EXPRESSION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION-5 IN ALUMINIUM CHLORIDE-INDUCED ANAEMIA BEARING WISTAR RAT TREATED WITH *ICACINA TRICHANTHA* AQUEOUS LEAVES EXTRACT

BY

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BMS2001170

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

OCTOBER, 2025.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS), UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISOR: DR. (MRS.) P.A OBAZELU

OCTOBER, 2025.

CERTIFICATION

This is to certify that this a Project work carried out by **ISIBOR, FAVOUR EBAIDE** with the matriculation number **BMS2001170** under the supervision of **DR. (MRS.) P. A. OBAZELU** in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfilment of the requirement for the Award of Bachelor of Medical Laboratory Science (BMLS) Degree.

DR. (MRS.) P.A OBAZELU	**DATE**
(Project Supervisor)	
DR. (MRS.) ZAINAB OMORUYI	**DATE**
(Head of Department)	
EXTERNAL EXAMINER	**DATE**

DEDICATION

I dedicate this project work to God Almighty for his strength and for the success of this work and his guidance throughout my course of study.

ACKNOWLEDGMENT

I want to express my deepest gratitude to God Almighty for granting me the strength, resources and wisdom needed for this work and for seeing me through this project work.

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ABSTRACT

Icacina trichantha is widely recognized for its medicinal properties, including antioxidative and hematoprotective effects. Over the past decade, interest has grown in exploring the impact of medicinal plants on gene regulation and hematological parameters.The aim of this study is to investigate the expression of the Signal transducer and activator of transcription-5 (STAT5) gene in aluminium chloride-induced anaemia bearing Wistar rats treated with *Icacina trichantha* aqueous leaf extract. 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, aluminum chloride group, ferrous sulfate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* respectively. 5 milliliters (5ml) of blood sample were drawn from each rat, and haematological parameters and mRNA of STAT5 was determined using ERMA haematology autoanalyzer and polymerase chain reaction respectively. Data obtained was analyzed using the GraphPad prism software. The comparison of haematological parameters amongst the study groups showed that Mean cell volume (MCV) (μm3) was significantly lower in group F (54.64±0.96) when compared to group C (58.22±0.49) ($p<0.05$). Mean cell haemoglobin (MCH) (pg) was significantly lower in group F (18.72±0.23) when compared to group C (19.66±0.07) ($p<0.05$). Platelet distribution width (PDW %) was significantly lower in group E (8.96±0.27) when compared to group D (10.6±0.31) ($p<0.05$). Groups B, C, D, E, and F showed significantly higher mRNA expression of STAT5 when compared to group A ($p<0.05$). Groups D, E and F had a higher mRNA expression of STAT5 when compared to group A and B ($p<0.05$). Group F also had a higher mRNA expression of STAT5 when compared to group D ($p<0.05$). In conclusion, administration of aluminum chloride and *Icacina trichantha* aqueous leaf extract led to significant effects on some haematological parameters although the mRNA expression showed dose-dependent alterations in the gene expression of STAT5.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Medicinal herbs have long been used in traditional systems to treat a wide range of illnesses, demonstrating their complete therapeutic potential (Sun *et al.,* 2022; Obazelu and Ogiza, 2024). Worldwide, the usage of medicinal plant-based medicines is still growing quickly, and many people are increasingly turning to these products for the prevention and treatment of a variety of illnesses (Romano *et al.,* 2021). Because many plants contain a variety of phytoconstituents, including flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, it is advised to treat diseases with medicinal plants. These plants are a rich source of bioactive chemicals that have potent pharmacological actions and no unfavorable side effects (Salehi *et al.,* 2019). These phytochemicals have the potential to be used as medications, and if rigorously validated, their recognized pharmacological action provides the scientific foundation for their use in contemporary medicine (Obazelu and Evwaire, 2024).

Indigenous to West and Central Africa, *Icacina trichantha Oliv.* (Icacinaceae) is a drought-resistant shrub belonging to the tropical forest tree family. Native communities in Nigeria, for example, utilize it as a medicinal herb. The plant's tubers and leaves, also known as fake yam and Raynal, are said to have aphrodisiac properties. Asthma and hypertension may be treated by crushing and macerating the leaves and seeds with local gin. Alkaloids, tannins, phenols, and saponins have been detected in the *Icacina trichantha* leaf extract using phytochemical screening. The components of fatty acids, including erucic, oleic, and stearolic acids, were found (Che *et al.,* 2016).

Icacina trichantha leaves ethanol and water extracts are well-known for their phytochemical and antibacterial properties against *Klebsiella pneumonia, Staphylococcus aureus, streptococcus species, Candida albicans*, and *Escherichia col*i (Otun *et al.,* 2015). Research has also shown that when the plant extract was administered without negative interference, there was an improvement in all haematological indicators (Ojatula *et al.,* 2022).

A third of the world's population suffers from anemia, which also impairs neurological development, lowers work productivity, and increases illness and death. Red blood cell (RBC) counts and/or hemoglobin (Hb) concentrations that are below normal and inadequate to support a person's physiological demands are known as anemia (Chaparro and Suchdev, 2019).

Anemia, encephalopathy, aluminosis, osteomalacia, and osteoporosis are among the negative health consequences of aluminum, an environmental contaminant that affects the skeletal, neurological, and hematopoietic systems. Additionally, it disrupts cellular metabolism and iron homeostasis in the hematopoietic system, which may result in anemia (Valenzuela-Briseño *et al.,* 2022). It is believed to disrupt heme synthesis, either by blocking iron incorporation or by preventing iron from binding to transferrin, which could result in issues with erythropoiesis. This is caused by peroxidative changes in the erythrocyte's membrane, which cause hemolysis (Adewuyi *et al.,* 2024; Klein, 2019). A variety of hematopoietic problems may arise from disturbances in this delicate system. For instance, abnormalities in red blood cell differentiation may lead to insufficient development and maturation, which can result in diseases like anemia (Cha, 2024). Numerous variables, including as hormones, cytokines, growth factors, signaling

pathways, and transcription factors, tightly govern the complicated process of hematopoiesis (Tang and Wang, 2023). By regulating the expression of genes involved in different phases of red blood cell formation, transcriptional controls contribute to the regulation of the development of hematopoiesis. The timely activation and repression of certain genes necessary for progenitor cell proliferation, differentiation, and maturation are ensured by this control. Transcriptional regulation tightly controls the balance between hematopoietic stem cell self-renewal and differentiation through intricate signaling pathways and molecular mechanisms, guaranteeing the consistent production of functional blood cells to satisfy physiological demands (Obazelu and Gaius-Igboanugwo, 2024). Signal transducer and activator of transcription 5 (STAT5), a protein belonging to the STAT family, is one of them.

The twin functions of the STAT protein family—transducing signals from cytokine receptors to the nucleus and triggering gene transcription—give it its name. STAT1, STAT2, STAT3, STAT4, STAT5a and STAT5b (together known as STAT5), and STAT6 are the seven highly related homologous proteins that make up the family (Smith *et al.,* 2023). Two proteins that are transcribed from different genes, STAT5A and STAT5B, and have 94% structural similarity are referred to as signal transducer and activator of transcription 5 (STAT5) genes. However, they are both essential in erythroid differentiation (Tóthová *et al.,* 2021). STAT5 proteins play crucial roles in controlling physiological processes like haematopoiesis or hepatocyte function and unrestricted STAT5 activation leads to pathological conditions such as cancer promotion and progression, myelo-proliferative diseases, inflammation, or auto-immunity (Kornfeld *et al.,* 2008).

1.2 Justification of Study

Research into practical and affordable treatment options is necessary due to aluminum's growing environmental impact and proven connection to hematotoxicity, which includes anemia. With its long ethnobotanical history and documented phytochemical composition, *Icacina trichantha* is a potential medicinal plant for treating anemia, especially in light of the increased interest in natural products for illness management. Since STAT5 is a key regulator of erythropoiesis and its dysregulation is directly linked to anemia, it is crucial to clarify how the expression of the STAT5 gene functions in aluminum chloride-induced anemia. This work intends to provide molecular insights into the pathophysiology of AlCl3-induced anemia and the possible remedial mechanisms of *Icacina trichantha* by examining the expression of STAT5. If successful, *Icacina trichantha's* aqueous leaf extract may provide a supplementary treatment or less expensive, easily accessible, and perhaps safer option for aluminum-induced anemia, especially in areas with limited access to traditional medical care. Additionally, this study will provide important scientific evidence in favour of *Icacina trichantha* traditional applications and might lead to the creation of new plant-based erythropoietic medicines. Additionally, albino Wistar rats are often used as experimental models in science since they enable ethical and regulated experiments.

1.3 Aim of study

The purpose of this research is to ascertain the expression of the Signal transducer and activator of transcription 5 (STAT5) gene in Aluminium chloride-induced anaemia bearing Wistar rats treated with *Icacina trichantha* aqueous leaves extract.

1.4 Specific Objectives

- To determine the expression of STAT5 gene in Aluminium chloride-induced anaemia bearing Wistar rats treated with Icacina trichantha aqueous leaf extract.
- To determine the effect of the aqueous leaves extract of *Icacina trichantha* on some haematological parameters in Aluminum chloride-induced anaemia bearing Wistar rats.
- To observe the effect of the aqueous leaves extract of *Icacina trichantha* on the morphology of blood cells in Aluminum chloride-induced anaemia bearing wistar rats.

1.5. Research Questions

- Does the aqueous leaves extract of *Icacina trichantha* have any effect on STAT5 gene expression in Aluminum chloride-induced anaemia bearing Wistar rats?
- Does the aqueous leaves extract of *Icacina trichantha* have any effect on haematological parameters in Aluminum chloride-induced anaemia bearing Wistar rats?
- Does the aqueous leaves extract of *Icacina trichantha* cause changes on the morphology of blood cells in Aluminum chloride-induced anaemia bearing wistar rats?

1.6. Research Hypothesis

Null Hypothesis (H0)

- The aqueous leaves extract of *Icacina trichantha* does not have any effect on STAT5 gene expression in Aluminum chloride-induced anaemia bearing Wistar rats.

- The aqueous leaves extract of *Icacina trichantha* does not have any effect on haematological parameters in Aluminum chloride-induced anaemia bearing Wistar rats.
- The aqueous leaves extract of *Icacina trichantha* does not cause changes in the morphology of blood cells in Aluminum chloride-induced anaemia bearing Wistar rats.

Alternate Hypothesis (HA)

- The aqueous leaves extract of *Icacina trichantha* has an effect on STAT5 gene expression in Aluminum chloride-induced anaemia bearing Wistar rats.
- The aqueous leaves extract of *Icacina trichantha* has an effect on haematological parameters in Aluminum chloride-induced anaemia bearing Wistar rats.
- The aqueous leaves extract of *Icacina trichantha* causes changes in Aluminum chloride-induced anaemia bearing wistar rats.

1.7. Scope of Study

The purpose of the research was to examine how the aqueous leaf extract of *Icacina trichantha* affected changes in blood cell shape, haematological parameters, and the expression of the Signal transducer and activator of transcription 5 (STAT5) gene.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of *Icacina trichantha*

The tropical forest tree family, Icacinaceae, was first recognized by Miers in 1864 and then revised on the basis of DNA sequencing as given by Karehed in 2001, *Icacina*

trichantha Oliver belongs to the family *Icacinaceae* and it is one of the five species of the genus *Icacina* found only in Africa (Ojatula *et al.,* 2023). *Icacina trichantha* (Icacinaceae, Oliv.) is a flowering shrub native to forested vegetation areas of southern Nigeria. It is known as "Urumbia" or "Eriagbo" (referring to its emetic effect) among the Igbos of Nigeria, or "Gbegbe" (meaning to cleanse) by the Yoruba of western Nigeria (che *et al.,* 2016). This plant which is native to West and Central found in Ivory Coast, Benin and Nigeria (Bánki *et al*., 2025; Alawode, 2018). *Icacina trichantha* is a perennial shrub up to 2 m with scandent growth above. It is commonly found in field crops, forest regrowths and waste areas in most part of Nigeria (kabri *et al.,* 2015). The leaves are simple, alternate and broadly-elliptic and the stem straggling, semi-wood, round in cross-section, has soft brown hairs and arises from an underground tuber that also has soft brown hairs (kabri *et al.,* 2015). In Nigeria, *Icacina trichantha* is used as a common household medicine for emergency and first-aid treatment for food poisoning and is valued for its traditional uses, apart from the medicinal properties and as a food energy source, it is still underutilized and could serve as a raw material for bioplastic production in Nigeria (Wahua and Awogbayila, 2024).

It is used infolks medicine as antiinflammatory, anti-convulsion, anti-diabetic and antimalarial in different part of African, some already isolated compounds from different parts of this plant has been reported to possess anti-inflammatory, anti-diabetic, antimicrobial, anti-oxidant, anti-gene toxic, anti-cytotoxic and hepatoprotective activity (Akoh and Mac-Kalunta, 2021).

Figure 2.1: Leaves of *Icacina trichantha* plant (Wahua and Awogbayila, 2024).

Table2.1: Some Species of *Icacina* genus (Ojatula *et al.*, 2023)

S/N	Species
1	*Icacina trichantha*
2	*Icacina claessensii*
3	*Icacina guessfeldtii*
4	*Icacina mannii*
5	*Icacina oliviformis J.Raynal*

Table2.2: Taxonomy of *Icacina trichantha* (Akoègninou *et al.*, 2006).

Domain	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Streptophyta*
Class	*Equisetopsida*
Subclass	*Magnoliidae*
Order	*Icacinales*
Family	*Icacinaceae*
Genus	*Icacina*
Specie	*Trichantha*

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

- **Tuber**: Traditional healers utilize the tubers of *Icacina trichantha* to produce emesis and abortion, as well as to cure a variety of illnesses, including as constipation, poisoning, malaria, rheumatism, and toothaches. The mumps may be treated with the tuber juice. Termites were shown to be killed by an aqueous extract of the tuber in a way that was dependent on both time and concentration (Che *et al.,* 2016).
- **Seeds:** The nutritional contents of the seeds include: 13% moisture, 72% carbohydrate, 8 – 10% proteins, 0.1% fat which can serve as a source of food for animals (wahua and Awogbayila 2024). The seeds when crushed can be used in the for the treatment of hypertension (che *et at.,* 2016).
- **Leaves:** *Icacina trichantha* leaves are used to treat skin diseases in ethnomedicine. Additionally, the leaves have antibacterial properties against *Klebsiella oxytoca*, *Pseudomonas aeruginosa,* and *Escherichia coli* (Alawode *et al.,* 2021). The leaves when crushed and macerated in local gin, can be used for the treatment of hypertension and asthma (che *et at.,* 2016)**.**

2.1.2. Biochemical constituents of *Icacina trichantha*

The phytates found to be present in the flour of *Icacina trichantha* includes carbohydrate (Starch), proteins, lipids and other mineral elements such as potassium, sodium and calcium (Udofia *et al.,* 2014). False yam, Icacina trichantha tubers analyzed for proximate composition revealed presence of carbohydrate 91.93% and proteins 5.25% (Sunday *et al.,* 2016) and also the nutritional contents of the seeds include: 13% moisture, 72% carbohydrate, 8 – 10% proteins, 0.1% fat (Fay, 1991).

2.1.3. Phytochemical constituents *Icacina trichantha*

Phytochemicals are non-nutritive substances that are present in plants and contribute significantly to their flavor and color (Kwon *et al.,* 2023). Phytochemicals can ameliorate diseases by altering the composition and/or diversity of the gut microbiota, and they increase the abundance of some gut microbiota that produce beneficial substances (Rudzińska *et al.,* 2023). Phytochemicals are responsible for the broad range of pharmacological activities displayed by plants, and thus, the extracts are expected to exhibit varying bioactivities due to differences in their phytoconstituents (Alawode *et al.,* 2021). Phytochemical studies indicate that the tuber extracts of *Icacina trichantha* contained alkaloids, terpenes, flavonoids, glycosides, steroids, saponins and tannins (Monday and Uzoma, 2013).

Phytochemical screening of the leaf extract of *Icacina trichantha* has shown the presence of alkaloids, tannins, phenols, saponins and some fatty acid components such as stearolic acid, oleic acid and erucic acids were also identified, novies of novel (9βH)-pimarane and (9βH)-17-nor-pimarane structures have been isolated from the tuber of *Icacina trichantha* (Che *et al.,* 2016).

2.1.4. Anti-microbial Activity of *Icacina trichantha*

The antimicrobial chemicals found in medicinal plants may offer a substantial therapeutic benefit in the treatment of resistant microbial strains and may work via different mechanisms than currently employed antimicrobials to suppress the development of bacteria, fungus, viruses, and protozoa (Vaou *et al.,* 2021). It has been shown that the wide range antibacterial properties of *Icacina trichantha* leaves are highly reliant on the extraction solvent. The methanol extract's ability to compare well with antibiotics that have previously been developed, including pefloxacin, at higher doses (Timothy and Idu, 2021). *Icacina trichantha* leaves antibacterial activity in vitro was first shown in *Escherichia coli* and *Pseudomonas aeruginosa* (Che *et al.,* 2016). When Icacina trichantha leaf extract was tested using three different fractions (hexane, ethyl acetate, and methanol), the hexane fraction had the highest efficacy against all test microorganisms. According to Alawode et al. (2021), it even showed modest effectiveness against gram-negative bacteria, including *Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli*. Additionally, the plant demonstrated a strong in vitro antimicrobial profile against *Streptococcus pyrogenes, Klebsiella pneumonia, Shigella dysenteriae, Candida albicans, Candida krusei,* and MRSA (Methicillin-resistant Staphylococcus aureus), which are known to cause infections that are challenging to treat because of their multiple drug resistance. Additionally, the extract demonstrated efficacy against some fungal species and both gram-positive and gram-negative bacteria (Ojatula et al., 2023).

2.1.5. Anti-diabetic Activity of *Icacina trichantha*

The anti-diabetic potential of *Icacina trichantha* leaf extract was studied in alloxan-induced diabetic mice (Che *et al.,* 2016). A study conducted by Ezeigbo (2010) in alloxan-induced diabetic mice reveal that orally administered methanolic extracts of *Icacina trichantha* leaves at the dose of 300 mg/kg produced significant fasting blood glucose lowering activity in 6 and 12 hour samples compared with the control and an increase in the dose (450 mg/kg) showed even a more observable hypoglycemic activity in the diabetic mice. A study conducted by Monday et al (2013) indicated that *Icacina trichantha* tuber extracts have good antidiabetic activity. On Days 7, 14, and 21, the quantities of 400 mg/kg and 600 mg/kg of tuber extract caused a larger reduction ($P<0.01$), which was statistically significant. Blood sugar levels decreased in a dose-dependent manner after three weeks of daily therapy with different concentrations of Icacina trichantha extract. According to metrics like triglycerides, serum LDL, and HDL, they may also help those with diabetes mellitus. When compared to the diabetic control group, the 400 mg/kg and 600 mg/kg extracts demonstrated a slight improvement in serum triglyceride levels on Day 21, which was statistically significant ($P<0.01$) (Monday *et al.,* 2013).

2.1.6. Antioxidant Activity of *Icacina trichantha*

Antioxidants oxidants are a diverse reactive oxygen species which neutralize free radicals by donating electrons, thereby stabilizing them group of compounds that play a crucial role in protecting cells from oxidative damage and stress caused by free radicals and and preventing further damage to body cells (Obazelu and Willams, 2024). The in vitro methods for evaluation of antioxidant activity have been developed to measure the

efficiency of natural antioxidants either as pure compounds or as plant extracts resulting in measurements of antioxidant capacity of plant materials using highly sensitive methods while the antioxidant activity of *Icacina trichantha* extract may be attributed to the presence of some phytochemicals such as flavonoids, tannins, cardiac glycosides and terpenoids (Ojatula *et al.,* 2023). In vitro screening of the leaf, wood, and root parts of *Icacina trichantha* have reportedly exhibited moderate levels of antioxidant activity with respect to 2, 2-diphenyl-picryl-hydrzyl radical(DPPH) (Ojah and kachi, 2020). Antioxidant activity of the leaf was correlated to total phenol contents and a hexane extract was also claimed to be active in three almost identical reports (che *et al.,* 2016). It has also been shown that the n-hexane extract of the leaf of the plant has higher antioxidant activity against DPPH than the hexane and methanol extract due to the presence of high content of phenolics, which could be the most effective in protecting the body against different oxidative stressors (Otun *et al.*, 2015). Ojatula *et al* (2023) also studied the plant and it showed efficient resolution of in vitro oxidative stress (anti-oxidant activity) by scavenging DPPH, and improving the concentrations of other radicals.

2.1.7. Anti-inflammatory Activity of *Icacina trichantha*

Inflammation is the body's normal immune response against any harmful stimuli such as tissue injury, toxins or pathogens that can cause infection in the body (Sohrab *et al.,* 2023; Obazelu and Abadaike-Elvis, 2024). *Icacina trichantha* leaves most likely contain substances that have the ability to stabilize membranes. It is well known that these types of substances disrupt the first phases of inflammatory responses and stop phospholipase from being released, which initiates the production of inflammatory mediators. *Icacina*

trichantha leaves contain flavonoids and saponins, two families of chemicals that have been shown to have anti-inflammatory properties, according to phytochemical screening of the methanol and ethylacetate extracts of the leaves (Alawode *et al.,* 2018). At dosages of 100, 200, and 400 mg/kg, the aqueous leaf extract of Icacina trichantha considerably reduced paw edema, exhibiting an inhibitory impact throughout the first, second, and continuous stages of inflammation. The extract's antihistamine properties, which may hinder carrageenan-induced microvascular leakage, are suggested by the inhibition of the first phase (Timothy *et al.,* 2015). One important prepared mediator that mast cells produce is histamine, which significantly raises vascular permeability. Many symptoms of acute allergic responses, such as urticaria, edema, and in severe instances, anaphylactic shock, are brought on by this increase in vascular permeability (Ashina *et al.,* 2015). The extract's action in the second phase raises the possibility that prostanoid production is inhibited. Since it closely mimics human arthritis, the suppression of edema in rats caused by formalin is one of the best test methods to screen for anti-arthritis and anti-inflammatory drugs (Timothy *et al.,* 2015).

2.2 Signal Transducer and Activator of Transcription 5 (STAT5) Gene

The STAT (Signal Transducer and Activator of Transcription) protein family is a significant family of evolutionarily conserved transcription factors that are essential for a variety of biological processes, most notably the development and function of blood and immune cells (Awasthi *et al.,* 2021). The transcription factors known as STAT (signal transducers and activators of transcription) proteins are phosphorylated on tyrosine residues in response to cytokine activation. According to Onishi et al. (1998), there are seven members of the STAT family, including the closely related STAT5A and STAT5B,

which are activated by different cytokines. The twin functions of the STAT protein family—transducing signals from cytokine receptors to the nucleus and triggering gene transcription—give it its name. Numerous cytokines and growth factors cause STAT5 to become active. When activated, it is essential for hematopoiesis, namely the growth, division, and survival of lymphocytes (Smith *et al.,* 2024). When growth factors and cytokines are present, STAT5 proteins alter gene transcription (Kornfeld *et al.,* 2008). With pleiotropic functions in hematopoietic stem cell (HSC), hematopoietic progenitor cell (HPC), and mature cell populations, STAT5 is the primary regulator of normal hematopoiesis. IL-3, thrombopoietin (TPO), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), and erythropoietin (EPO), a key STAT5 activator in the erythroid lineage, are among the early acting cytokines that activate STAT5 (Wang and Bunting, 2013). In hematopoietic cell types, including erythrocytes, megakaryocytes, Natural Killer (NK) cells, CD4+ and CD8+ T cells, and B cells, STAT5B is more strongly expressed than STAT5A. Only CD34+ hematopoietic stem cells exhibit increased expression of STAT5A (Smit *et al.,* 2024).

2.2.1. Structure and location of STAT5 gene

The STAT5 gene is located on chromosome 17. STAT5 gene contains the following conserved domains: an N-terminal region (associated with cooperative DNA binding between dimers), a coiled-coil domain (plays a role during the DNA binding process and provides a site for interactions with other proteins), a DNA binding domain (allows function as transcription factors and targets specific DNA sites) , a linker region and SH2 domain (modulates the interaction through phospho-tyrosines) and a C-terminal transactivation domain (Smith *et al.,* 2023). In contrast to STAT5A, which is made up of

794 amino acids, the 694th amino acid is a tyrosine phosphorylation site. The 699th amino acid is the tyrosine phosphorylation site for STAT5. The ability of purified STAT5B to bind DNA is greater than that of STAT5A. While STAT5B binds to DNA in the form of dimers, STAT5A may also form tetramers (Hu *et al.,* 2021).

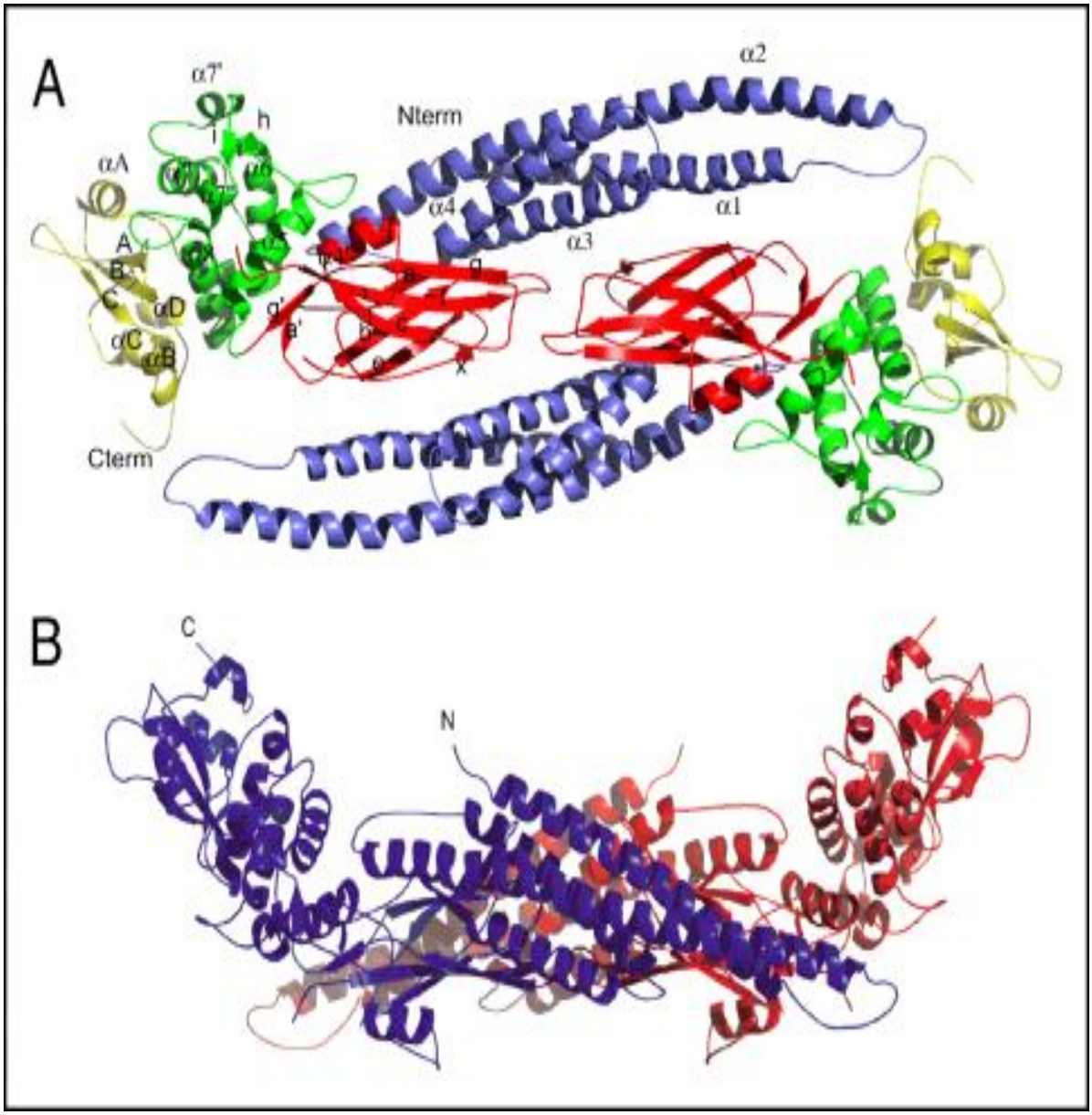

Figure 2.2. Structure of STAT5 Protein (Neculai *et al.*, 2008)

2.2.2 Role of STAT5 in development and growth regulation

It has been shown that signaling via the JAK2/STAT5 cascade is essential for secretory mammary epithelial cell specification, proliferation, differentiation, and survival (Rädler *et al.,* 2017). Initially known as prolactin-induced mammary gland factor, STAT5a was discovered to neutralize the β-casein gene. Mammary gland development and milk secretion are severely impaired in STAT5-knockout mice, whereas growth hormone synthesis is impaired in STAT5b-/-mice (Hu *et al.,* 2021).

2.2.3. Role of STAT5 in immune system regulation

Since the production of a constitutively active version of STAT5b (Stat5b-CA) significantly restores B cell formation in Il7r-/-mice, STAT5 plays a particularly significant role in this process. The crucial involvement of STAT5 in B cell formation was validated by further research using animals in which STAT5 could be specifically removed from growing B cells (Heltemes-Harris and Farrar, 2012). Survival depends on STAT5 dimers; animals lacking STAT5a and STAT5b show significant abnormalities in lymphatic development as well as neonatal mortality. While mice lacking the STAT5a-STAT5b tetramer are still alive, they have fewer T cells, NK cells, and CD8+ T cells, as well as a reduced ability to proliferate and mature NK cells (Hue *et al.,* 2021). Because STAT5 controls the rearrangement of the T-cell receptor (TCR) γ gene, studies using

STAT5 knockout mice showed that STAT5 is essential for the generation of γδ T cells. Similarly, the growth of double-negative thymocytes depends on STAT5 (Owen and Farrar, 2017).

2.2.4. Role of STAT5 in Cancer

STAT5 has a role in lymphoid cell survival and proliferation. Thus, the association between STAT5 activity and lymphocyte transformation may not be unexpected. For instance, the BCR-ABL fusion gene is known to include STAT5 as a downstream target. About half of human acute lymphoblastic leukemia (ALL) samples have high levels of activated STAT5, according to Weber-Nordt and colleagues (Heltemes-Harris and Farrar, 2012). Due to uncontrolled activation of other signaling molecules or intracellular or extracellular stimuli, STAT proteins are often constitutively phosphorylated or constitutively active in melanoma cells. In order to activate or inhibit the transcription of certain target genes, phosphorylated STATs enter the nucleus as dimers and attach to particular regulatory regions (Rah *et al.,* 2022). Through receptors or receptor-associated tyrosine kinases, STAT5 is constitutively active in leukemias and some solid tumors, aiding in the survival and growth of cancerous cells (Wittig and Groner, 2005). Carcinomas of the breast, prostate, ovary, head, and neck also exhibit Stat5 activation (Moriggl *et al.,* 2005).

2.2.5. Role of STAT5 in Haematopoiesis

Multilineage progenitors that are limited to either the myeloid or lymphoid lineages (the common myeloid progenitor and the common lymphoid progenitor) are created during the process of hematopoietic differentiation. These pluripotent cells can then differentiate further to produce mature erythroid, megakaryocytic, myeloid, or lymphoid cells

(Schuringa *et al.,* 2004). Signal transducer and activator of transcription 5 (STAT5) is extensively expressed in committed erythroid, myeloid, and lymphoid cells, as well as stem and progenitor cells (Wierenga *et al.,* 2008). The activation of Janus tyrosine kinases (JAKs) by hematopoietic cytokines such erythropoietin (Epo), thrombopoietin, and GM-CSF causes a brief phosphorylation of STAT5 at a single conserved tyrosine residue. By regulating the growth and differentiation of T and B cells via a variety of cytokines, such as IL-2 and IL-7, STAT5 functions as a master regulator of lymphopoiesis (Fahrenkamp *et al.,* 2016). Multiple hematological abnormalities are seen in Stat5a and Stat5b double-deficient knockout mice (Stat5−/−). Hematopoietic progenitors, T, NK, erythroid, and myeloid cells, as well as cytokine-mediated proliferation and survival, are all compromised in Stat5−/− cells (Moriggl *et al.,* 2005). Lastly, the total deletion of Stat5a and Stat5b utilizing Cre-LoxP techniques showed that STAT5a and STAT5b are definitely necessary for the formation of lymphocytes since Stat5a/b -/-mice exhibited significant lymphocyte development blocks that resembled those shown in Il7r -/-mice (Owen and Farrar, 2017).

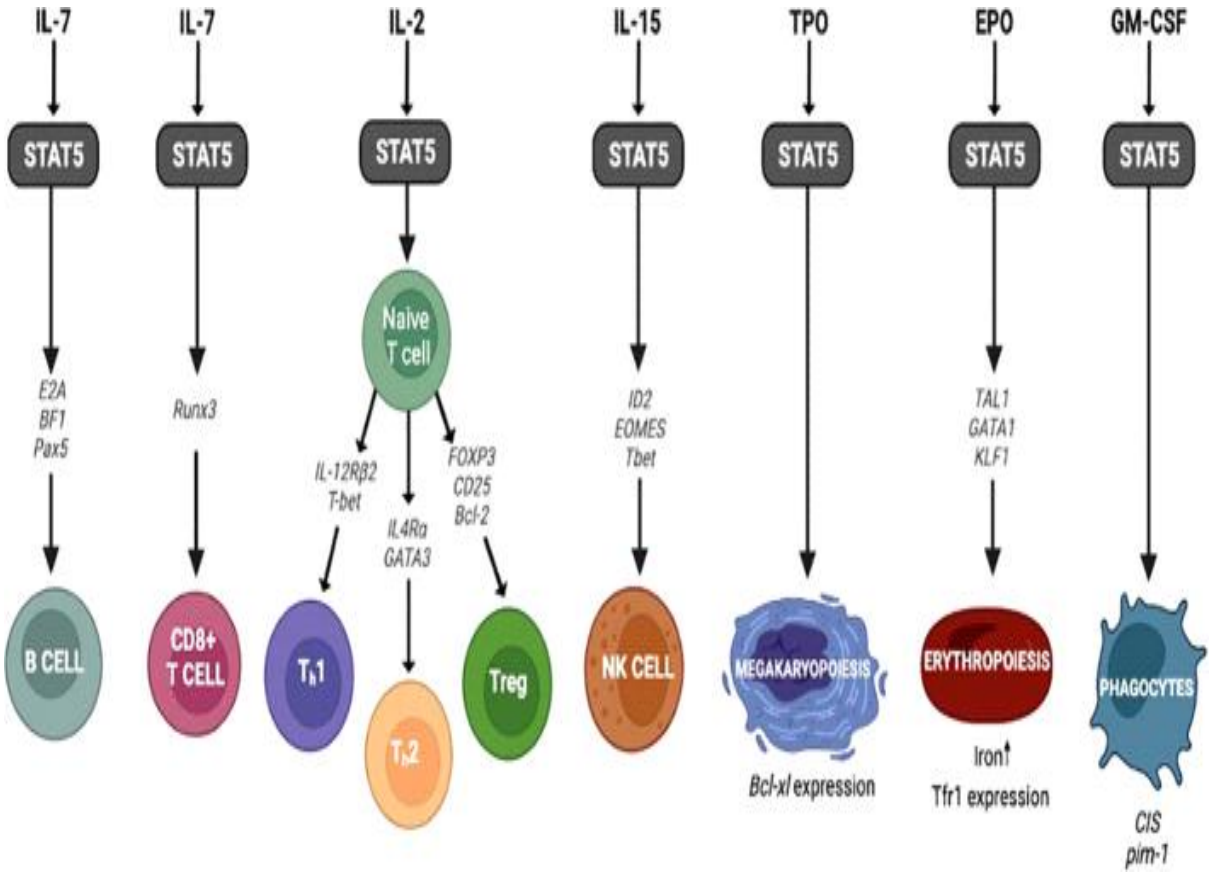

Figure 2.3. Role of STAT5 in Haematopoiesis (Smith *et al.*, 2023).

2.2.6. JAK-STAT5 Signaling pathway

The JAK-STAT signaling pathway transmits extracellular signals to the nucleus and regulates a variety of cellular activities including apoptosis, differentiation, proliferation, and immunological responses, this pathway consists of receptor-associated Janus kinases (JAKs), signal transducers and activators of transcription (STATs), and a cytokine or hormone receptor (Able *et al.,* 2017). The JAKs are a family of tyrosine kinases that are activated by the binding of ligands that includes growth factors, hormones, interferons, and a variety of cytokines to their specific receptors (Able *et al.,* 2017). The JAK protein family contains four members: JAK1, JAK2, JAK3, and TYK2 (Xue *et al.,* 2023). Upon ligand binding to the receptor, JAKs tyrosine-phosphorylate themselves as well as the receptor, creating a binding site for the SH2 (Src Homology 2) domain containing STAT proteins (Abel *et al.,* 2017). The JAK-STAT5 signaling pathway is then activated via tyrosine phosphorylations that occur in response to cytokines (IL-2, -3, -5, -7, -9, -15, -21) and growth factors stimulations including granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO), thrombopoietin (TPO), and Fms-like tyrosine kinase 3 (Flt3) (Smith *et al.,* 2023). STAT dissociates from the receptor and forms homodimers or heterodimers (dimerization) through SH2-domain–phosphotyrosine interactions and these dimers translocate to the nucleus of the cell where they bind to specific promoter regions of DNA to regulate the transcription of target genes (Hu *et al.,* 2021; Abel *et al.,* 2017). The JAK-STAT pathway is regulated by positive and negative effectors, activators include signal-transducing adapter molecules (STAMs) and proteins containing SH2 domains, while inhibitors include suppressors of cytokine signaling (SOCS) and tyrosine phosphatases (Abel *et al.,* 2017).

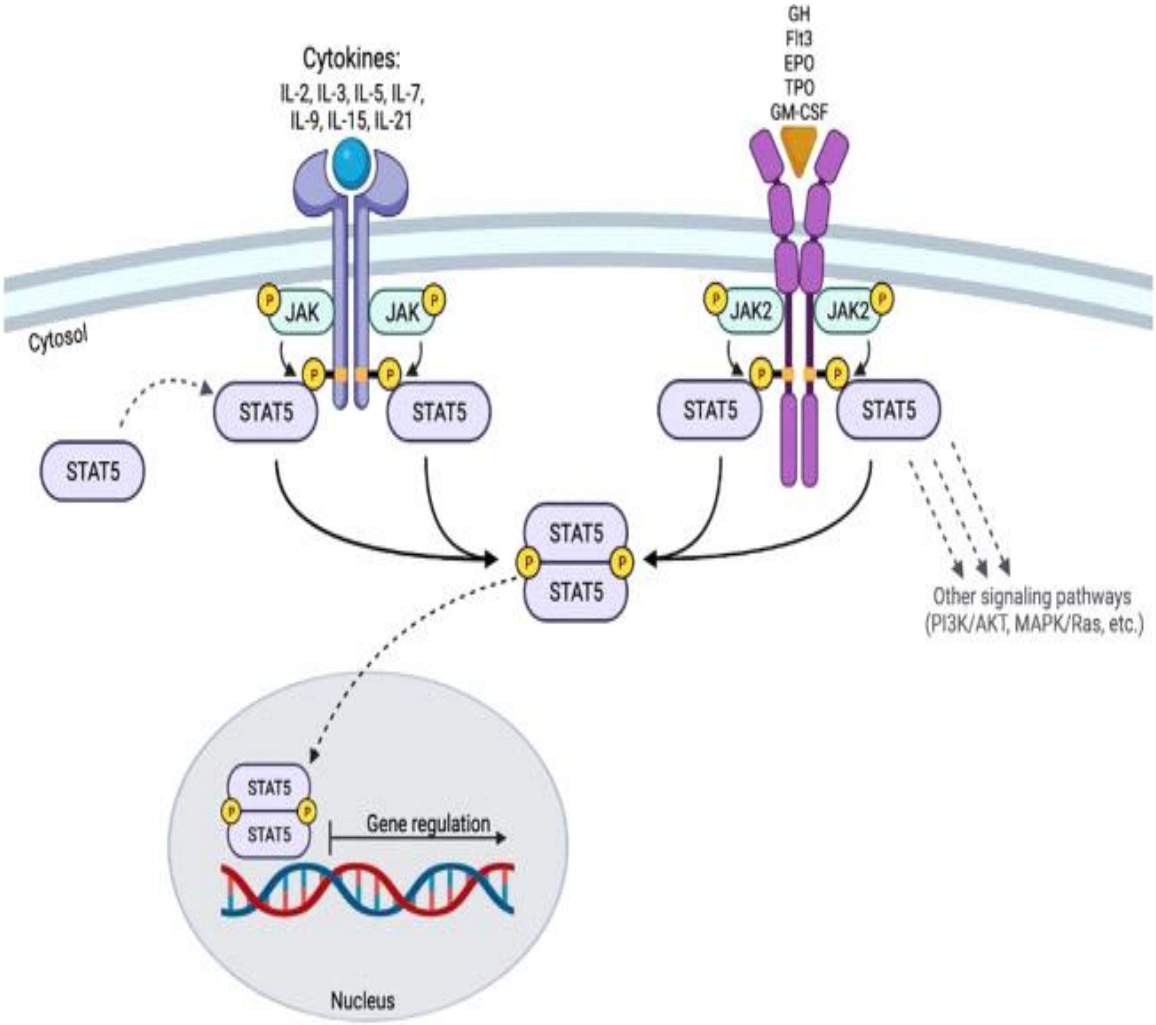

Figure 2.4. JAK-STAT5 Pathway (Smith *et al.*, 2023).

2.3. Haematological Parameters

In clinical practice, reference ranges for haematological and immunological parameters are often employed to evaluate health and illness states. The reference ranges may also serve as valuable indicators for evaluating the course of the illness or the effectiveness of treatment. Age, gender, ethnicity, environment, and genetic background may all affect these characteristics. The science of haematology has benefited greatly from recent advancements in peripheral blood cell counts, which have also increased the availability and accessibility of such data for clincians throughout the globe (Kone *et al.,* 2017). The work-up should include laboratory testing, such as measurements of hemoglobin, red blood cell indices, and haematocrit, as well as physical examinations to determine the type of anemia and create a differential diagnosis. The cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) should all be included in the red blood cell indices. The most often used metric to assess the pathophysiological effects of anemia is the hemoglobin concentration (Sgnaolin *et al.,* 2013).

2.3.1. Red Blood Cells

The most abundant cells in mammals is the red blood cell (RBC) (Ducamp and Ostuni, 2023). Despite lacking the organelles and nucleus found in most cells, the mature red blood cell (RBC) is exquisitely shaped to carry out the vital task of transporting oxygen and eliminating carbon dioxide from every other cell while withstanding the shear stress caused by negotiating tiny vessels and sinusoids (Risinger and Kalfa, 2020). The form of RBCs has a big impact on how they work. RBCs typically have a biconcave discoid shape, however they may also take on various forms, such spiculated echinocytes or cup-

shaped stomatocytes (Mesarec *et al.,* 2019). The bulk of the four months that mature RBCs live are spent moving through the microcirculation (Rogers and Doctor, 2020). The normal range for RBC in men and women is 4.5 to 5.5 × 1012/L and 4.0 to 5.0 × 1012/L, respectively (Cheesebrough 2006).

2.3.2. Haemoglobin

Erythrocytes contain the oxygen-binding protein hemoglobin, which carries oxygen from the lungs to tissues. The four polypeptide globin chains that make up each hemoglobin molecule form a tetramer. A heme moiety made up of an organic protoporphyrin ring and a ferrous iron ion (Fe2+) is present in every globin subunit. Each heme moiety contains an iron molecule that has the ability to bind and unbind oxygen, enabling the organism to transport oxygen. HbA, which is made up of two alpha and two beta hemoglobin subunits, is the most prevalent form of hemoglobin in adults. Each kind of globin subunit is encoded by a distinct globin gene (Farid *et al.,* 2023). The unit of measurement for the quantity of hemoglobin in whole blood is grams per deciliter, or g/dl. Males typically have a hemoglobin level of 14–18 g/dl, whereas females typically have 12–16 g/dl (Billett, 1990).

2.3.3. Haematocrit

The proportion of red blood cells relative to the volume of blood (red blood cells and plasma) is measured by the hematocrit, which is represented as a percentage (Schreijer *et al.,* 2010). Men typically have a haematocrit of 40–54%, whereas women typically have one between 36 and 48%. This value may be computed indirectly or directly using microhaematocrit centrifugation. By multiplying the mean cell volume (MCV, in femtoliters) by the red cell number (in millions/mm3), automated cell counters determine

the haematocrit. When tested in this manner, it is susceptible to the whims of obtaining a precise measurement of the MCV (Billett, 1990).

2.3.4. Red Cell Indices

2.3.4.1. Mean Corpuscular Volume (MCV)

MCV, which is measured in femtoliters, determines the size of red blood cells (Sarma, 1990). Together with other factors like hemoglobin and hemocrit, MCV divides anemia into three primary types: microcytic, normocytic, and macrocytic. Microcytic anemia is defined by MCV values that are below, within, and above the normal range, respectively. Additionally, the red blood cell distribution width (RDW) computation is guided by MCV (Maner *et al.,* 2024).

It can be calculated as:

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

A normal MCV ranges between 80 and 100 fL (Cheesbrough, 2006).

2.3.4.2. Mean Corpuscular Haemoglobin (MCH)

MCH quantifies the amount of haemoglobin per red blood cell (Sarma, 1990). It is expressed in pictograms (pg). It is calculated as thus:

$$\textbf{MCH} = \frac{\text{(Haemoglobin in g/10ml of blood)pg/cell}}{\text{Red blood cell count in millions/ml}}$$

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

2.3.4.3. Mean Corpuscular Haemoglobin Concentration (MCHC)

The quantity of hemoglobin per unit volume is indicated by MCHC. Unlike MCH, MCHC establishes a correlation between the volume of the cell and the amount of

hemoglobin. It may be reported as a percentage or as g/dl or g/l of red blood cells (Sarma, 1990).

$$\textbf{MCHC} = \frac{\text{(Haemoglobin in g/10ml of blood)x 100g/l}}{\text{Red blood cell count in millions/ml}}$$

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

2.3.4.4. Red Cell Distribution Width (RDW)

RDW is a percentage that stands for the coefficient of variation of the red blood cell volume distribution (size) (Sarma, 1990). The size variation of circulating erythrocytes (anisocytosis) is measured by red cell distribution width (RDW). Numerous clinical situations, including hemolysis, post-transfusion reactions, and inadequate red cell production which may be brought on by iron, vitamin B12, or folate deficiencies can result in elevated RDW levels. Additionally, RDW is elevated in certain clinical conditions, including thrombotic thrombocytopenic purpura, inflammatory bowel disease, and pregnancy (Isik *et al.,* 2012). RDW typically has a value of 13 ± 1.5% (Sarma, 1990). RDW represents the coefficient of variation of the red blood cell volume distribution

2.3.5. Platelets

The megakaryocyte is the initial source of the platelet, a tiny, anucleated cell that comes from the hematopoietic lineage. It is believed that the bone marrow or, more recently, the lung, are the sites of the systematic and controlled process that produces platelets from megakaryocytes. The platelet's lifespan is restricted to 5–7 days after formation and separation from the megakaryocyte, largely because of the severe shear forces it is subjected to in the vessel and the limitations imposed by its lack of a nucleus (Holinstat,

2017). Adults typically have 140–400 × 109/L of platelets, whereas children typically have 150–450 × 109/L (Fischbach, 2003).

2.3.6. White Blood cells (WBC)

WBCs, often referred to as leukocytes, are a vital part of the human immune system and help the body fight against foreign substances and infectious illnesses (Ahmad et al., 2023). The human immune system uses white blood cells (WBCs) to fight off infections and shield the body from dangerous substances outside. Each of its components—neutrophils, eosinophils, basophils, monocytes, and lymphocytes—makes up a different proportion and has a particular purpose (Tamang et al., 2022). The differential WBC count shows the absolute amount or percentage of neutrophils, eosinophils, basophils, lymphocytes, and monocytes, whereas the WBC count represents important data on the total WBC. Granulocytes and agranulocytes are two classifications for these five types of WBCs (Tamang et al., 2022). According to Riley and Rupert (2015), the normal range for adults is 4,500 to 11,000 per mm3 (4.5 to 11.0 × 109 per L), whereas for children it is around 5,000 to 20,000 per mm3 (5.0 to 20.0 × 109 per L). WBCs, also known as leukocytes, are an essential component of the human immune system, assisting the body in fighting infectious diseases and foreign substances (Ahmad *et al.,* 2023). White blood cells (WBCs) in the human immune system defend against infection and protect the body from external hazardous objects. They are comprised of neutrophils, eosinophils, basophils, monocytes, and lymphocytes, whereby each accounts for a distinct percentage and performs specific functions (Tamang *et al.,* 2022). The WBC count represents essential parameters on the total WBC, and the differential WBC count displays the absolute number or percentage of neutrophils, eosinophils, basophils, lymphocytes and

monocytes. These five categories of WBCs can be categorized as granulocytes and agranulocytes (Tamang *et al.,* 2022). Normal range for children is approximately 5,000 to 20,000 per mm3 (5.0 to 20.0 × 10^9 per L), and for adults is 4,500 to 11,000 per mm3 (4.5 to 11.0 × 10^9 per L) (Riley and Rupert, 2015).

2.3.6.1. Neutrophils

Neutrophils are the most abundant leukocytes in the circulation, and have been regarded as first line of defense in the innate arm of the immune system against most bacterial and fungal pathogens that occurs before the complex humoral and lymphocyte cellular processes of acquired immunity can be brought to bear on an infection (Rosales, 2018; Malech *et al.,* 2014). The bone marrow produces a lot of them—about 1011 cells per day. Neutrophils enter the bloodstream, go to tissues, do their tasks, and are eventually removed by macrophages when homeostatic conditions are met. Once pathogens are detected, they use phagocytosis, intracellular degradation, granule release, and neutrophil extracellular trap creation to catch and eliminate invasive bacteria. Additionally, neutrophils mediate inflammation (Rosales, 2018)

Neutrophilia and neutropenia are abnormally high and low neutrophil count (Hoffbrand et al., 2016). Reference range for Adults: 40% to 60% of total white blood cells and for children: 25% to 40% of total white blood cells (Ficshbach, 2003).

2.3.6.2. Lymphocytes

T, B, and natural killer cells are examples of lymphocytes, which are white blood cells with a consistent appearance but a variety of functions. These cells are in charge of producing antibodies, controlling the immune response, and directly destroying tumor and virus-infected cells (Larosa and Orange, 2008). When it comes to fighting infections,

lymphocytes are crucial. The functions of lymphocytes and their byproducts include directing cytotoxic action, activating macrophages, and neutralizing infections with certain antibodies. Adaptive immunological responses are mediated by B and T cells. Natural killer cells, NK, are also regarded a lymphocytic lineage; nonetheless, their development is fundamentally distinct from that of lymphocytes. NK cells are in charge of innate, not adaptive, responses against tumor cells and virus-infected cells. They also exhibit a distinct collection of receptors compared to those produced by B and T lymphocytes (Camara *et al.,* 2012). 20% to 40% of total white blood cells is the guideline range for both adults and children (Ficshbach, 2003).

2.3.6.3. Monocytes

Monocytes are important cell types of the innate immune system (Karlmark *et al.,* 2012). They regulate inflammatory and immune responses by interacting with lymphocytes and serve as antigen-presenting cells by differentiating into dendritic cells, upon response to activating signals such as chemokines and cytokines, activated monocytes adhere and migrate to the sites of infection or inflammation through diapedesis (Mangaonkar *et al.,* 2021). Normal absolute monocyte count ranges in adults ranges in between 0.2–0.8 × 10^9/L (Mangaonkar *et al.,* 2021).

2.3.6.4. Basophils

Basophils are the least abundant leucocytes primarily found in the circulation which comprise only a small percentage (~ 0·5%) of circulating blood cells under steady-state condition (Min *et al.,* 2012). Basophils play an active role in allergic response and inflammation, autoimmunity, hyper sensitivity reactions and haematological

malignancies (Chirumbolo *et al.,* 2018; Shah *et al.,* 2021). Normal range for basophils is 0.02-0.05 x 10^9/L (Fischbach, 2003).

2.3.6.5. Eosinophils

Eosinophils are bone marrow–derived cells of the granulocyte lineage and are innate immune cells (Kovalszki and Weller, 2016; Akuthota and Weller, 2012). These cells are involved in combating some parasitic, bacterial, and viral infections and certain cancers (Wechsler *et al.,* 2021). Eosinophilia is defined as elevation of eosinophils in the bloodstream (450-550 cell/μL) and there are many reasons for eosinophilia to exist, including parasitic disease, allergic disease, autoimmune, connective tissue disease, rheumatologic disease, primary eosinophilia such as hypereosinophilic syndrome, and as part of a malignant state (Kovalszki and Weller, 2016). Normal range of eosinophils is 0-0.7 x 10^9/L (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 and the study carried was out at the Animal Housing wing of the Department of Anatomy, University of Benin (Obazelu and Efosa, 2025).

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 by Dr. A. H. Akinnibosun 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 duration of two weeks. Further drying was then carried out using a hot air oven at 50ºC for 24 hours. 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 (Obazelu and Anyafulu, 2025).

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 with 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 Ezeonyebuchi, 2025).

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 Agbikimi, 2025).

3.5.1. Inclusion Criteria

- Apparently healthy albino 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/05210723 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 (Obazelu and Anyafulu, 2025).

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 and 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 Agbikimi, 2025).

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 the 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 aluminum chloride intraperitoneally.

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

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

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

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

3.8.1. Dosage of Ferrous Sulphate Administered

40mg/kg.bw

40mg - 1000g

? - 150g (Mean weight of rats)

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

1000mg of ferrous sulphate powder = 50mls

6mg = X

$= \frac{50 \times 6}{1000} = 0.3ml$

X= 0.3ml 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

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

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 aluminum chloride intraperitoneally

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

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

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

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

Calculating dose of extract for each group using;

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

Group D

Average weight of 10 rats = 150kg

Dose = 100mg/kg

Stock = 100mg/ml

150g to kg = 0.150kg

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

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

Group E

Average weight of 10 rats = 150kg

Dose = 200 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

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

=$\frac{0.15 \times 200}{100}$ = 0.3ml

Group F

Average weight of 10 rats = 150kg

Dose = 400 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

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

= $\frac{0.15 \times 400}{100}$ = 0.6ml

3.8.3. Administered Doses *Icacina trichantha* Leaves Extract

Group A (control) received only standardized feed and clean water ad libitum. Group B (aluminum chloride treated group) were administered 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days. Group C (ferrous sulphate drug solution treated group) were administered 0.1ml of aluminum 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 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of aqueous leaves extract of *Icacina trichantha* orally using a gavage tube every 24 hours for 28 days. Group E were administered with 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of aqueous leaves extract of *Icacina trichantha* orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of aqueous leaves extract of *Icacina trichantha* orally using a gavage tube every 24 hours for 28 days.

3.9. Physical Examination of Animals

Animals were weighed to check for any increase or decrease in body weight throughout the course of the experiment (Obazelu and Osarinmwian, 2025).

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 (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. After anaesthetizing (using chloroform) and cervical dislocation, a midline incision was made through the ventral wall of the rats. Five milliliters (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. 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 (Obazelu and Osarinmwian, 2025).

3.11. Laboratory Analysis

3.11.1. Haematological Profile

The full blood count (FBC) parameters were analysed immediately after sample collection using the automated three parts ERMA Haematology Auto analyser PCE-525 (Diamond Diagnostic; Holliston, USA). Calibration and standardization of the Auto amslyzer, processing and analysis of the samples were done strictly according to the manufacturer's instructions (Obazelu and Osarinmwian, 2025).

3.11.1.1. Detection Principle of Haematology Autoanalyzer

The instrument counts and sizes blood 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 Ohm's formula: U=IR (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 collected from the sacrified animals were 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

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 container.
- 100 ml of distilled water was added to the glass container and the mixture was stirred until the powder was completely dissolved.
- Solution was labelled as "Eosin Y stock solution".

Stock solution of Methylene blue:

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 jar.
- The contents of the staining jar were mixed thoroughly using a glass stirring rod and then allowed to ripen under room temperature 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 one third of a clean grease free glass slide.
- The spreader was placed at a 45-degree angle against the blood drop while allowing it to spread along the contact lines after which a smooth firm 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 and 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 also for abnormalities.

3.12. Signal transducer and activator of transcription 5 (STAT5) mRNA Assay

3.12.1. 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).

3.12.2. 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).

3.12.3. PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest 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 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The 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).

3.12.4. Primers

STAT5

Forward primer: ACATGTACCCACCGAACCCC

Reverse primer: AGTCCAGCGTTCAGGACAAG

GAPDH

Forward primer: CTCCCTGGAGAAGAGCTATGA

Reverse primer: AGGAAGGAAGGCTGGAAGA

3.13. Statistical Analysis

Data obtained from this research was presented and analyzed using GraphPad prism 8.0 (California, 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, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* respectively.

Mean cell volume (MCV) (μm3) was significantly lower in group F (54.64±0.96) when compared to group C (58.22±0.49) ($p<0.05$). Mean cell haemoglobin (MCH) (pg) was significantly lower in group F (18.72±0.23) when compared to group C (19.66±0.07) ($p<0.05$). There was no significant difference in Red blood cell count (RBC μL), Haemoglobin concentration (g/dL), Haematocrit (HCT %), Mean cell haemoglobin concentration (MCHC g/dL), Red cell distribution width – Coefficient of Variation (RDW-CV %) and Red cell distribution width – Standard Deviation (RDW-SD μm3) ($p>0.05$).

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

Parameters	Group A (Control) (n=10)	Group B (Alcl3 only) (n=10)	Group C (Alcl3 + Ferrous sulfate) (n=10)	Group D (Alcl3 + 100mg/kg) (n=10)	Group E (Alcl3 + 200mg/kg) (n=10)	Group F (Alcl3 + 400mg/kg) (n=10)	f value	P value
RBC Count (x109/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 (L/L)	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: Table presented in mean±SEM. $p < 0.05$-Significant; $p > 0.05$- Not significant, Alcl3=Aluminum chloride. Superscript a represents significance with control, b represents significance with Alcl3 group, c represents significance with Alcl3 + Ferrous sulfate, d represents significance with Alcl3 + 100mg/kg, e represents significance with Alcl3 + 200mg/kg, f represents significance with Alcl3 + 400mg/kg.

RBC: Red Blood Cell

HCT: Haematocrit

MCV: Mean Cell Volume

MCH: Mean Cell Haemoglobin

MCHC: Mean Cell Haemoglobin Concentration

RDW-CV: Red cell distribution width – Coefficient of Variation

RDW-SD: Red cell distribution width – Standard Deviation

Table 4.2 shows the comparison of Mean±SD of white blood cell parameters of six groups namely; groups A, B, C, D, E and F, representing control, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* respectively. Total white blood cell count (TWBC μL), lymphocyte count (%), monocyte count (MID%) and granulocytes count (Gran%) showed no significant difference ($p>0.05$).

Table 4.2:Mean Comparison of White Blood Cell Parameters among the Studied Groups

Parameters	Group A (Control) (n=10)	Group B (Alcl3 only) (n=10)	Group C (Alcl3 + Ferrous sulfate) (n=10)	Group D (Alcl3 + 100mg/kg) (n=10)	Group E (Alcl3 + 200mg/kg) (n=10)	Group F (Alcl3 + 400mg/kg) (n=10)	f value	P value
Total WBC ($x10^9$/L)	6.4±0.38	7.5±0.91	4.92±0.51	6.28±0.46	5.88±1.17	5.98±0.57	1.308	0.2952
Lymphocyte Count (%)	92.15±0.40	89.2±2.15	91.4±1.00	92.2±0.77	92.92±1.06	92.02±1.47	0.9954	0.4423
MID (%)	6.15±0.32	8.52±1.68	6.78±1.08	6.16±0.65	5.42±0.72	6.18±1.19	1.013	0.4326
GRAN (%)	1.7±0.12	2.28±0.49	2.42±0.39	1.64±0.17	1.66±0.39	1.8±0.35	0.9438	0.4717

Key: Table presented in mean±SEM. $p < 0.05$-Significant; $p > 0.05$- Not significant, Alcl3=Aluminum chloride. Superscript a represents significance with control, b represents significance with Alcl3 group, c represents significance with Alcl3 + Ferrous sulfate, d represents significance with Alcl3 + 100mg/kg, e represents significance with Alcl3 + 200mg/kg, f represents significance with Alcl3 + 400mg/kg.

TWBC: Total white blood cell count

MID: Monocyte count

GRAN: Granulocytes count

Table 4.3 shows the comparison of Mean±SD of platelet parameters of six groups namely; groups A, B, C, D, E and F, representing control, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg Icacina trichantha, aluminum chloride + 200mg/kg Icacina trichantha and aluminum chloride + 400mg/kg Icacina trichantha respectively. Platelet distribution width (PDW %) was significantly lower in group E (8.96±0.27) when compared to group D (10.6±0.31) ($p<0.05$). There was no significant difference in all other platelet parameters (platelet count, Mean platelet volume, plateletcrit and Platelet-Large cell ratio) ($p>0.05$).

Table 4.3: Mean Comparison of Platelet Parameters among the Studied Groups

Parameters	Group A (Control) (n=10)	Group B (Alcl3 only) (n=10)	Group C (Alcl3 + Ferrous sulfate) (n=10)	Group D (Alcl3 + 100mg/kg) (n=10)	Group E (Alcl3 + 200mg/kg) (n=10)	Group F (Alcl3 + 400mg/kg) (n=10)	f value	P value
Platelet Count ($X10^9/L$)	824.8±115.4	764±58.77	758.4±61.46	816.8±47.72	586.2±123.4	817.2±109.2	1.032	0.4224
MPV (fL)	7.725±0.11	7.78±0.27	7.58±0.30	7.38±0.10	7.16±0.20	7.14±0.14	1.717	0.1708
PDW (%)	10.43±0.07	10.18±0.36	9.3±0.56	10.6±0.31	8.96±0.27d	9.14±0.38	3.728	0.0128
PCT (%)	0.63±0.1	0.59±0.07	0.57±0.05	0.59±0.04	0.43±0.09	0.58±0.08	0.9816	0.4501
P-LCR (%)	9.72±1.20	9.48±2.22	9.9±2.08	6.34±1.11	5.66±0.89	6.02±1.43	1.629	0.1923

Key: Table presented in mean±SEM. $p < 0.05$-Significant; $p > 0.05$- Not significant, Alcl3=Aluminum chloride. Superscript a represents significance with control, b represents significance with Alcl3 group, c represents significance with Alcl3 + Ferrous sulfate, d represents significance with Alcl3 + 100mg/kg, e represents significance with Alcl3 + 200mg/kg, f represents significance with Alcl3 + 400mg/kg.

PLT: Platelet Count

MPV: Mean Platelet Volume

PCT: Plateletcrit

PDW: Platelet Distribution Width

PLCR: Platelet-Large cell ratio

Table 4.4 shows the blood morphology of the studied groups namely; groups A, B, C, D, E and F, representing control, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg Icacina trichantha, aluminum chloride + 200mg/kg Icacina trichantha and aluminum chloride + 400mg/kg Icacina trichantha respectively. All groups showed the presence of both small and large (atypical) lymphocytes. Small lymphocytes were highly present in group E and F and moderately present in groups A, B, C and D. Large lymphocytes were mildly present in group A, B and C and moderately present in groups D, E and F. Eosinophils was midly present in group A and absent in all other groups. Monocytes and basophils were absent in all groups. Nomocytic and normochromic red blood cells were highly present in all groups. Polychromatic and crenated red blood cells were mildly present in all groups. Platelets were highly present in all groups.

Table 4.4. Blood Morphology of the Studied Groups

	LYMPH% (Small)	LYMPH % (Large)	EOS %	MON%	BAS%	NORMOCT CELLS %	NORMOCM CELLS%.	POLYCMT CELLS%	CRENATED CELLS%	PLT%
Group A	++	+	+	-	-	+++	+++	+	+	+++
Group B	++	+	-	-	-	+++	+++	+	+	+++
Group C	++	+	-	-	-	+++	+++	+	+	+++
Group D	++	++	-	-	-	+++	+++	+	+	+++
Group E	+++	++	-	-	-	+++	+++	+	+	+++
Group F	+++	++	-	-	-	+++	+++	+	+	+++

LYMPH-Lymphocytes, EOS-Eosinophils, MON-Monocytes, BAS-Basophils, NORMOCT-normocytic cells, NORMOCM-Normochromic cells, POLYCMT-Polychromatic cells, PLT-Platelet. - = 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, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg Icacina trichantha, aluminum chloride + 200mg/kg Icacina trichantha and aluminum chloride + 400mg/kg Icacina trichantha respectively measured at day 0 and 28. The weight of group A, B, E and F significantly increased at day 28 when compared to day 0. There was a slight increase in group C and D at day 28 compared to day 0.

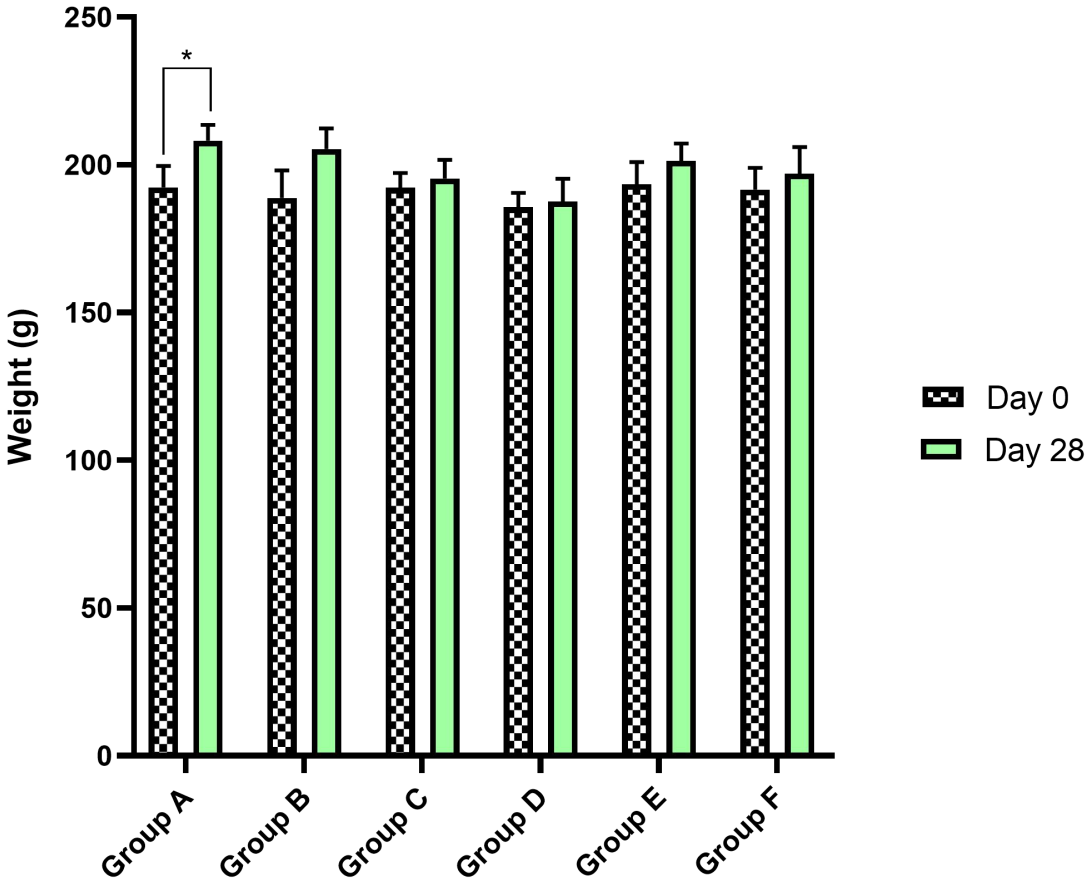

Figure 4.1 : Body weights of Groups A, B, C, D, E and F measured at Day 14 and Day 28.

Key:

Group A represents Control group

Group B represents Alcl3 group

Group C represents Alcl3 + Ferrous sulfate

Group D represents Alcl3 + 100mg/kg

Group E represents Alcl3 + 200mg/kg

Group F represents Alcl3 + 400mg/kg.

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 Signal Transducer and Activator of Transcription 5 (STAT5) of groups A, B, C, D, E and F, representing control, aluminum chloride group, Ferrous sulfate group, aluminum chloride + 100mg/kg Icacina trichantha, aluminum chloride + 200mg/kg Icacina trichantha and aluminum chloride + 400mg/kg Icacina trichantha respectively, represented on different bars on the bar chart. There was a significant increase in the mRNA expression of STAT5 in groups B, C, D, E, and F when compared to group A ($p<0.05$). Groups D, E and F had a higher mRNA expression of STAT5 when compared to group A and B ($p<0.05$). Group F also had a higher expression of STAT5 when compared to group D ($p<0.05$).

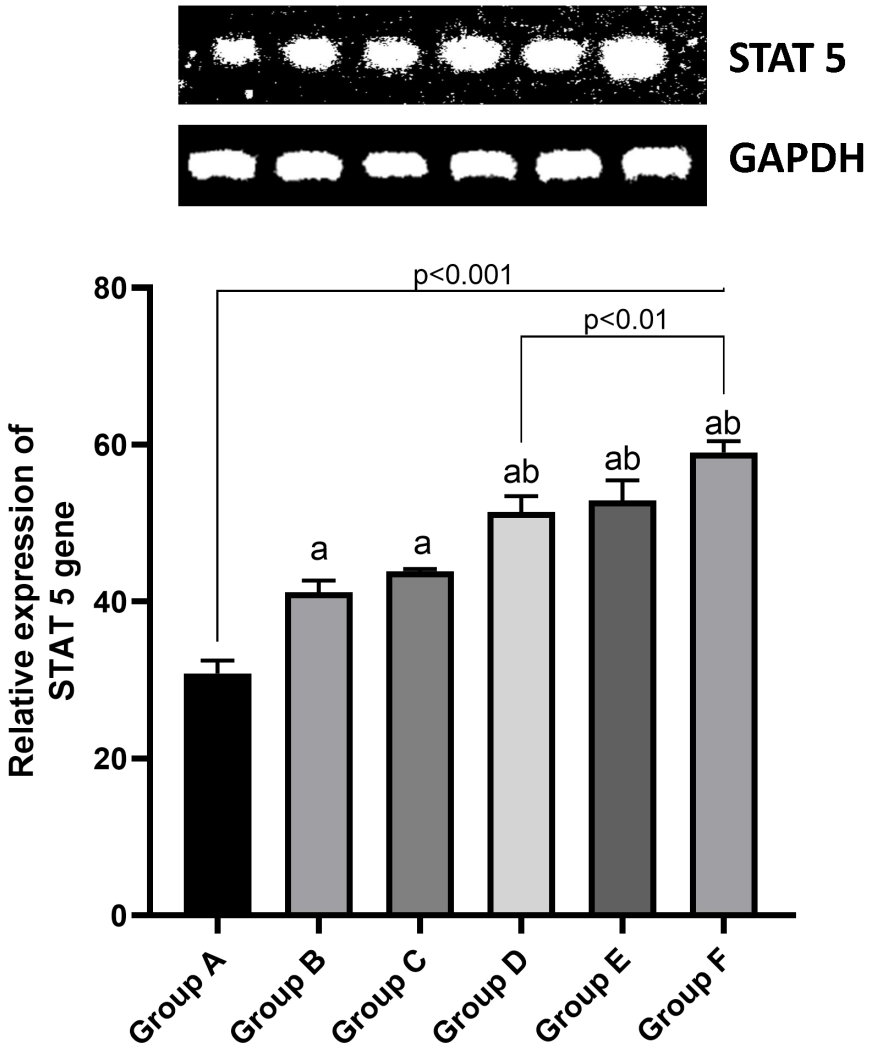

Figure 4.2: mRNA Expression of Signal Transducer and Activator of Transcription 5 (STAT5) of the Studied Groups.

Key: Figure shows mean±SEM. Error bar represents triplicate of each groups. $p<0.05$ was considered significant. a represents significance with group A, b represents significance with group B.

GADPH=Glyceraldehyde-3-Phosphate Dehydrogenase

Group A represents Control group

Group B represents Alcl3 group

Group C represents Alcl3 + Ferrous sulfate

Group D represents Alcl3 + 100mg/kg

Group E represents Alcl3 + 200mg/kg

Group F represents Alcl3 + 400mg/kg.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Medicinal and aromatic plants, especially those with ethnopharmacological uses, have been utilized as a natural source of remedies and healthcare (Chaachouay and Zidane, 2024).The search for natural plant-derived remedies to mitigate the toxic effects of heavy metals and environmental pollutants has gained increasing momentum in recent years (Obazelu and Odionyenma, 2024). Numerous researchers have explored the therapeutic potential of medicinal plants due to their bioactive phytochemicals, antioxidant capacity, and ability to modulate key molecular pathways involved in cellular injury and repair (Obazelu and Odionyenma, 2024). *Icacina trichantha*, a medicinal plant widely used in African traditional medicine, has been reported to contain alkaloids, flavonoids, and phenolic compounds with pharmacological activities that could support blood health and tissue repair (Che *et al.,* 2016).

Aluminium chloride ($AlCl_3$) is a common environmental toxicant known to disrupt haematological parameters and impair erythropoiesis through oxidative stress mechanisms (Buraimoh *et al.,* 2012). The Signal Transducer and Activator of Transcription 5 (STAT5) play a vital role in mediating erythropoietin signaling and regulating the proliferation and survival of erythroid progenitor cells (Yu *et al.,* 2009). Alterations in STAT5 expression can therefore provide molecular insight into the effects of toxicants and therapeutic agents on blood formation. In this study, the aqueous leaf extract of *Icacina trichantha* was investigated for its effect on haematological parameters

and STAT5 gene expression in Aluminium Chloride ($AlCl_3$)-induced anaemia in Wistar rats.

In this study, administration of $AlCl_3$ statistically did not result in significant changes in RBC count, haemoglobin (Hb), or haematocrit (HCT) compared to the control group ($p > 0.05$). This finding is in contrast to several studies which have reported that $AlCl_3$ exposure induces anaemia through mechanisms involving oxidative stress, impaired erythropoiesis, and increased haemolysis (Al-Doaiss *et al.,* 2024). The lack of marked RBC suppression in this study could be attributed to the dosage and exposure duration, which may not have been sufficient to cause severe bone marrow suppression. Ferrous sulfate treatment caused an increase significantly in mean cell volume (MCV) and mean cell haemoglobin (MCH) when compared with the group administered 100mg/kg of *Icacina trichantha* extract. This agrees with previous studies reporting that ferrous sulfate supplementation leads to improvements in MCV and MCH among individuals with iron-deficiency anemia (Okam *et al.,* 2017). This increase is likely due to the fact that iron deficiency leads to the production of microcytic, hypochromic cells (smaller red cells with reduced hemoglobin content). Administration of ferrous sulfate replenishes iron stores, enabling the bone marrow to produce red blood cells that are larger in size and richer in haemoglobin, thereby improving MCV and MCH (Lopez *et al.,* 2016).

While the groups administered 100mg/kg and 400mg/kg of *Icacina trichantha* extract showed no significant difference in all the blood parameters, there was a significant reduction in Mean cell volume (MCV) and Mean cell haemoglobin (MCH) in the group administered 400mg/kg of *Icacina trichantha* leaves extract. This finding is in contrast to other studies which have noted increase in red blood cell parameters after administration

of plant extracts (Obazelu and Osazee, 2024). These reductions in MCV and MCH may indicate the onset of microcytic, hypochromic anaemia, which is consistent with iron metabolism disruption often linked to aluminium toxicity (Exley, 2013).

This study showed that aluminium chloride exposure did not cause any statistically significant changes in total white blood cell (TWBC) count, lymphocyte count, monocyte count, or granulocyte count across the groups. This suggests that, at the administered dose and exposure period, aluminium chloride did not exert a marked myelosuppressive or stimulatory effect on white cell production. This finding aligns with earlier reports by Qu *et al.* (2002) and Schnatter *et al.* (2010), where moderate exposure to certain toxicants did not significantly alter total leukocyte counts, likely due to the absence of severe bone marrow damage or systemic infection during the experimental period. This is in disagreement with previous study by Obazelu and Faluyi (2023) which found that plant extracts could produce dose-dependent alterations in TWBC, either increasing counts via stimulation of immune cells or reducing them through suppression of pro-inflammatory mediators. In the present study, the minimal variation between groups suggests that Icacina trichantha did not cause immune suppression, a desirable feature in therapeutic interventions.

In this study platelet count, mean platelet volume (MPV), plateletcrit (PCT) and platelet large cell ratio (P-LCR) showed no significant differences between groups, although a significant change was observed in Platelet Distribution Width (PDW), which was markedly reduced in the 200mg/kg *Icacina trichantha* group compared to other groups. PDW is an indicator of platelet size variability and is often elevated in conditions involving platelet activation or bone marrow stress. The observed reduction here may

reflect a stabilising effect on platelet production or maturation, possibly due to bioactive compounds influencing megakaryocyte activity. These findings differ from Chmielnicka *et al.* 1996 who reported increased platelets after aluminium exposure, but the difference may be as a result of the mitigating influence of Icacina trichantha in the current study. The reduction in PDW at this dose also contrasts with the 100mg/kg and 400mg/kg groups, suggesting a dose-specific effect that may relate to phytochemical concentration and bioavailability.

The peripheral blood film analysis revealed that aluminum chloride exposure did not affect the morphology of blood cells. All groups when compared to aluminum chloride group did not show any difference. Small lymphocytes was highly present in the groups administered 200mg/kg and 400mg/kg of leave extract, this may be due to the administration of *Icacina trichantha* leave extract as aluminum chloride did not cause any effect. All other group showed normal blood cell morphology.

This research showed body weight gain across different test groups. The control group demonstrated high significant weight gain compared to the other groups by Day 28. This may due to the absence of heavy metals like aluminum chloride which resulted in normal physiological growth and weight gain. However, aluminum chloride group experienced increase in body weight which may be as a result of the volume of the aluminum chloride administered not been able to cause hematoxicity. 200mg/kg and 400kg/mg administered group experienced increase in weight gain compared to 100mg/kg administered group which shown lesser weight gain, possibly due to the concentration of extract as higher concentration lead to weight gain. This finding is in contrast to previous studies which

observed decline in weight gain at the end of the experiment compared to the beginning (Obazelu and Omoregie, 2024).

Analysis of STAT5 mRNA expression revealed a marked increase in all experimental groups when compared to the control group, indicating that both aluminium chloride exposure and the administered treatments influenced STAT5 gene activity. Aluminium chloride exposure alone resulted in elevated STAT5 expression, consistent with its capacity to trigger oxidative and inflammatory stress pathways that activate erythropoietic signaling (Yu et al., 2009). Groups administered 100mg/kg, 200mg/kg and 400mg/kg of *Icacina trichantha* also showed increased STAT5 expression compared to both the control and the aluminium chloride group. 100mg/kg administered group had lower expression when compared with 400mg/kg administered group suggesting a possible dose-dependent response to the leaves extract. However, it aligns with findings from other studies where certain plant-derived compounds enhanced STAT5 activity to promote erythropoiesis and immune cell differentiation (Wierenga *et al.,* 2008; Asuzu and Gray, 1999).

This suggests that *Icacina trichantha* extract, may upregulate STAT5-mediated pathways. The extract with increasing dose could be stimulating erythropoietin signaling or other cytokine-mediated cascades that require STAT5 activation, potentially as part of a reparative or compensatory response to aluminium-induced haematotoxicity.

5.2. Conclusion

Data from this study has shown that no significant changes in white blood cells parameter while red blood cell and platelets parameters showed significant difference in MCV, MCH and PDW only. Treatment with the aqueous leaves extract of *Icacina trichantha* increased with increasing dose of extract resulting in a dose-dependent increase in STAT5 in mRNA expression, with the highest dose showing the highest mRNA expression.

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APPENDICES

APPENDIX I

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.

University of Benin

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

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**..... 23/6/25

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

.....

ATTESTATION BY INVESTIGATOR(S)

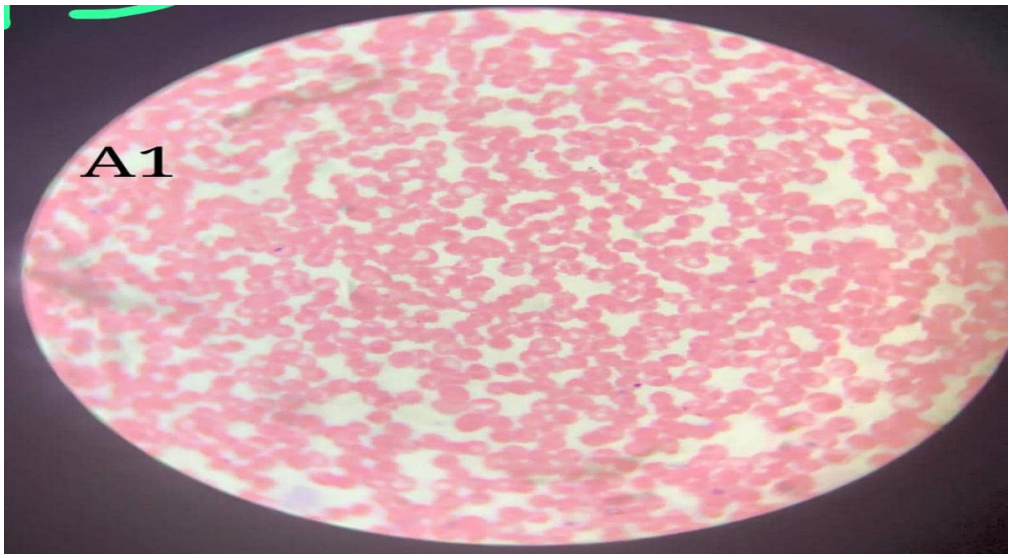
No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the 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.

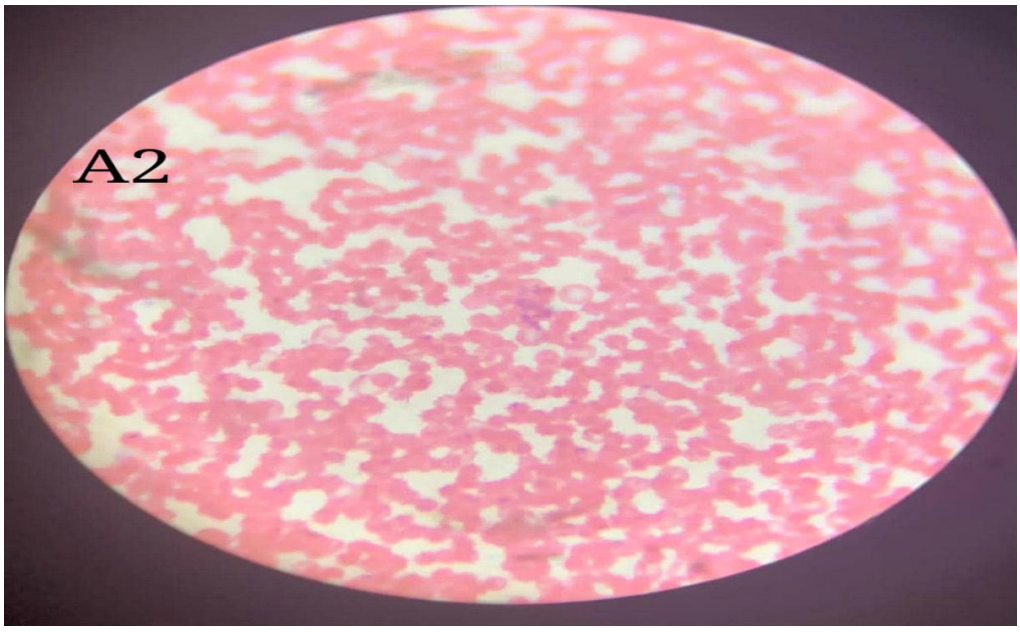
Signature & Date..... 15/07/2025

edohrec@edostate.gov.ng

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

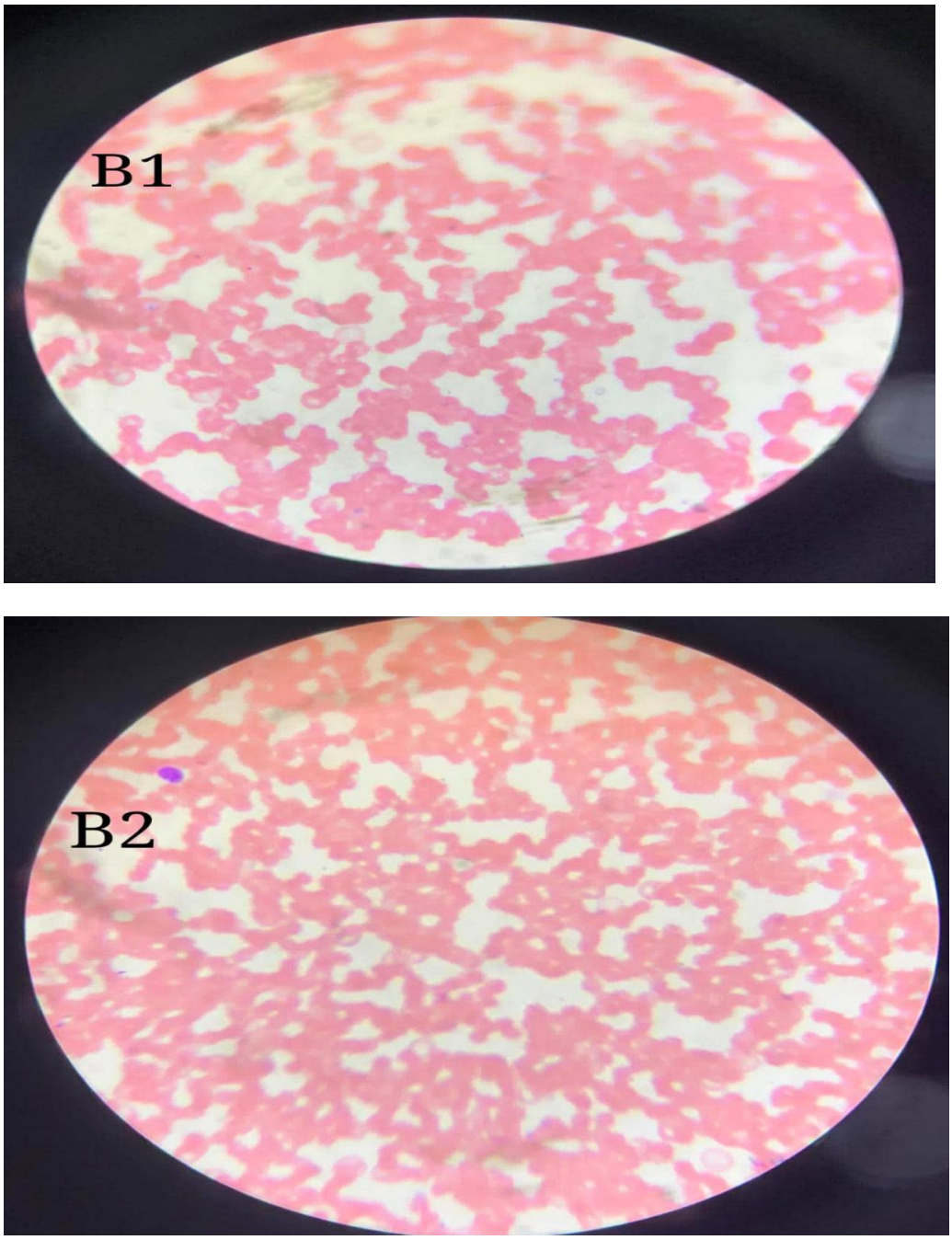
APPENDIX III





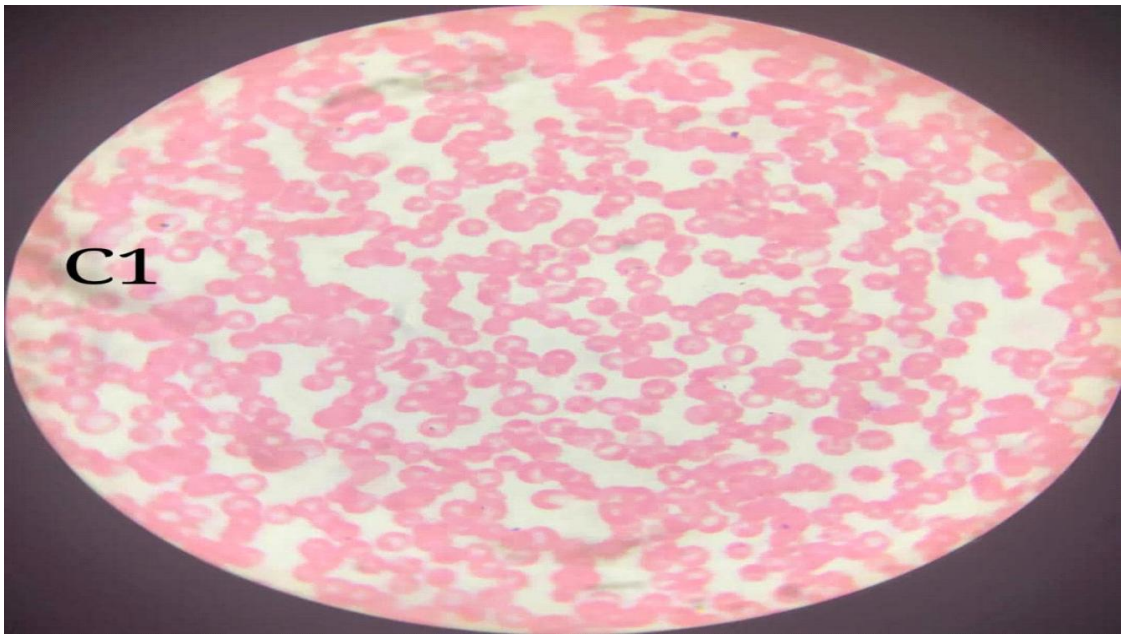
Slides Showing the Blood Cell Morphology of Group A Experimental Animals.

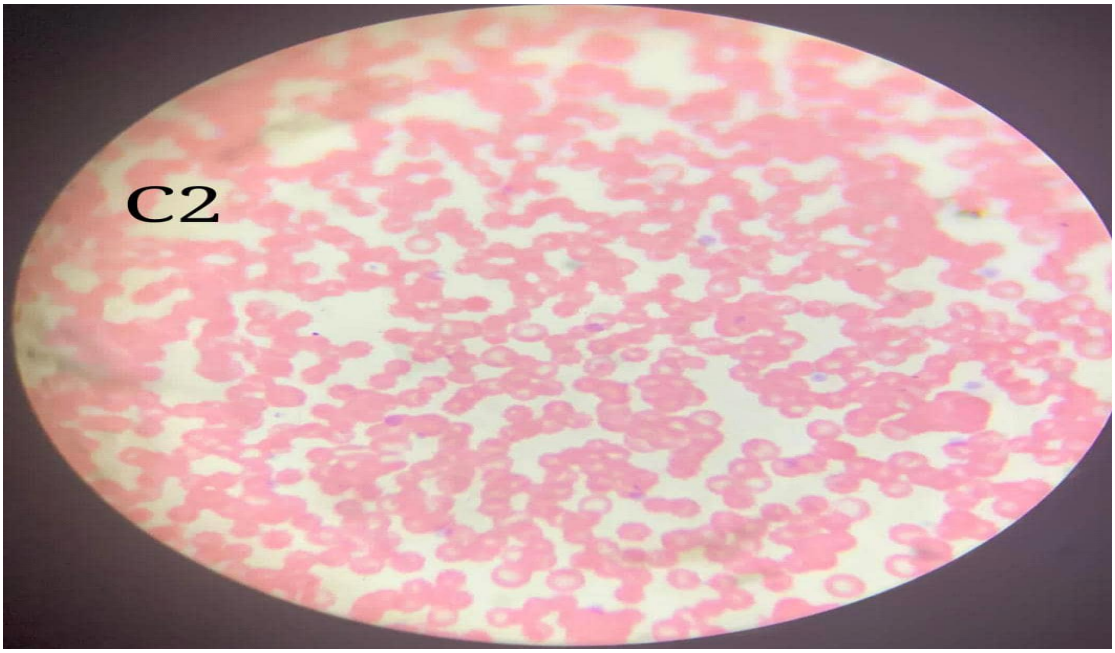
APPENDIX IV



Slides Showing the Blood Cell Morphology of Group B Experimental Animals.

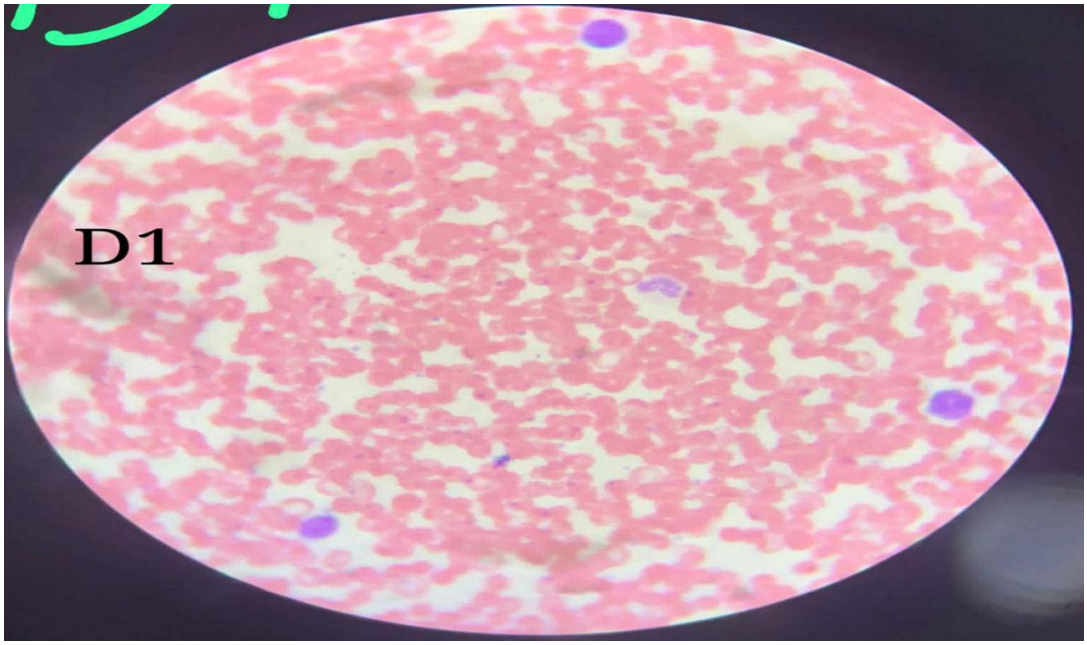
APPENDIX V

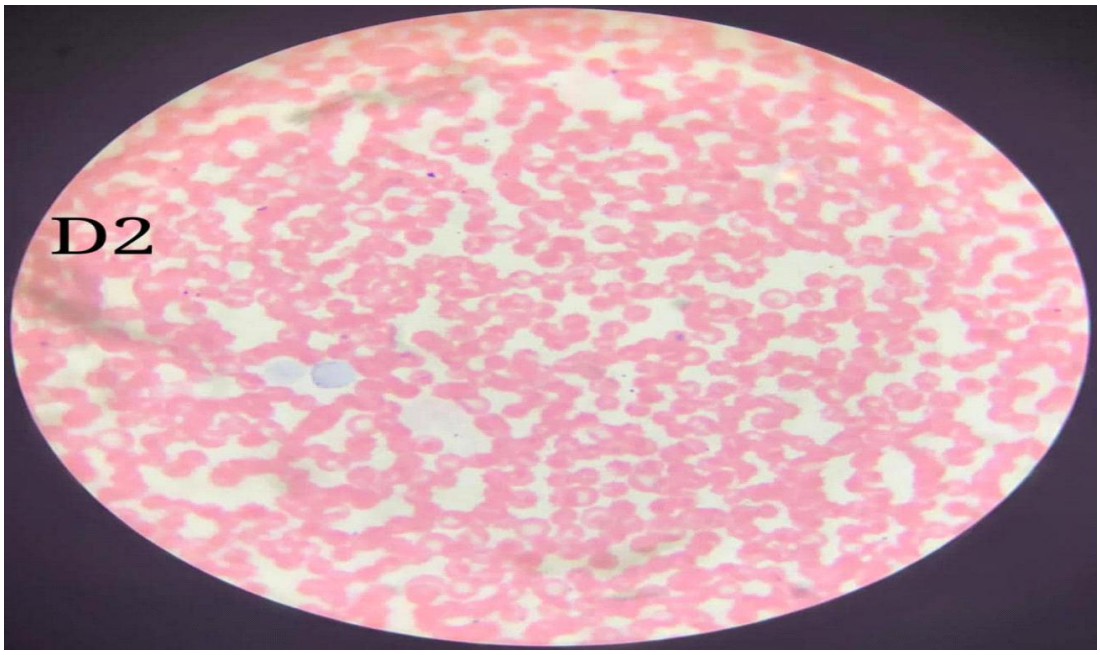




Slides Showing the Blood Cell Morphology of Group C Experimental Animals.

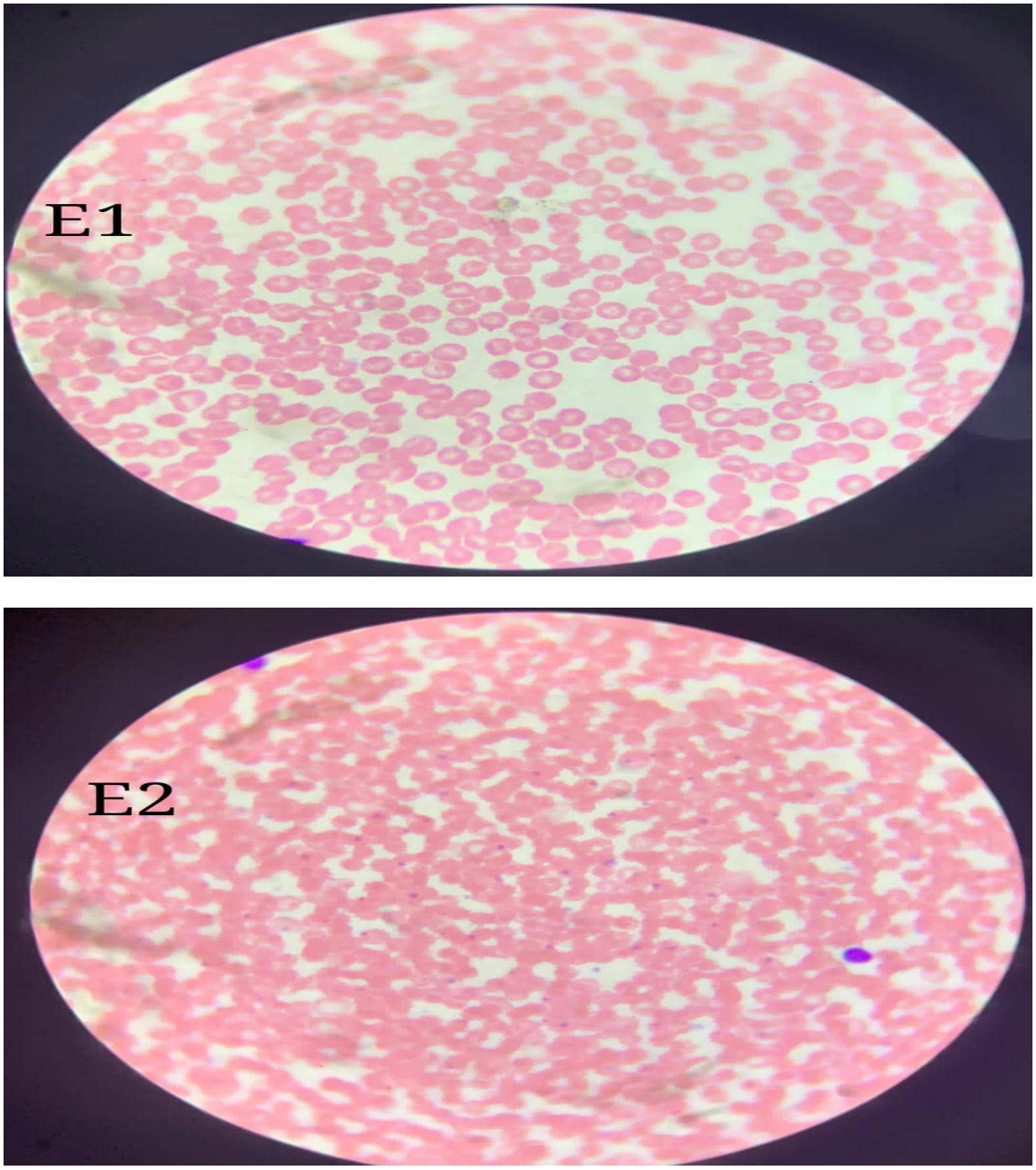
APPENDIX VI





Slides Showing the Blood Cell Morphology of Group D Experimental Animals.

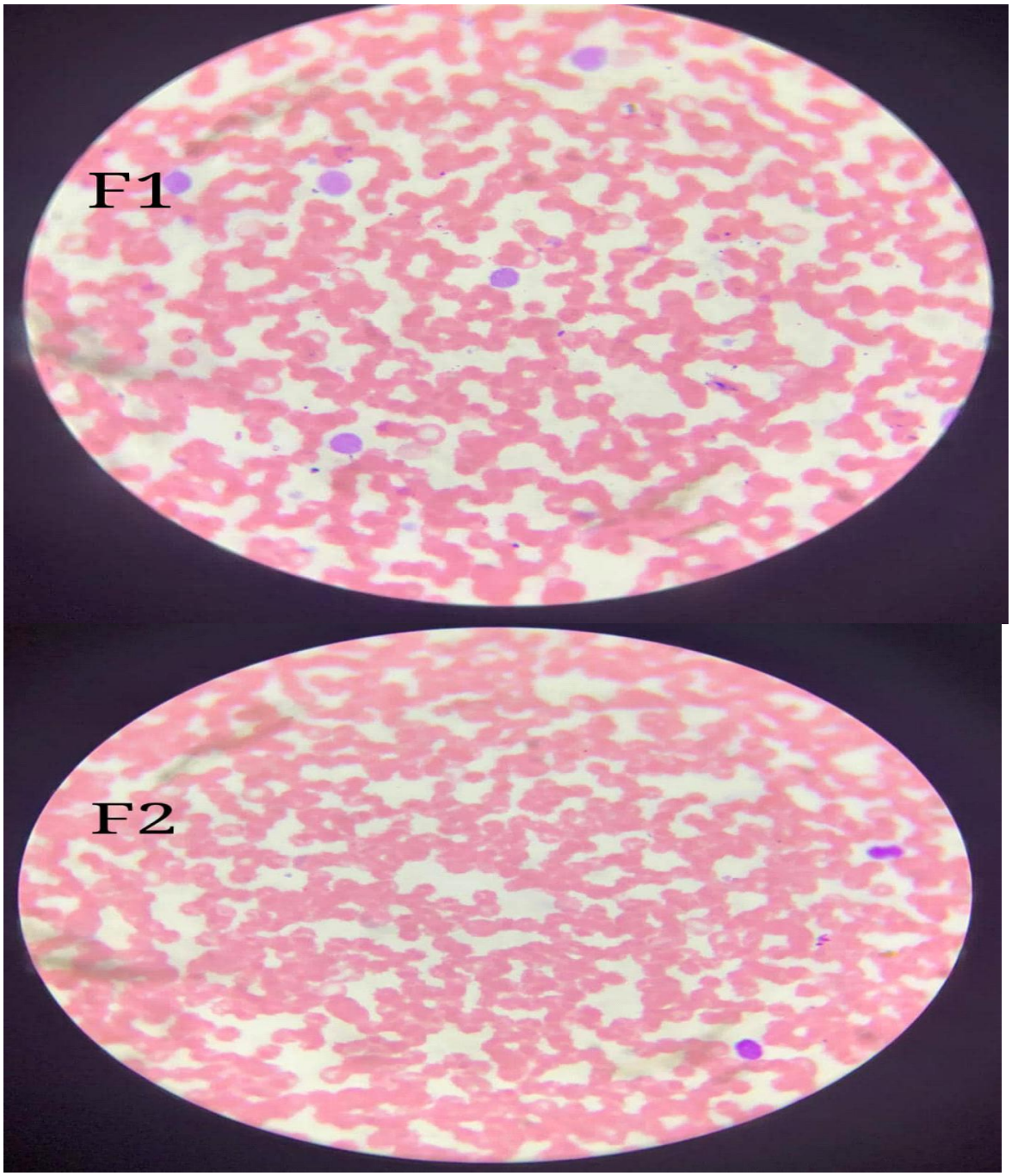
APPENDIX VII



Slides Showing the Blood Cell Morphology of Group E Experimental Animals.

APPENDIX

VIII



Slides Showing the 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