): 1195-1202.

Daher, R., Manceau, H. and Karim, Z. (2017). Iron metabolism and the role of the iron-regulating hormone hepcidin in health and disease. *La Presse Médicale*. 46(12): 272-278.

Drakesmith, H., Nemeth, E. and Ganz, T. (2015). Ironing out ferroportin. *Cell Metabolism*. 22(5): 777-787.

EI, A. and Agianaku, O. F. (2015). Composition, Structure and conservation potential of vegetation adjoining a wire-mesh perimeter fence at University of Benin farm site, Benin city, Nigeria. *Nigerian Journal of Weed Science*. 28(1): 1-5.

Ekeke, C., Joseph, T. O. and Mensah, S. I. (2021). Morphological, anatomical and histological studies on the genus Icacina (Icacinaceae) from Nigeria, West Africa. *Phytologia Balcanica*. 27(1): 1-5.

Ekpo, B. A., Bala, D. N., Essien, E. E. and Adesanya, S. A. (2008). Ethnobotanical survey of Akwa Ibom state of Nigeria. *Journal of Ethnopharmacology*. 115(3): 387–408.

Elekofehinti, O. O., Lawal, A. O., Ejelonu, O. C., Molehin, O. R. and Famusiwa, C. D. (2020). Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of Annona muricata Annonaceae: in silico and in vivo studies. *Journal of Diabetes and Metabolic Disorders*. 19(1): 197-204.

El-Gamal, R. A. E. R., Abdel-Messih, I. Y., Habashy, D. M., Zaiema, S. E. G. and Pessar, S. A. (2020). Erythroferrone, the new iron regulator: evaluation of its levels in Egyptian patients with beta thalassemia. *Annals of Hematology*. 99(1): 31-39.

Enebeli-Ekwutoziam, K. C., Aruah, C. B., Ogbonna, B. O., Eze, U. J., Egedeye-Fubura, F. S., Nwankwo, C. F. and Orji, C. M. (2021). A review of ethnomedicinal plant resources in southern Nigeria. *Ethnobotany Research and Applications*. 22(1): 1-40.

Engwa, G. A., Nweke, F. N. and Nkeh-Chungag, B. N. (2022). Free radicals, oxidative stress-related diseases and antioxidant supplementation. *Alternative Therapies in Health and Medicine*. 28(1): 1-5.

Etim, N. N., Williams, M. E., Akpabio, U. and Offiong, E. E. (2014). Haematological parameters and factors affecting their values. *Agricultural Science*. 2(1): 37-47.

Ezeigbo, I. (2010). Antidiabetic potential of methanolic leaf extracts of Icacina trichantha in alloxan-induced diabetic mice. *International Journal of Diabetes in Developing Countries*. 30(3): 150.

Farooq, U., Idris, M., Sajjad, N., Lashari, M. H., Ahmad, S., Rehman, Z. U. and Hameed, S. (2023). Investigating the potential of packed cell volume for deducing hemoglobin: Cholistani camels in perspective. *PloS One*. 18(5): e0280659.

Fillebeen, C., Charlebois, E., Wagner, J., Katsarou, A., Mui, J., Vali, H. and Pantopoulos, K. (2019). Transferrin receptor 1 controls systemic iron homeostasis by fine-tuning hepcidin expression to hepatocellular iron load. *Blood, The Journal of the American Society of Hematology*. 133(4): 344-355.

Fischbach, F. (2003). A Manual of Laboratory and Diagnostic Tests.7th Edition. Lippincott Williams and Wilkins Publishers. Chicago. Pp.1-422.

Forejtnikova, H., Vieillevoye, M., Zermati, Y., Lambert, M., Pellegrino, R. M., Guihard, S. and Verdier, F. (2010). Transferrin receptor 2 is a component of the erythropoietin receptor complex and is required for efficient erythropoiesis. *Blood, The Journal of the American Society of Hematology*. 116(24): 5357-5367.

Frazer, D. M. and Anderson, G. J. (2005). Iron imports. I. Intestinal iron absorption and its regulation. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 289(4): 631–635.

Fuhrmann, D. C., Mondorf, A., Beifuß, J., Jung, M. and Brüne, B. (2020). Hypoxia inhibits ferritinophagy, increases mitochondrial ferritin, and protects from ferroptosis. *Redox Biology*. 36: 101670.

Gammella, E., Buratti, P., Cairo, G. and Recalcati, S. (2017). The transferrin receptor: the cellular iron gate. *Metallomics*. 9(10): 1367-1375.

Ganz, T. (2019). Erythropoietic regulators of iron metabolism. *Free Radical Biology and Medicine*. 133: 69-74.

Ganz, T. (2019). Erythropoietic regulators of iron metabolism. *Free Radical Biology and Medicine*. 133(1): 69-74.

Georgatzakou, H. T., Antonelou, M. H., Papassideri, I. S. and Kriebardis, A. G. (2016). Red blood cell abnormalities and the pathogenesis of anemia in end-stage renal disease. *PROTEOMICS–Clinical Applications*. 10(8): 778-790.

Gulcin, İ. and Alwasel, S. H. (2025). Fe3+ reducing power as the most common assay for understanding the biological functions of antioxidants. *Processes*. 13(5): 1296.

Gulec, S., Anderson, G. J. and Collins, J. F. (2014). Mechanistic and regulatory aspects of intestinal iron absorption. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 307(4): G397-G409.

Hanna, R. M., Streja, E. and Kalantar-Zadeh, K. (2021). Burden of anemia in chronic kidney disease: beyond erythropoietin. *Advances in Therapy*. 38(1): 52-75.

Hentze, M. W., Muckenthaler, M. U., Galy, B. and Camaschella, C. (2010). Two to tango: regulation of Mammalian iron metabolism. *Cell*. 142(1): 24-38.

Hoffbrand, A.V., Moss, P. A. H. and Petit, J. E. (2016). Haematological changes in systemic diseases. Essential Haematology, 7th Edition. Blackwell publishing, Oxford. Pp.1-150.

Humphry, E. and Armstrong, C. E. (2022). Physiology of red and white blood cells. *Anaesthesia and Intensive Care Medicine*. 23(2): 118-122.

Hussien, R. S., Jabuk, S. I., Altaee, Z. M. and Al-Maamori, A. M. (2023). Review of anemia: types and causes. *European Journal of Research Development and Sustainability*. 4(7): 1-3.

Idu, M., Sarah, I. O. and Oghale, O. U. (2017). Indigenous plants used by the Otuo tribe of Owan East Local Government Area, Edo State, Nigeria. *Journal of Medicinal Plants for Economic Development*. 1(1): 1–10.

Igbokwe, I. O., Igwenagu, E. and Igbokwe, N. A. (2019). Aluminium toxicosis: a review of toxic actions and effects. *Interdisciplinary Toxicology*. 12(2): 45-70.

Imam, M. U., Zhang, S., Ma, J., Wang, H. and Wang, F. (2017). Antioxidants mediate both iron homeostasis and oxidative stress. *Nutrients*. 9(7): 671.

Isaac, L. J., Abah, G., Akpan, B. and Ekaette, I. U. (2013, September). Haematological properties of different breeds and sexes of rabbits. *In* Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria. Pp. 24-27.

Jabara, H. H., Boyden, S. E., Chou, J., Ramesh, N., Massaad, M. J., Benson, H. and Geha, R. S. (2016). A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. *Nature Genetics*. 48(1): 74-78.

Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*. 7(1): 1-7.

Kadhum, S. A. H. (2017). Effects of Aluminum Chloride on some blood parameters and histological spleen in white male rats. *Journal of University of Babylon for Pure and Applied Sciences*. 25(5): 1886-1895.

Kaushik, B., Sharma, J., Kumar, P. and Shourie, A. (2021). Phytochemical properties and pharmacological role of plants: secondary metabolites. *Biosciences Biotechnology Research Asia*. 18(1): 23.

Kautz, L., Jung, G., Du, X., Gabayan, V., Chapman, J., Nasoff, M. and Ganz, T. (2015). Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of β-thalassemia. *Blood, The Journal of the American Society of Hematology*. 126(17): 2031-2037.

Kumar, V. and Gill, K. D. (2014). Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *Neurotoxicology*. 41(1): 154-166.

Lane, D. J. and Richardson, D. R. (2014). The active role of vitamin C in mammalian iron metabolism: much more than just enhanced iron absorption! *Free Radical Biology and Medicine*. 75(1): 69-83.

Langer, A. L. and Ginzburg, Y. Z. (2017). Role of hepcidin-ferroportin axis in the pathophysiology, diagnosis, and treatment of anemia of chronic inflammation. *Hemodialysis International*. 21(1): S37-S46.

Malyszko, J., Malyszko, J. S. and Matuszkiewicz-Rowinska, J. (2019). Hepcidin as a therapeutic target for anemia and inflammation associated with chronic kidney disease. *Expert Opinion on Therapeutic Targets*. 23(5): 407-421.

Mleczko-Sanecka, K. and Silvestri, L. (2021). Cell-type-specific insights into iron regulatory processes. *American Journal of Hematology*. 96(1): 110-127.

Nairz, M., Theurl, I., Swirski, F. K. and Weiss, G. (2017). "Pumping iron"—how macrophages handle iron at the systemic, microenvironmental, and cellular levels. *Pflügers Archiv-European Journal of Physiology*. 469: 397-418.

Nemeth, E. and Ganz, T. (2021). Hepcidin-ferroportin interaction controls systemic iron homeostasis. *International Journal of Molecular Sciences*. 22(12): 6493.

Nemeth, E. and Ganz, T. (2023). Hepcidin and iron in health and disease. *Annual Review of Medicine*. 74(1): 261-277.

Nemkov, T., Reisz, J. A., Xia, Y., Zimring, J. C. and D'Alessandro, A. (2018). Red blood cells as an organ? What a deep omics characterization of the most abundant cell in the human body highlights other systemic metabolic functions beyond oxygen transport. *Expert Review of Proteomics*. 15(11): 855-864.

Noreen, S., Bashir, S., Bano, S., Fatima, T., Sani, A., Imran, S. and Yaseen, M. (2020). Anemia and its consequences on human body; A comprehensive overview. *NUST Journal of Natural Sciences*. 5(2): 1-5.

Obazelu, P. A. and Abadaike-Elvis, N. O. (2024). Proinflammatory Effect of a bi-herbal formula of Picralima nitida and Cymbopogon citratus aqueous leaf extracts in Phenyl hydrazine induced Anaemia in Albino Wistar Rats. *Bayero Journal of Medical Laboratory Science*. 9(1): 187-194.

Obazelu, P. A. and Evwaire, O. P. (2024). Effect of a Bi-Herbal Formula of Picralima Nitida and Cymbopogon Citratus aqueous leaf extracts on Glucose-6-Phosphate Dehydrogenase and Krüppel-Like Factor-1 Genes in Phenyl-Hydrazine induced Anaemia in Albino Wistar Rats. *African Journal of Laboratory Haematology and Transfusion Science*. 3(2): 138-146.

Obazelu, P. A. and Evwaire, O. P. (2024). Effect of a Bi-Herbal Formula of Picralima Nitida and Cymbopogon Citratus aqueous leaf extracts on Glucose-6-Phosphate Dehydrogenase and Krüppel-Like Factor-1 Genes in Phenyl-Hydrazine induced Anaemia in Albino Wistar Rats. *African Journal of Laboratory Haematology and Transfusion Science*. 3(2): 138-146.

Obazelu, P. A. and Faluyi, O. (2023). Effect of Launaea taraxacifolia Aqueous Leaves Extracts on ICAM-1 and VCAM-1 Gene Expressions in Benzene-Induced Haematotoxicity in Albino Wistar Rats. *Journal of Applied Sciences and Environmental Management*. 27(12): 2845-2852.

Obazelu, P. A. and Gaius-Igboanugwo, C. O. (2024). Effect of a bi-herbal formula extract on ETS Variant-6 and Nuclear Factor Erythroid-2 genes expression in Phenyl-Hydrazine induced anaemia in Albino Wistar rats. *Journal of Medicine and Biomedical Research*. 23(1): 45-52.

Obazelu, P. A. and Ogiza, F. O. (2024). Effect of Bi-Herbal Formula of Picralima Nitida and Cymbopogon Citratus Aqueous Leaf Extracts on Cyclooxygenase-2 and Interleukin-10 Gene Expressions in Phenyl-Hydrazine Induced Anaemia in Albino Wistar Rats. *Journal of Applied Sciences and Environmental Management*. 28(6): 1765-1770.

Obazelu, P. A. and Olorunda, F. U. (2024). Effect of Aqueous Extract of Launaea Taraxacifolia Leaves on Beta-Catenin and Bcl-2 Expressions in Benzene-Induced Haematotoxicity in Albino Wistar Rats. In Book of Conference. University of Benin. Pp. 136.

Obazelu, P. A. and Omoregie, A. P. (2024). Antioxidant Effects of Aqueous Leaves Extracts of Launaea Taraxacifolia in Benzene-Induced Haematotoxicity in Albino Wistar Rats. *Sokoto Journal of Medical Laboratory Science*. 9(1): 1-5.

Obazelu, P. A. and Osazee, O. O. (2024). Effect of Aqueous Leaves Extracts of Launaea Taraxacifolia on Caspase 3 and Caspase 9 Gene Expressions in Benzene-Induced

Haematotoxicity in Albino Wistar Rats. *Bayero Journal of Medical Laboratory Science (BJMLS).* 9(1): 111-120.

Obazelu, P. A. and Wasa, F. E. (2023). Proinflammatory Effect of Launaea taraxacifolia Aqueous Leaves Extracts in Benzene-Induced Haematotoxicity in Male Albino Wistar Rats. *Nigerian Journal of Pharmaceutical and Applied Science Research*. 12(4): 34-40.

Obazelu, P. A. and Williams, D. E. (2024). Antioxidant effect of bi-herbal formula of Picralima nitida and Cymbopogon citratus extracts in phenyl-hydrazine induced Anaemia in albino Wistar rats. *Nigerian Journal of Pharmaceutical and Applied Science Research*. 13(1): 1-7.

Obode, O. C., Adebayo, A. H., Omonhinmin, C. A. and Yakubu, O. F. (2020). A systematic review of medicinal plants used in Nigeria for hypertension management. *International Journal of Pharmaceutical Research*. 12(4): 2-4.

Ogbuagu, O. O., Mbata, A. O., Balogun, O. D., Oladapo, O., Ojo, O. O. and Muonde, M. (2022). Novel phytochemicals in traditional medicine: Isolation and pharmacological profiling of bioactive compounds. *International Journal of Medical and All Body Health Research*. 3(1): 63-71.

Oke, J. M. and Hamburger, M. O. (2002). Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2, diphenyl-picryl-hydrazyl radical. *African Journal of Biomedical Research*. 5(1-2): 1-5.

Okieimen, E. F., Owolabi, O. J., Iyekowa, O. and Jonathan, E. M. (2018). Uterine Contractile Activity of Extract of Icacina Trichantha on Albino Non-Pregnant Rat Uterus. *Tropical Journal of Natural Product Research.* 2(3): 118-121.

Olayemi, O., Onakpa, M. and Jegede, O. (2022). In vitro Anthelmintic Activity of Icacina trichantha Leaf Crude Extract and Fractions on Haemonchus contortus. *Veterinarski Arhiv*. 92(3): 331–337.

Olde, P. M. (2021). Grevillea trichantha, a third species with hairy flowers in the Triloba Group (Proteaceae: Grevilleoideae: Hakeinae) from the Marchagee Track, south-west Western Australia. *Telopea*. 24(2): 303-309.

Olumegbon, L. T., Lawal, A. O., Oluyede, D. M., Adebimpe, M. O., Elekofehinti, O. O. and I. Umar, H. (2022). Hesperetin protects against diesel exhaust particles-induced cardiovascular oxidative stress and inflammation in Wistar rats. *Environmental Science and Pollution Research*. 29(35): 52574-52589.

Orimisan, O. A., Olakunle, T. F. and Morenikeji, E. S. (2023). Studies on In-vitro Bioactivity of Icacina trichantha Methanol Seed Extract. *International Journal of Science for Global Sustainability (IJSGS)*. 9(1): 1-5.

Otun, K. O., Onikosi, D. B., Ajiboye, A. T. and Jimoh, A. A. (2015). Chemical composition, antioxidant and antimicrobial potentials of Icacina trichantha Oliv. leaf extracts. *Research Journal of Phytochemistry*. 9(4): 161-174.

Otun, K. O., Onikosi, D. B., Ajiboye, A. T. and Jimoh, A. A. (2015). Chemical composition, antioxidant and antimicrobial potentials of Icacina trichantha Oliv. leaf extracts. *Research Journal of Phytochemistry*. 9(4): 161-174.

Pagani, A., Nai, A., Silvestri, L., and Camaschella, C. (2019). Hepcidin and anemia: a tight relationship. *Frontiers in Physiology*. 10(1): 1294.

Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q. and Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-Based Complementary and Alternative Medicine*. 2014(1): 525340.

Pantopoulos, K., Porwal, S. K., Tartakoff, A. and Devireddy, L. (2012). Mechanisms of mammalian iron homeostasis. *Biochemistry*. 51(29): 5705-5724.

Papanikolaou, G. and Pantopoulos, K. (2017). Systemic iron homeostasis and erythropoiesis. *IUBMB Life*. 69(6): 399-413.

Parrow, N. L., Li, Y., Feola, M., Guerra, A., Casu, C., Prasad, P. and Fleming, R. E. (2019). Lobe specificity of iron binding to transferrin modulates murine erythropoiesis and iron homeostasis. *Blood, The Journal of the American Society of Hematology*. 134(17): 1373-1384.

Peto, M. V. (2010). Aluminium and iron in humans: bioaccumulation, pathology, and removal. *Rejuvenation Research*. 13(5): 589-598.

Pietrangelo, A. (2017). Ferroportin disease: pathogenesis, diagnosis and treatment. *Haematologica*. 102(12): 1972.

Piperno, A., Pelucchi, S., and Mariani, R. (2020). Inherited iron overload disorders. *Translational Gastroenterology and Hepatology*. 5(1): 25-30.

Ramirez Cuevas, K., Schobinger, C., Gottardo, E., Voss, S. C., Kuuranne, T., Tissot, J. D. and Leuenberger, N. (2020). Erythroferrone as a sensitive biomarker to detect stimulation of erythropoiesis. *Drug Testing and Analysis*. 12(2): 261-267.

Rapa, S. F., Di Iorio, B. R., Campiglia, P., Heidland, A. and Marzocco, S. (2019). Inflammation and oxidative stress in chronic kidney disease—potential therapeutic role of minerals, vitamins and plant-derived metabolites. *International Journal of Molecular Sciences*. 21(1): 263-268.

Reichert, C. O., da Cunha, J., Levy, D., Maselli, L. M. F., Bydlowski, S. P. and Spada, C. (2017). Hepcidin: homeostasis and diseases related to iron metabolism. *Acta Haematologica*. 137(4): 220-236.

Richardson, C., Yan, S. and Vestal, C. G. (2015). Oxidative stress, bone marrow failure, and genome instability in hematopoietic stem cells. *International Journal of Molecular Sciences*. 16(2): 2366-2385.

Salvagno, G. L., Sanchis-Gomar, F., Picanza, A. and Lippi, G. (2015). Red blood cell distribution width: a simple parameter with multiple clinical applications. *Critical Reviews in Clinical Laboratory Sciences*. 52(2): 86-105.

Sanajou, S., Şahin, G. and Baydar, T. (2021). Aluminium in cosmetics and personal care products. *Journal of Applied Toxicology*. 41(11): 1704-1718.

Schmidt, P. J. (2015). Regulation of iron metabolism by hepcidin under conditions of inflammation. *Journal of Biological Chemistry*. 290(31): 18975-18983.

Shagal, M. H. and Kubmarawa, D. (2013). Antimicrobial and phytochemical screening of Icacina trichantha. *American Journal of Biomedical Life Sciences*. 1(2): 37-40.

Sharma, A., Patel, S. K. and Singh, G. S. (2021). Traditional knowledge of medicinal plants among three tribal communities of Vindhyan highlands, India: an approach for their conservation and sustainability. *Environmental Sustainability*. 4(4): 749-783.

Sofidiya, M. O., Odukoya, O. A., Familoni, O. B. and Inya-Agha, S. I. (2006). Free radical scavenging activity of some Nigerian medicinal plant extracts. *Journal of Natural Products*. 78(11): 2731-2737.

Srole, D. N., and Ganz, T. (2021). Erythroferrone structure, function, and physiology: Iron homeostasis and beyond. *Journal of Cellular Physiology*. 236(7): 4888-4901.

Strzelak, K., Rybkowska, N., Wiśniewska, A. and Koncki, R. (2017). Photometric flow analysis system for biomedical investigations of iron/transferrin speciation in human serum. *Analytica Chimica Acta*. 995(1): 43-51.

Tacke, F., Nuraldeen, R., Koch, A., Strathmann, K., Hutschenreuter, G., Trautwein, C. and Strnad, P. (2016). Iron parameters determine the prognosis of critically ill patients. *Critical Care Medicine*. 44(6): 1049-1058.

Tang, P. and Wang, H. (2023). Regulation of erythropoiesis: emerging concepts and therapeutic implications. *Hematology*. 28(1): 2250645.

Tang, X., Fang, M., Cheng, R., Zhang, Z., Wang, Y., Shen, C. and Lai, R. (2020). Iron-deficiency and estrogen are associated with ischemic stroke by up-regulating transferrin to induce hypercoagulability. *Circulation Research*. 127(5): 651-663.

Tekle, E., Gelaw, Y. and Asrie, F. (2022). Hematological profile changes among oral contraceptive users: a narrative review. *Journal of Blood Medicine*. 2(1): 525-536.

Timothy, O., Idu, M., Olorunfemi, D. I. and Ovuakporie-Uvo, O. (2014). Cytotoxic and genotoxic properties of leaf extract of Icacina trichantha Oliv. *South African Journal of Botany*. 91(1): 71-74.

Tomasz, G., Ewa, W. and Jolanta, M. (2021). Biomarkers of iron metabolism in chronic kidney disease. *International Urology and Nephrology*. 53(5): 935-944.

Tomc, J. and Debeljak, N. (2021). Molecular pathways involved in the development of congenital erythrocytosis. *Genes*. 12(8): 1150.

Tsiftsoglou, A. S. (2021). Erythropoietin (EPO) as a key regulator of erythropoiesis, bone remodeling and endothelial transdifferentiation of multipotent mesenchymal stem cells (MSCs): implications in regenerative medicine. *Cells*. 10(8): 2140.

Udeh, N. E. and Nwaehujor, C. O. (2011). Antioxidant and hepatoprotective activities of ethyl acetate leaf extract of Icacina trichantha on paracetamol-induced liver damage in rats. *Continental Journal of Animal and Veterinary Research*. 3(1): 11-15.

Vota, D. M., Crisp, R. L., Nesse, A. B. and Vittori, D. C. (2012). Oxidative stress due to aluminum exposure induces eryptosis which is prevented by erythropoietin. *Journal of Cellular Biochemistry*. 113(5): 1581-1589.

Wang, J. and Pantopoulos, K. (2011). Regulation of cellular iron metabolism. *Biochemical Journal*. 434(3): 365-381.

Wang, W., Wang, L., Huang, Y., Xie, Z. and Jing, X. (2016). Nanoscale metal–organic framework–hemoglobin conjugates. *Chemistry–An Asian Journal*. 11(5): 750-756.

Wojciechowska, M., Wisniewski, O. W., Kolodziejski, P., and Krauss, H. (2021). Role of hepcidin in physiology and pathophysiology. Emerging experimental and clinical evidence. *Journal of Physiology and Pharmacology*. 72(1): 1-5.

Wojtaszek, E., Glogowski, T. and Malyszko, J. (2020). Iron and chronic kidney disease: still a challenge. *Frontiers in Medicine*. 7(1): 565135.

Xu, P., Wong, R. S. and Yan, X. (2024). Early erythroferrone levels can predict the long-term haemoglobin responses to erythropoiesis-stimulating agents. *British Journal of Pharmacology*. 181(16): 2833-2850.

Yanatori, I. and Kishi, F. (2019). DMT1 and iron transport. *Free Radical Biology and Medicine*. 133(1): 55-63.

Yun, S. and Vincelette, N. D. (2015). Update on iron metabolism and molecular perspective of common genetic and acquired disorder, hemochromatosis. *Critical Reviews in Oncology/Hematology*. 95(1): 12-25.

Zhang, D. L., Ghosh, M. C. and Rouault, T. A. (2014). The physiological functions of iron regulatory proteins in iron homeostasis-an update. *Frontiers in Pharmacology*. 5(1): 124.

Zhao, B., Mei, Y., Yang, J. and Ji, P. (2016). Erythropoietin-regulated oxidative stress negatively affects enucleation during terminal erythropoiesis. *Experimental Hematology*. 44(10): 975-981.

APPENDIX I

Plant Identification Certification

University of Benin

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Icacina trichantha* Oliv.

Family: Icacinaceae

Common Name: False Yam, Raynal

Voucher Number: UBH-I185

Student Name: Audu Winnifred Omoye

Plant Identification and Voucher Number Issued by:

03/04/2025

Prof. **Akinnibosun** Henry Adewale (FLS, MRSB; London, LMBOSON, MAEIAN; MFBAN, MECOSON; Nigeria)

APPENDIX II

Ethical Approval

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/25/D/05210723 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/25/D/06180723**
TITLE OF RESEARCH PROPOSAL	**EFFECT OF AQUEOUS LEAVES EXTRACT OF *ICACINA TRICHANTHA* ON SOME GENES IN ALUMINIUM CHLORIDE-INDUCED ANAEMIA IN ALBINO WISTAR RATS**
PRINCIPAL INVESTIGATOR (S)	**OBAZELU PROGRESS ARHENRHEN**
DATE CONSIDERED	**18TH JUNE, 2025**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 18/06/2025 TO 18/06/2026. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS.) OMONYEMEN B. BELLO (MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**.....Bello 23/6/25.....

SUPERVISOR(S) .....Dr. Mrs. P.A. Obazelu.....

.....

ATTESTATION BY INVESTIGATOR(S)

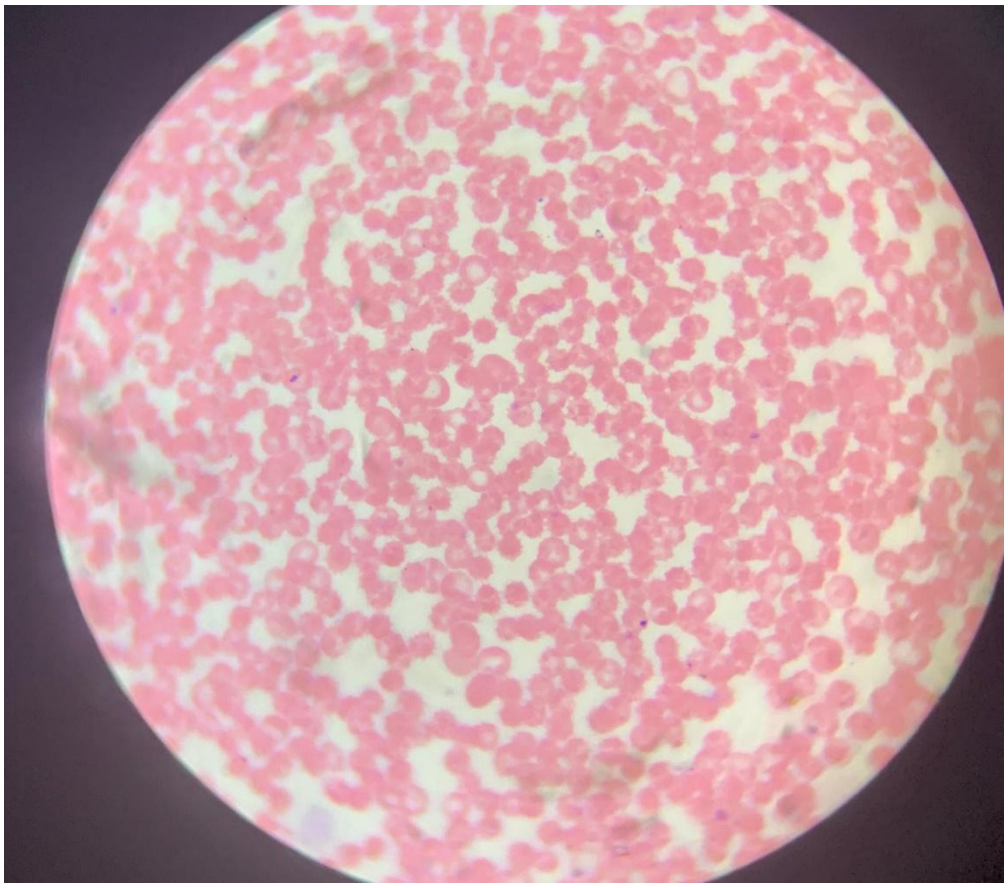
No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date.....15/07/2025

edohrec@edostate.gov.ng

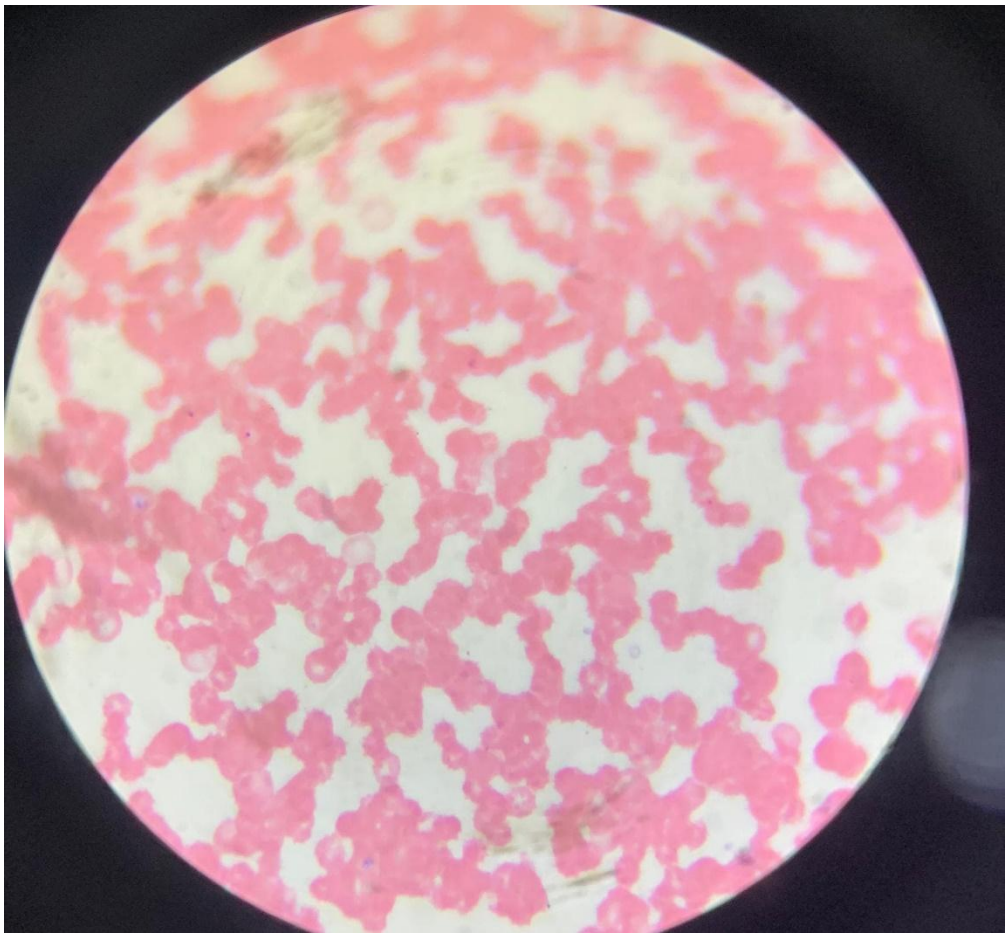
Room 16, Block D, 2nd floor, State secetariat building.

APPENDIX III



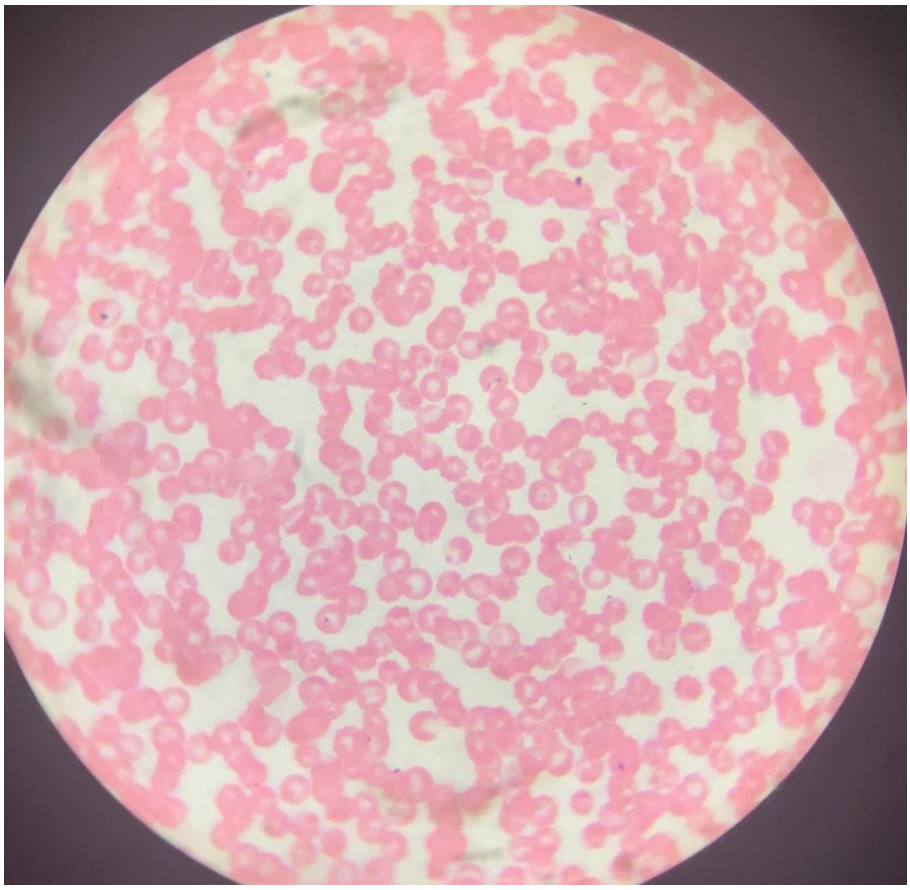
Blood cell morphology of group A experimental animals.

APPENDIX IV



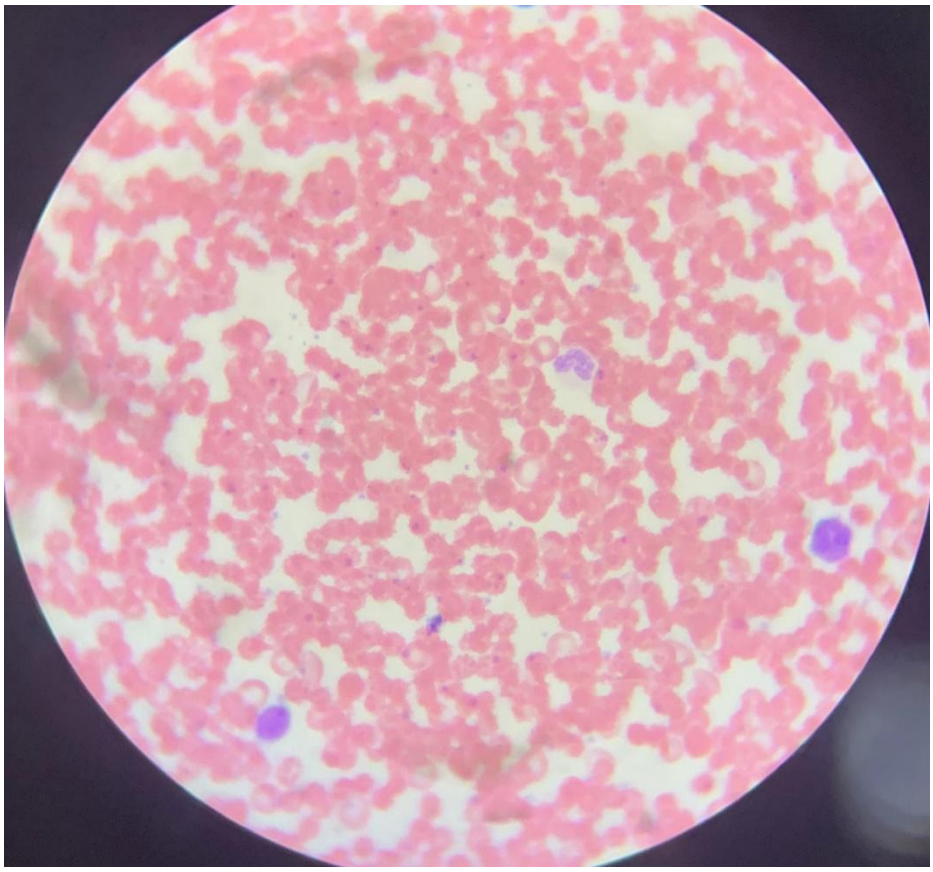
Blood cell morphology of group B experimental animals.

APPENDIX V



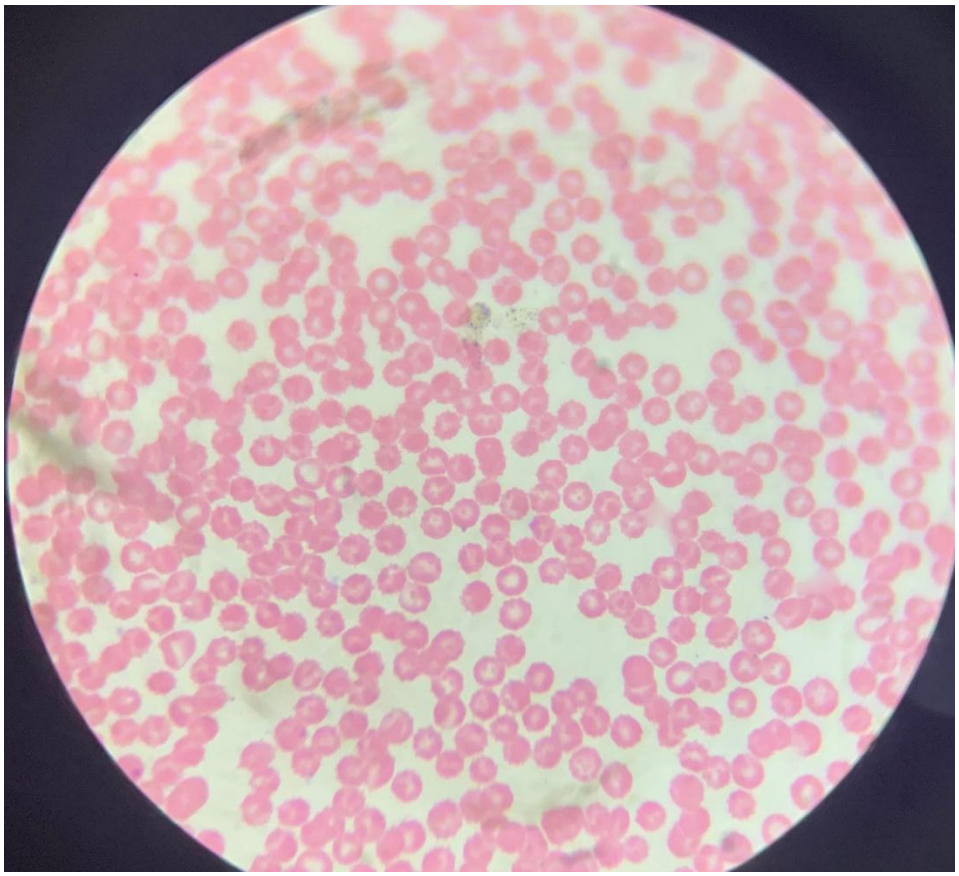
Blood cell morphology of group C experimental animals.

APPENDIX VI



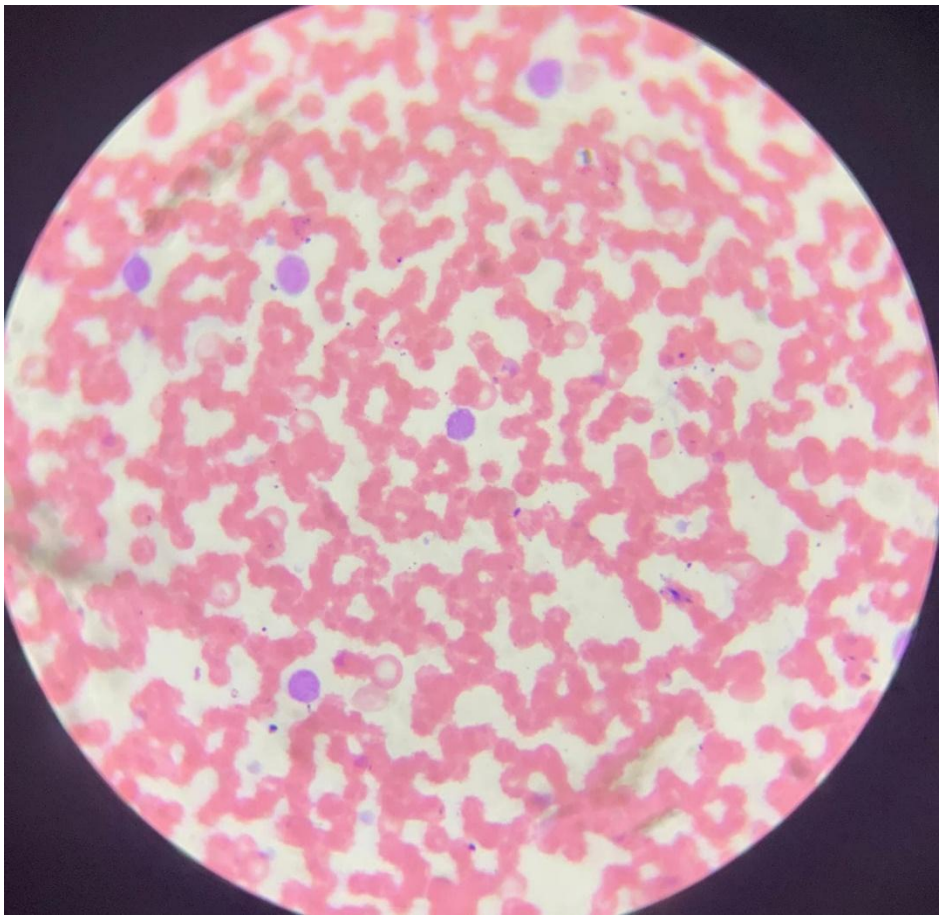
Blood cell morphology of group D experimental animals.

APPENDIX VII



Blood cell morphology of group E experimental animals.

APPENDIX VIII



Blood cell morphology of group F experimental animals.

APPENDIX IX
MATERIALS AND REAGENTS USED

MATERIALS USED

Forceps

Eppendorf Container

Gavage tube

Glass slides

Staining racks

Immersion oil

Microscope

Timer

Sorvall biofuge

Germany eppendor mastercycler

Germany Labnet Electrophoresis system

USA micro pipettes

Hisense Microwave

A & E

UV-visible

Spectrophotometer

Water Bath

REAGENTS USED

Trizol

Chloroform

Buffer solution

Distilled Water

Leishman Stain

Primers used were synthesized by Inqaba Biotec, South Africa.

Zymo DNA extraction kit.

Loading dye.

EZ-Vision.

TBE buffer.

Nuclease Free Water.

Agarose.

All purchased from Inqaba Biotec