EXPRESSION OF HMGB-1 GENE IN ALUMINUM CHLORIDE-INDUCED ANAEMIA BEARING WISTAR RATS TREATED WITH AQUEOUS LEAVES EXTRACT OF *Icacina trichantha*

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OCTOBER, 2025

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THIS PROJECT IS SUBMITTED TO:

THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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SUPERVISOR:

DR (MRS) P.A.OBAZELU

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **IGORU OGHENEREKE LAUREN (MISS)** with matriculation number: **BMS2001165** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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(Ag. Head of Department)

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to God Almighty for his strength and help in making this work a huge success.

ACKNOWLEDGEMENT

I am grateful to God Almighty for his faithfulness over my life including my Academics. God has been so good to me. There is no way I could have come this far without his help.

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ABSTRACT

Icacina trichantha is a medicinal plant traditionally used in West Africa for its hematinic and immunomodulatory properties. The study is aimed to evaluate the effect of aqueous leaf extract of *Icacina trichantha* on the expression of the High-Mobility Group Box-1 (HMGB-1) gene in Wistar rats with aluminum chloride–induced anaemia. Sixty (60) adult male Wistar rats were divided into six (6) groups: Group A (Control), Group B ($AlCl_3$ only), Group C ($AlCl_3$ + 40 mg/kg ferrous sulphate), and Groups D, E, and F ($AlCl_3$ + 100 mg/kg, 200 mg/kg, and 400 mg/kg I. trichantha extract, respectively). Blood samples were analyzed for white blood cell parameters using an ERMA haematology autoanalyzer, and HMGB1 mRNA expression was determined by polymerase chain reaction (PCR) with GAPDH as the internal control. Total WBC count was highest in Group B (7.5 ± 0.91) and lowest in Group C (4.92 ± 0.51), with extract-treated groups showing intermediate values (6.28 ± 0.46, 5.88 ± 1.17, and 5.98 ± 0.57 for Groups D, E, and F, respectively), though differences were not statistically significant ($p > 0.05$). Lymphocyte, MID, and granulocyte percentages showed mild variations across groups but without statistical significance. Significant weight gain was observed in Groups D, E, and F at day 28 compared to day 0 ($p < 0.05$). HMGB1 mRNA expression was significantly elevated in Groups C, D, and E compared to Groups A and B ($p < 0.05$), indicating activation of immune-related molecular pathways. In conclusion, while I. trichantha did not significantly alter WBC indices in $AlCl_3$-induced anaemia, its administration was associated with notable upregulation of HMGB1 expression and improved body weight, suggesting potential modulatory and restorative effects that warrant further mechanistic studies.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Erythropoiesis, the process by which red blood cells are produced, is a finely orchestrated biological phenomenon crucial for maintaining oxygen transport and tissue oxygenation (Obazelu and Gaius-Igboanugwo, 2024). Anaemia on the other hand is a global public health concern characterized by a reduced capacity of the blood to carry oxygen, primarily due to decreased hemoglobin levels or erythrocyte count (Ameh and Alafi, 2018). Experimental models frequently induce Anaemia in laboratory animals using aluminum chloride ($AlCl_3$), a neurotoxic and hematotoxic compound known to cause oxidative stress and erythrocyte membrane damage (Bouasla, Bouasla, and Boumendjel, 2014). Aluminum disrupts iron metabolism, inhibits erythropoiesis, and induces lipid peroxidation in red blood cells (Ige and Aiyeola, 2017). In Wistar rats, $AlCl_3$ exposure has been shown to result in normocytic normochromic Anaemia accompanied by oxidative alterations in hepatic and hematologic parameters (Adedosu *et al*., 2018). At the molecular level, oxidative stress induced by aluminum triggers the upregulation of various pro-inflammatory mediators, including High Mobility Group Box 1 (HMGB1) protein (Akpanyung *et al*., 2020). HMGB1 is a nuclear non-histone protein involved in DNA architecture and gene transcription, but under stress conditions, it translocates to the extracellular space where it functions as a danger-associated molecular pattern (DAMP) (Cheng *et al*., 2020). Elevated HMGB1 expression is a hallmark of cellular stress, apoptosis, and immune activation in inflammation-associated disorders (Yang *et al*., 2013). Studies suggest that HMGB1 gene expression increases in response to redox imbalance and DNA damage, both of which are implicated in

$AlCl_3$ toxicity (Zhang *et al*., 2016). The practice of using plants for medicinal purposes has a rich history that dates back thousands of years, with many cultures relying on botanicals to treat health issues and improve overall well-being (Obazelu and Efosa, 2025), *Icacina trichantha* , a medicinal plant native to West Africa, has been traditionally used for treating fever, gastrointestinal ailments, and general body weakness (Sofidiya *et al*., 2021). Phytochemical investigations of *Icacina trichantha* leaf extracts have revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which possess antioxidant, anti-inflammatory, and immunomodulatory properties (Okonkwo *et al*., 2019). These bioactive constituents are hypothesized to attenuate oxidative damage and downregulate pro-inflammatory gene expression, including HMGB1, during toxicological insults (Adelakun *et al*., 2020). By modulating cellular oxidative stress and cytokine release, aqueous extracts of *Icacina trichantha* may protect against $AlCl_3$-induced erythrocyte degeneration and inflammatory gene activation in Wistar rats (Kassa *et al*., 2023).

1.2 Justification of the Study

Aluminum chloride ($AlCl_3$) is a well-documented environmental toxicant known for its deleterious effects on hematological parameters, particularly in inducing Anaemia through oxidative damage, inhibition of erythropoiesis, and disruption of iron metabolism. HMGB-1 is a non-histone chromatin-binding protein that plays a crucial role in DNA organization and transcription under normal physiological conditions. However, under stress or injury, it translocates to the extracellular space where it acts as a potent pro-inflammatory mediator. Its overexpression is strongly associated with oxidative stress, apoptosis, and inflammatory responses, making it a valuable molecular biomarker in various toxicological and pathological conditions. The search for natural agents with therapeutic potential against oxidative and inflammatory conditions has directed attention toward medicinal plants.

Icacina trichantha , a tropical West African shrub, has a rich history in ethnomedicine for treating ailments such as fever, digestive disorders, and general body weakness. Phytochemical investigations of *I. trichantha* have revealed the presence of flavonoids, alkaloids, saponins, and phenolic compounds bioactive molecules known for their antioxidant, anti-inflammatory, and hematopoietic activities. This study is justified in its aim to explore the protective role of *Icacina trichantha* in $AlCl_3$-induced Anaemia. Moreover, investigating its effect on HMGB-1 gene expression could provide novel insights into the molecular basis of its protective properties. The use of Wistar rats as a model organism further enhances the scientific validity of the study due to their well-characterized response to toxicological and hematological challenges. Altogether, this research may contribute valuable data toward the development of alternative therapies for Anaemia and deepen the understanding of inflammation-related gene expression in oxidative stress models.

1.3 Aim of the Study

The study is aimed to evaluate the effect of aqueous leaf extract of *Icacina trichantha* on the expression of the High-Mobility Group Box-1 (HMGB-1) gene in Wistar rats with aluminum chloride–induced anaemia.

1.4 Specific Objectives

1. To determine the effect of aqueous leaves extract of *Icacina trichantha* on the expression of HMGB-1 genes in aluminum chloride–induced anaemia in albino Wistar rats.
2. To determine the effect of *Icacina trichantha* aqueous leaves extract on hematological parameters in aluminum chloride–induced anaemia in albino Wistar rats.

3. To determine the effect of *Icacina trichantha* aqueous leaves extract on the blood morphology of albino Wistar rats with aluminum chloride–induced anaemia.

1.5 Research Hypotheses

1.5.1 Null Hypotheses (H_0):

1. Aqueous leaves extract of *Icacina trichantha* has no significant effect on the expression of the HMGB-1 gene in aluminum chloride–induced *anaemia* in albino Wistar rats.
2. Aqueous leaves extract of *Icacina trichantha* has no significant effect on the hematological parameters of albino Wistar rats with aluminum chloride–induced *anaemia*.
3. Aqueous leaves extract of *Icacina trichantha* has no significant effect on the blood morphology of albino Wistar rats with aluminum chloride–induced *anaemia*.

1.5.2 Alternate Hypotheses (H_a):

1. Aqueous leaves extract of *Icacina trichantha* has a significant effect on the expression of the HMGB-1 gene in aluminum chloride–induced *anaemia* in albino Wistar rats.
2. Aqueous leaves extract of *Icacina trichantha* has a significant effect on the hematological parameters of albino Wistar rats with aluminum chloride–induced *anaemia*.
3. Aqueous leaves extract of *Icacina trichantha* has a significant effect on the blood morphology of albino Wistar rats with aluminum chloride–induced *anaemia*.

1.6 Research Question

1. What is the effect of aqueous leaves extract of *Icacina trichantha* on the expression of the HMGB-1 gene in aluminum chloride–induced *anaemia* in albino Wistar rats?
2. How does *Icacina trichantha* aqueous leaves extract influence hematological parameters **i**n albino Wistar rats with aluminum chloride–induced *anaemia*?
3. What changes occur on the blood morphology of albino Wistar rats with aluminum chloride–induced *anaemia* following treatment with *Icacina trichantha* aqueous leaves extract?

1.7 Scope of the Study

This study is specifically designed to investigate the potential modulatory effects of *Icacina trichantha* aqueous leaf extract on gene expression patterns in albino Wistar rats exposed to aluminium chloride-induced toxicity.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of *Icacina trichantha*

Icacina trichantha is a perennial shrub that belongs to the family Icacinaceae (Abu *et al.,* 2023). It is widely distributed across tropical regions of West Africa, especially in countries such as Nigeria, Ghana, Cameroon, and Côte d'Ivoire (Hazarika *et al.,* 2023). The plant is typically found growing in savanna zones, forest edges, and fallow farmlands (Busari *et al.,* 2019), where it thrives in well-drained, loamy soils (Akintunde *et al*., 2024) It is highly tolerant of drought conditions and poor soil fertility (Abu *et al.,* 2023), making it ecologically suited for regions with limited rainfall and subsistence farming systems (Busar*i et al., 2019)*. Locally, *I. trichantha* is known by several indigenous names, such as "Urumbia" or "Gbengbengwu" in southeastern Nigeria (Akintunde *et al*., 2024) These vernacular names reflect its cultural integration and long-standing use in traditional medicine and nutrition (Otun *et al.,* 2023). Its presence in diverse agro-ecological zones across West Africa indicates broad environmental adaptability (Hazarika *et al.*, 2023). The plant is commonly gathered from the wild for both food and therapeutic purposes, particularly among rural and indigenous populations (Okeke *et al.*, 2023). *I. trichantha* has historically been classified as a wild edible plant and a "famine food," due to its utility during periods of food scarcity (Busari *et al.,* 2019). The tubers and leaves are consumed during droughts or agricultural shortfalls, often serving as emergency nutritional support (Abu *et al.,* 2023). Despite its wide distribution, the species remains underutilized in commercial agriculture (Otun *et al.*, 2023), though recent pharmacological studies suggest increasing interest in its cultivation for medicinal use (Okeke *et al.,* 2023).

2.1.1. Botanical Description of *Icacina trichantha*

Icacina trichantha is a robust, tuberous, perennial shrub belonging to the family **Icacinaceae** and is commonly found in savanna and forest transition zones of West and Central Africa. Morphologically, the plant is characterized by a stout woody stem and extensive subterranean tuber system, which serves as both a water and nutrient reservoir, allowing the plant to thrive under arid and nutrient-poor conditions (Hazarika *et al.,* 2023; Alawode, 2024). The aerial stem is erect and sparsely branched, typically reaching up to 1–2 meters in height. The leaves are simple, alternate, and spirally arranged, displaying a broad ovate to elliptic shape with entire margins. Leaf blades are leathery and dark green with prominent venation on the abaxial surface. The petioles are short and stout, supporting the leaf blades with a firm attachment (Ojah, 2020). *Icacina trichantha* produces inconspicuous greenish-white flowers arranged in axillary or terminal inflorescences. The plant is monoecious, bearing both male and female flowers on the same individual. Its flowers are small, five-merous, and generally unisexual, with a short hypogynous disk. Stamens are five in number in the male flower, and the ovary is superior with two or three locules in the female flower (Onakpa *et al.,* 2016). Fruiting occurs in the form of drupes, which are oval to subglobose and about 1–2 cm in diameter. The fruits transition from green to reddish or purplish when mature and contain a hard endocarp enclosing a single seed. Below the soil, the plant develops large, starchy tubers which may weigh up to several kilograms. These tubers are often irregularly shaped, with a rough brown skin and white fibrous interior rich in carbohydrates (Otun *et al.,* 2015; Busari *et al.,* 2019).

Figure 2.1. leaf and fruit of *Icacina trichantha* (Onakpa *et al.*, 2016).

2.1.2. Ecological Distribution of *Icacina trichantha* in Africa

Icacina trichantha is widely distributed across the savanna and forest-edge ecosystems of West and Central Africa, particularly in countries such as Nigeria, Benin, Ghana, and Cameroon. This species typically thrives in semi-arid to sub-humid tropical climates, showing remarkable ecological plasticity that allows it to adapt to a wide range of rainfall and temperature variations (Ganglo, 2024). The species is commonly found in degraded farmlands, fallow fields, and lightly forested areas, where it plays a significant role in community-level biodiversity and nutritional resilience (Adesipo *et al.,* 2020).

The plant's adaptability is closely linked to its soil preference. It grows optimally in ferrallitic and loamy soils, which are common in West Africa's tropical zones. These soils are well-drained and often nutrient-deficient, yet *Icacina trichantha* shows considerable drought resistance and can thrive under low fertility conditions (Ganglo, 2024). This feature makes it particularly valuable in regions affected by seasonal droughts and increasing desertification due to climate change (Amprako, 2020).

From an ecological standpoint, *I. trichantha* contributes significantly to land restoration, especially in post-mining landscapes and disturbed habitats. Studies from gold-mined sites in southwestern Nigeria identified *Icacina trichantha* as part of a resilient floristic association capable of regenerating under environmental stress and anthropogenic disturbance (Adesipo *et al.,* 2020). Its presence in these areas highlights its importance in ecological succession and soil stabilization.

Ecological models also indicate that *I. trichantha* shows high tolerance to both elevation gradients and varied bioclimatic zones, making it an ideal candidate for conservation and sustainable land use planning (Padonou *et al.,* 2015). The plant’s role in native ecosystems

extends beyond soil restoration to include contributions to food security and traditional medicine, particularly during periods of agricultural scarcity (Akinola *et al.,* 2020).

2.1.3. Ethnomedicinal Significance in Traditional Practices

Icacina trichantha has long held a central place in the ethnomedicinal traditions of West and Central African communities, where it is valued for its pharmacological versatility. Known locally by various names such as "Urumbia" in southeastern Nigeria, the plant is frequently used by traditional healers to manage a broad spectrum of ailments including anaemia, diarrhoea, fever, inflammation, and microbial infections (Alawode, 2024; Otun *et al.,* 2015). The tubers, roots, and leaves are the most commonly utilized parts of the plant, often prepared as decoctions, infusions, or powders. In many rural areas, *I. trichantha* is used as a substitute or adjunct to conventional medicines due to its accessibility and reported efficacy. For example, decoctions made from the leaf extract are traditionally administered to treat internal heat, malaria symptoms, and digestive disorders (Onakpa *et al.,* 2016). The tuber is also grated and applied topically for the treatment of ulcers and skin infections, reflecting the plant's external and internal therapeutic utility (Busari *et al.,* 2019). Recent research has begun to validate many of these traditional uses. Studies demonstrate the presence of secondary metabolites such as flavonoids, saponins, alkaloids, and tannins, which possess antioxidant, antimicrobial, and anti-inflammatory properties (Alawode, 2024; Otun *et al.,* 2015). These bioactive compounds are thought to underpin the plant's effectiveness in treating infections and oxidative stress–related diseases. For instance, Otun *et al*. (2015) confirmed the antimicrobial efficacy of *I. trichantha* extracts against both Gram-positive and Gram-negative bacteria. Additionally, *I. trichantha* has been explored for its role in managing anaemia and haematological abnormalities, particularly in cases induced by heavy metal exposure such as aluminium chloride toxicity (Abu *et al.,* 2023). Such findings support the

plant's use in traditional systems for blood-related disorders and enhance its standing as a phytomedicinal resource worthy of further pharmacological investigation.

Table 2.1: Selected Species within the *Icacina* Genus

Scientific Name	Common Name(s)	Geographical Distribution	Notable Uses
Icacina trichantha	Bush banana, Urumbia	West and Central Africa	Traditional medicine, food (tuber), Anaemia
Icacina senegalensis	—	Senegal, Mali	Medicinal use, drought-resistant plant
Icacina mannii	—	Central Africa	Less studied; presumed similar use
Icacina oliviformis	False yam	Tropical Africa	Edible root, famine food
Icacina guessfeldtii	—	Equatorial Africa	Local medicinal use

Table 2.2 Taxonomy of *Icacina trichantha*

Taxonomic Rank	Classification
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Icacinales (or sometimes assigned to Garryales)
Family	Icacinaceae
Genus	*Icacina*
Species	*Icacina trichantha* Baker

2.2. Ethnobotanical and Therapeutic Uses of *Icacina trichantha*

Icacina trichantha has been widely recognized across West and Central Africa for its ethnobotanical and therapeutic importance. Traditionally, local communities have relied on this plant not only for nutritional sustenance during periods of famine but also as a multipurpose remedy in the treatment of various ailments (Alawode, 2024). The leaves, tubers, and roots are commonly used in decoctions, infusions, or dried and powdered form, reflecting the versatility of the plant within indigenous medical systems (Otun *et al.,* 2015). Ethnobotanically, the plant is classified as a "famine food" and has been used as an emergency nutritional resource, particularly in semi-arid regions where food security is threatened (Busari *et al.,* 2019). The tubers are rich in carbohydrates and trace minerals, which help sustain rural populations during dry seasons or agricultural shortfalls. In addition

to its food value, *I. trichantha* is used as livestock feed, particularly for goats and sheep, contributing to both human and animal health (Otun *et al.,* 2015). Therapeutically, *I. trichantha* is employed in managing ailments such as fever, internal heat, diarrhoea, respiratory tract infections, anaemia, ulcers, and various microbial infections (Onakpa *et al.,* 2016). Its antimicrobial and antioxidant properties have been attributed to the presence of flavonoids, tannins, saponins, and alkaloids, which act synergistically to neutralize free radicals and inhibit pathogenic organisms (Abu *et al.,* 2023;Alawode, 2024). The leaves, in particular, are used in decoctions for malaria symptoms and as a general detoxifying agent in traditional medicine. Recent pharmacological studies support many of these traditional applications. Abu *et al*. (2023) reported the cardio-protective and hepatoprotective properties of aqueous extracts of *I. trichantha* in chemically-induced toxicity models in rats. Additionally, antioxidant assays have demonstrated the plant's potential to counter oxidative stress, supporting its use in managing inflammatory and degenerative diseases (Onakpa *et al.,* 2016). These findings substantiate its traditional use in combating conditions linked to oxidative imbalance, including anaemia and liver damage. Despite its ethnomedicinal significance, *I. trichantha* remains underutilized in mainstream pharmacology. However, the increasing interest in plant-based therapeutics has renewed scientific attention toward its bioactive profile, with several studies calling for further characterization of its phytochemical compounds and molecular mechanisms of action (Otun *et al.,* 2015).

2.2.1. Traditional Applications in Treating Anaemia, Infections, and Inflammation

Icacina trichantha is extensively utilized in African ethnomedicine for its therapeutic potential in treating anaemia, infections, and inflammation. Among indigenous communities in Nigeria and surrounding regions, the plant is particularly valued for its efficacy in blood-building therapies and as a remedy for infectious and inflammatory conditions. In traditional

anaemia management, *I. trichantha* has been reported to improve red blood cell parameters when used as decoctions or infusions derived from its tubers and leaves. Although it lacks iron supplementation in the classical sense, it has shown restorative effects on haematological indices, likely through its antioxidant action that protects erythrocytes from oxidative damage (Adaka *et al.,* 2021). Bioactivity-guided fractionation of the tubers confirmed their efficacy in reversing Plasmodium berghei–induced anaemia in mice, supporting its antimalarial and blood-regenerative role. In the context of infectious diseases, the roots and leaves of *I. trichantha* are traditionally boiled and used as oral remedies or topical applications for managing gastrointestinal infections, wounds, and respiratory ailments. Its effectiveness is supported by its demonstrated antimicrobial activity, including inhibition of *Staphylococcus aureus* and *Escherichia coli* in vitro (Ezeala *et al.,* 2023). This aligns with its common use in rural settings for treating diarrhoea, skin infections, and bronchitis. The plant's anti-inflammatory applications are equally well documented. Traditional healers administer *I. trichantha* preparations for the management of joint pain, internal inflammation, and fevers. These uses are linked to the presence of flavonoids and tannins in the plant, which have been shown to inhibit key mediators of inflammation, such as prostaglandins and cytokines (Okeke *et al.,* 2023). In addition, it has shown promise in managing sickle cell anaemia-related inflammation and pain, as noted in folk practices across southwestern Nigeria.

2.2.2. Use in Detoxification and Blood Purification

In traditional African medicine, *Icacina trichantha* is widely recognized for its detoxifying properties and its role in purifying the blood. These applications are largely attributed to its rich phytochemical profile and antioxidant capacity, which help neutralize toxins and restore physiological balance. The aqueous extract of the plant’s leaves and tubers is commonly used in herbal preparations to cleanse the blood, especially in the context of toxin exposure,

inflammation, or febrile illnesses (Abu *et al.,* 2023). Recent experimental research supports these traditional claims. A study by Abu *et al*. (2023) investigated the cardio-protective potential of *I. trichantha* leaf extract in rats exposed to carbon tetrachloride (CCl_4), a known hepatotoxicant. The results demonstrated that the plant extract significantly reduced elevated levels of liver enzymes and improved antioxidant markers in the serum, indicating an enhancement in hepatic detoxification and systemic blood cleansing. This is consistent with its ethnomedicinal use in treating blood "dirtiness" and inflammatory overloads in traditional practice (Obazelu *et al* 2025). Similarly, ethnobotanical surveys confirm that *I. trichantha* is among the most cited plant species for detoxification and treatment of tropical diseases that impact blood and liver function. Okosodo *et al*. (2021) noted its frequent usage in rural medical systems in southwestern Nigeria, where herbalists employ decoctions of the plant as a systemic cleanser, often in combination with other herbs, to manage malaria, jaundice, and blood-related disorders. These findings are further supported by ecological and agricultural studies that highlight the resilience of the plant in poor soil conditions and its bioaccumulation of minerals and beneficial compounds (Amprako, 2020). The plant's adaptability to harsh environments may contribute to its high bioactive content, reinforcing its pharmacological strength in detoxification protocols.

2.2.3. Safety and Toxicological Considerations of Leaf Extracts

Despite the growing use of *Icacina trichantha* in ethnomedicine, there are increasing concerns about its toxicological safety, particularly when used in high doses or over extended periods. Several experimental studies have evaluated the acute and sub-chronic toxicity of *I. trichantha* leaf extracts to determine its safety profile for therapeutic application. A study by Ojatula and Ogunwande (2022) assessed both the physicochemical characteristics and biosafety of aqueous extracts of *I. trichantha* in albino Wistar rats. Administering doses of

100 mg/kg and 200 mg/kg for 14 consecutive days, the researchers observed no mortality or overt signs of toxicity. However, mild histopathological changes were noted in the liver and kidney at higher doses, suggesting that long-term or excessive intake may pose risks to hepatic and renal function Ojatula and Ogunwande, 2022. Similarly, Abu *et al*. (2023) explored the cardio-protective effects of *I. trichantha* extract against carbon tetrachloride-induced toxicity. While demonstrating protective antioxidant activity, the study emphasized the need for dose monitoring, noting that plant extracts can exert dual pharmacological and toxic effects depending on concentration and exposure duration Abu *et al.,* 2023. Earlier toxicological assessments also support cautious use. Timothy *et al*. (2018) conducted acute and sub-chronic studies with aqueous extracts of *I. trichantha*, showing no significant organ damage or changes in haematological parameters at therapeutic doses. Nonetheless, their findings also stressed that doses above the safety threshold could lead to hepatic enzyme elevation and mild necrotic lesions in rats Timothy *et al.,* 2018. These studies indicate that while *I. trichantha* is generally safe within traditional dosing limits, its use must be informed by controlled dosing, route of administration, and duration. Toxicological effects are often linked to the bioaccumulation of certain secondary metabolites, especially alkaloids and oxalates, which may induce nephrotoxicity or hepatotoxicity in high concentrations (Akinwumi *et al.,* 2011).

2.3. Phytochemical and Pharmacological Properties of *Icacina trichantha*

Phytochemical screening has consistently revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides in both aqueous and organic extracts of *I. trichantha* (Alawode, 2024). These phytoconstituents are known for their pharmacodynamic roles in disease modulation. For instance, flavonoids and phenolic compounds contribute significantly to the plant's antioxidant potential, acting as free radical scavengers that reduce

oxidative stress implicated in conditions such as anaemia and inflammation (Olubomehin *et al.,* 2024).

The pharmacological profile of *I. trichantha* has also been validated through antimicrobial testing. Otun *et al*. (2015) demonstrated that the ethanol and aqueous extracts of the leaf exhibited notable inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, confirming its use in traditional remedies for gastrointestinal and respiratory tract infections. Additionally, Ojah (2020) isolated ethyl acetate and methanol fractions from the stem bark that exhibited strong antibacterial effects, supporting the use of bark infusions in local healing systems. More recent evidence from essential oil analysis has shown that oils extracted from fresh and air-dried leaves of *I. trichantha* retain substantial antimicrobial and antioxidant properties, with the potential for natural therapeutic formulations (Olubomehin *et al.,* 2024). These properties correlate with earlier findings by Shagal and Kubmarawa (2013), who noted broad-spectrum antimicrobial activity from phytochemically rich extracts.

Furthermore, the antioxidant capacities of the plant have been supported by both DPPH and FRAP assays, with findings suggesting its suitability as a natural antioxidant agent (Otun *et al.,* 2015). These properties position *I. trichantha* as a candidate for managing oxidative-related diseases such as neurodegeneration and metabolic syndrome.

2.3.1. Phytochemical Constituents: Flavonoids, Alkaloids, Saponins, etc.

Icacina trichantha possesses a rich spectrum of phytochemicals that underpin its medicinal and therapeutic applications. Several recent studies have confirmed the consistent presence of flavonoids, alkaloids, saponins, tannins, glycosides, phenols, and steroids in different parts of the plant particularly the leaves and tubers highlighting its pharmacological potential (Obazelu and Osarinmwian 2025).

Flavonoids are one of the most abundant bioactive compounds in *I. trichantha*, contributing significantly to its antioxidant and anti-inflammatory effects. According to Alawode (2024), both the leaves and tubers exhibit high flavonoid concentrations, which scavenge free radicals and mitigate oxidative stress a key mechanism in the plant's therapeutic impact.

Alkaloids, known for their antimicrobial and analgesic properties, were also prominently identified in methanol and aqueous extracts. Ojah (2020) documented their presence in both the hexane and ethyl acetate fractions of the stem bark, supporting the plant's use in managing infections and pain-related disorders.

Saponins, another major constituent, contribute to the plant's antidiabetic, cholesterol-lowering, and immunomodulatory effects. These were identified in significant amounts in methanol extracts by Otun *et al*. (2015), suggesting their role in enhancing the permeability of biological membranes and improving nutrient absorption.

Tannins, with astringent and anti-inflammatory properties, are consistently present across studies. Shagal and Kubmarawa (2013) confirmed their presence in the roots and leaves, suggesting a role in wound healing and anti-parasitic applications.

Glycosides, including cardiac glycosides, were identified by Akoh and Mac-Kalunta (2021), potentially offering benefits in cardiac modulation and energy metabolism. These compounds are believed to contribute to the plant's regulatory effect on blood pressure and cardiac rhythm.

Phenolic compounds, particularly polyphenols, were also noted in appreciable concentrations. These compounds play crucial roles in enzyme modulation, antioxidant defense, and cellular signaling.

2.3.2. Antioxidant Properties and Free Radical Scavenging Activity

Icacina trichantha has gained considerable attention for its potent antioxidant capabilities, which are attributed to the high concentrations of bioactive phytochemicals such as flavonoids, phenols, and tannins. These constituents act as natural free radical scavengers, mitigating oxidative stress a key factor in the development of inflammation, cardiovascular diseases, and metabolic disorders. A recent study by Olubomehin *et al*. (2024) examined the essential oils derived from both fresh and air-dried leaves of *I. trichantha* and confirmed strong antioxidant activity through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assays. The authors emphasized that this antioxidant potential is crucial to the plant's traditional application in managing oxidative-related ailments such as anaemia and chronic infections. Isaac *et al*. (2022) also supported this finding, revealing that methanol extracts from *I. trichantha* leaves demonstrated high in vitro antioxidant activity. Their study, which included a comprehensive screen of 32 medicinal plants from Southern Nigeria, placed *I. trichantha* among the top candidates with significant free radical scavenging effects. Similarly, Orimisan and Olakunle (2023) evaluated the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging potential of methanol extracts from the seeds of *I. trichantha*. Their results indicated effective antioxidant performance, reinforcing the plant's systemic cleansing properties and its protective role in oxidative damage to tissues. Onakpa *et al*. (2016) provided foundational in vivo evidence, demonstrating that tuber extracts of *I. trichantha* administered to rats enhanced antioxidant enzyme activity while reducing lipid peroxidation. This highlights its protective effects against oxidative damage induced by chemical toxins.Further validating these findings, Abu *et al*. (2023) reported elevated activity of endogenous antioxidants like superoxide dismutase (SOD) and catalase in rats treated with *I. trichantha* following exposure to CCl_4. These

biochemical markers confirmed the plant's role in maintaining oxidative balance and preventing organ damage.

2.4. Aluminium Chloride-Induced Anaemia: Mechanism and Toxicodynamics

Aluminium chloride ($AlCl_3$) is a widely studied environmental toxicant with well-documented haematological toxicity, particularly its capacity to induce anaemia through oxidative and inflammatory pathways. The toxicodynamics of aluminium-induced anaemia involves multifaceted mechanisms that affect erythropoiesis, red blood cell integrity, and systemic oxidative balance. Recent literature indicates that exposure to $AlCl_3$ leads to the development of microcytic hypochromic anaemia characterized by reductions in haemoglobin levels, red blood cell count, and mean corpuscular volume. According to Bojanić *et al*. (2020), chronic aluminium intoxication results in bone marrow suppression and impaired iron metabolism, both of which culminate in the defective synthesis of haemoglobin and erythrocyte precursors. This aligns with findings by Igbokwe *et al*. (2019), who emphasized that aluminium disrupts iron absorption and utilization, leading to iron-deficiency-like anaemia. Mechanistically, aluminium exerts its toxicity through the generation of reactive oxygen species (ROS), which damage cell membranes, DNA, and enzymes. The accumulation of ROS in erythrocytes leads to lipid peroxidation, oxidative stress, and reduced erythrocyte lifespan. Obani and Anyachor (2023) supported this oxidative pathway, stating that aluminium induces haemolysis and erythrocyte membrane disintegration, thereby contributing to the progression of anaemia. Additionally, aluminium interferes with the regulation of hepcidin and ferroportin key proteins in iron homeostasis resulting in disrupted iron transport and decreased availability for erythropoiesis. The systemic toxicity of aluminium also extends to hepatic and renal systems, where it further impairs detoxification and erythropoietin synthesis (Saad *et al.,* 2018).

2.4.1. Aluminium Toxicity and Systemic Absorption

Aluminium toxicity is largely dependent on its systemic absorption, distribution, and accumulation in tissues, which ultimately lead to oxidative damage and organ dysfunction. When ingested orally through contaminated water, food additives, or pharmaceutical sources aluminium can be absorbed via the gastrointestinal tract and accumulate in organs such as the liver, kidneys, brain, and bone.

Gastrointestinal absorption of aluminium is generally low (approximately 0.1–1%) under normal conditions, but it increases when aluminium binds to organic acids like citrate, which enhances solubility and intestinal permeability (Fernandes *et al.,* 2021). This mechanism allows aluminium ions to bypass enterocyte defense mechanisms and enter systemic circulation. Once absorbed, aluminium binds to plasma proteins such as transferrin and albumin, facilitating transport to distant tissues.

Upon systemic circulation, **tissue accumulation** occurs in the liver, brain, and skeletal tissues. Notably, studies have confirmed that chronic exposure leads to a buildup of aluminium in the brain, contributing to neurodegenerative changes, as well as in the hematopoietic system, where it disrupts erythropoiesis (Igbokwe *et al.,* 2019). This bioaccumulation is compounded by aluminium's long biological half-life and inefficient renal clearance.

The **toxicodynamics** of aluminium primarily involve oxidative stress pathways. Aluminium induces reactive oxygen species (ROS) generation, lipid peroxidation, and mitochondrial dysfunction, which are implicated in tissue injury, enzyme inactivation, and DNA damage. Samir and Rashed (2018) reported increased oxidative stress biomarkers and DNA fragmentation in individuals with chronic aluminium exposure, supporting this mechanism.

Novaes *et al*. (2018) also documented that aluminium interferes with micronutrient balance and causes structural remodeling in cardiac and hepatic tissues, providing evidence of systemic bioaccumulation effects. Importantly, the degree of aluminium toxicity is dose-dependent and modulated by factors such as age, nutritional status, and renal function.

2.4.2. Mechanisms of Aluminium-Induced Oxidative Stress

Upon entering systemic circulation, aluminium catalyzes redox reactions that produce superoxide anions, hydrogen peroxide, and hydroxyl radicals reactive molecules capable of damaging lipids, proteins, and DNA. Studies have demonstrated that aluminium directly impairs mitochondrial respiratory chain enzymes, leading to reduced ATP synthesis and heightened ROS generation. Sharma *et al*. (2013) found that aluminium exposure modulates the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a key regulator of mitochondrial biogenesis, thus diminishing mitochondrial capacity and resilience to oxidative stress (Sharma *et al.,* 2013).

Moreover, aluminium significantly reduces the activity of primary antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Kumar and Gill (2014) demonstrated that mitochondrial SOD (MnSOD) is particularly susceptible to oxidative modifications caused by aluminium, exacerbating the ROS cascade and mitochondrial damage (Kumar and Gill, 2014).

These biochemical disruptions are associated with structural and functional degeneration in multiple tissues. For example, El-Gendy (2011) observed elevated malondialdehyde (MDA) and decreased reduced glutathione (GSH) levels in liver tissues of rats exposed to aluminium, confirming lipid peroxidation and impaired detoxification capacity (El-Gendy, 2011).

Additionally, Chowra *et al*. (2017) reported that continuous aluminium stress in plant models like *Vigna mungo* (black gram) caused cellular damage and metabolic shifts through sustained ROS exposure, providing a comparative model for understanding redox imbalance across biological kingdoms (Chowra *et al.,* 2017)

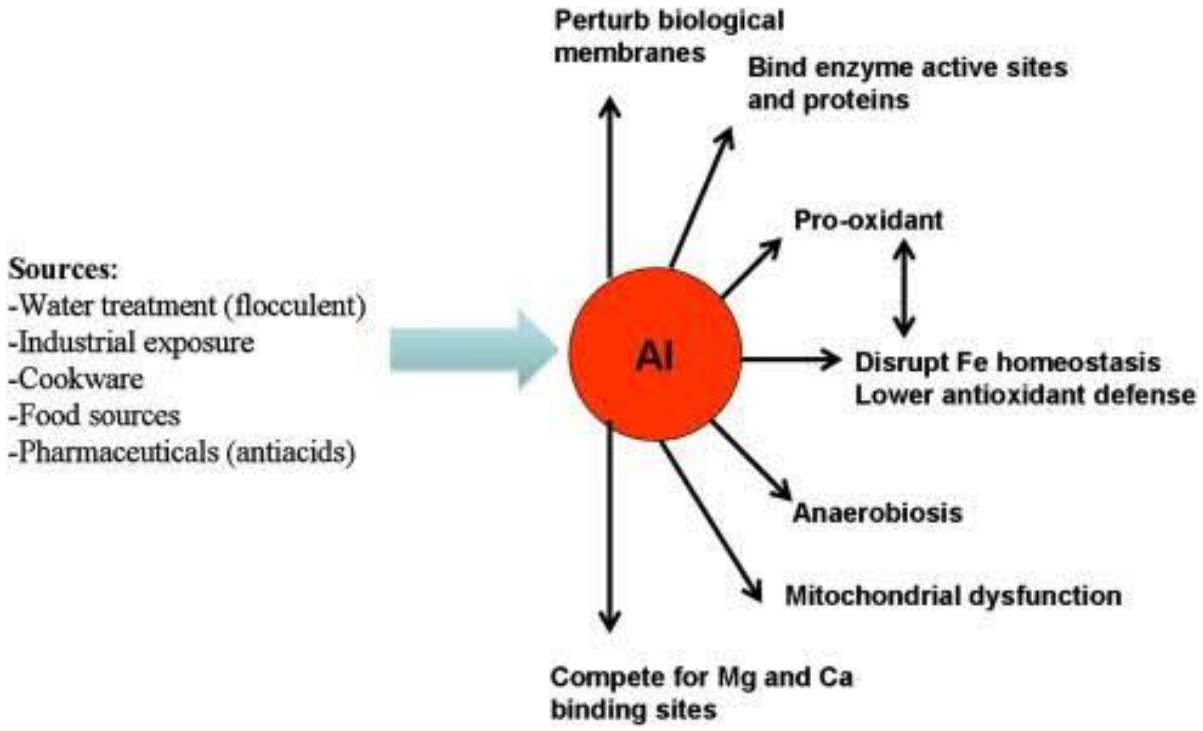


Figure 2.2. Mechanisms of Aluminium-Induced Oxidative Stress (Kumar and Gill, 2014)

2.4.3. Disruption of Erythropoiesis and Bone Marrow Function

Aluminium exposure has been shown to significantly impair erythropoiesis and alter the function of bone marrow, resulting in various forms of anaemia. The mechanisms by which aluminium exerts these effects are multifactorial, involving oxidative damage to progenitor cells, suppression of erythropoietin synthesis, and direct inhibition of erythroid colony formation.

Recent evidence indicates that aluminium disrupts the proliferation and differentiation of erythroid progenitor cells within the bone marrow. Igbokwe *et al*. (2019) reported that exposure to aluminium significantly reduced colony-forming units-erythroid (CFU-E) in bone marrow, suggesting a direct cytotoxic effect on early-stage erythropoietic cells. This finding aligns with earlier reports by Vota *et al*. (2012), which demonstrated that aluminium-induced oxidative stress leads to eryptosis (programmed erythrocyte death), further impairing red blood cell survival and contributing to anaemia.

Zhang *et al*. (2020) added a mechanistic dimension to these findings by demonstrating that aluminium disrupts iron metabolism and erythropoiesis both dependently and independently through effects on bone cells and haematopoiesis. Their data support a model in which heavy metal exposure, including aluminium, affects bone marrow niches by altering the availability of essential micronutrients such as magnesium and zinc, ultimately impairing erythropoietic signaling.

In vivo and in vitro assays also reveal that aluminium exposure increases the production of reactive oxygen species in bone marrow cells, leading to oxidative DNA damage and apoptosis of hematopoietic stem cells. This mechanism was detailed in the study by Vota *et al*. (2012), where erythropoietin supplementation was shown to partially reverse these effects, emphasizing the role of oxidative imbalance in aluminium-mediated haematotoxicity.

2.4.4. Aluminium's Effect on Red Blood Cell Morphology and Lifespan

Aluminium exposure significantly alters red blood cell (RBC) morphology and reduces their lifespan, contributing to anaemia and impaired oxygen transport capacity. Morphologically, aluminium disrupts erythrocyte membrane integrity, leading to cell deformation, increased osmotic fragility, and reduced mechanical stability.

Igbokwe *et al.,* (2019) provided recent evidence that aluminium induces oxidative damage to erythrocyte membranes, resulting in altered shapes (poikilocytosis), increased fragility, and hemolysis. This corresponds to a shortened red blood cell lifespan, as damaged RBCs are rapidly cleared from circulation by splenic macrophages (Igbokwe *et al.,* 2019).

Further corroborating this, Mahieu *et al*. (2000) observed aluminium-induced reductions in erythrocyte count, hemoglobin concentration, and hematocrit, with concurrent morphological changes such as spherocytosis and membrane blebbing (Mahieu *et al.,* 2000). These structural abnormalities were attributed to aluminium's pro-oxidant behavior, which disrupts membrane phospholipid bilayers and compromises cytoskeletal protein function.

Additionally, Turgut *et al*. (2006) reported that low-dose aluminium exposure in rats leads to decreased RBC deformability and lifespan, suggesting a role in systemic hypoxia and fatigue in chronic aluminium exposure scenarios (Turgut *et al.,* 2006).

2.5. Molecular Markers and Gene Expression in Anaemia

2.5.1. Genes Involved in Erythropoiesis

Erythropoiesis the process by which red blood cells (RBCs) are produced relies on a tightly coordinated network of transcription factors and signaling molecules. Disruptions to this pathway, whether due to genetic or environmental factors like aluminium toxicity, often result in anaemia. Several critical genes involved in this process include GATA-1, erythropoietin (EPO), erythropoietin receptor (EPOR), and hypoxia-inducible factor 1-alpha (HIF-1α).

GATA-1 is a zinc-finger transcription factor that regulates the expression of genes essential for erythroid differentiation. It acts in tandem with other erythroid-specific regulators to promote terminal maturation of erythroblasts. Studies have shown that reduced GATA-1 expression results in impaired erythropoiesis, underscoring its essential role in both steady-state and stress-induced erythroid lineage commitment (Nikpour *et al.,* 2010).

EPO is the primary cytokine regulating erythropoiesis, especially under hypoxic conditions. It is mainly produced in the kidneys and binds to its receptor EPOR on erythroid progenitor cells, triggering a cascade that promotes proliferation, survival, and differentiation of these cells. A 2019 study by Mello *et al*. profiled EPOR gene expression in bone marrow and showed that its levels are highest at intermediate and late stages of erythroid differentiation, highlighting the importance of EPO-EPOR signaling throughout maturation (Mello *et al.,* 2019).

HIF-1α serves as a critical oxygen sensor that regulates the transcription of EPO under hypoxic conditions. Stabilization of HIF-1α in response to low oxygen tension upregulates EPO production, thereby enhancing erythropoiesis. As reported by Papanikolaou and Pantopoulos (2017), HIF-1α also coordinates the response of iron homeostasis genes with erythropoietic demand (Papanikolaou and Pantopoulos, 2017).

Disruptions to the expression or signaling of any of these genes due to aluminium toxicity or other stressors can impair red blood cell production and contribute to anaemia. For example, oxidative stress may destabilize GATA-1 protein or inhibit HIF-1α signaling, thereby suppressing erythropoietic responses even during tissue hypoxia.

2.5.2. Role of Caspases in Programmed Cell Death (Apoptosis)

Caspases, a family of cysteine proteases, are central mediators of apoptosis the genetically regulated form of programmed cell death. Their role becomes particularly crucial in pathological contexts such as aluminium-induced oxidative stress and anaemia, where cellular homeostasis is disrupted. Caspases are categorized into initiator (e.g., caspase-8, -9) and effector (e.g., caspase-3, -6, -7) groups that orchestrate the cascade of apoptotic events leading to cell dismantling.

In the context of aluminium exposure, oxidative stress serves as a potent trigger for the activation of both intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) apoptotic pathways. Kumar and Gill (2014) highlighted that aluminium induces mitochondrial dysfunction, which results in the release of cytochrome c, activation of caspase-9, and subsequent cleavage of caspase-3, marking the execution phase of apoptosis (Kumar and Gill, 2014).

Similarly, Igbokwe *et al*. (2019) noted increased erythrocyte apoptosis (eryptosis) following aluminium-induced oxidative stress, with evidence pointing to caspase-3 activation as a key factor in erythrocyte membrane blebbing and hemoglobin leakage. These effects reduce red blood cell lifespan and contribute to the development of anaemia (Igbokwe *et al.,* 2019).

Dey and Singh (2022) further expanded the understanding of aluminium's apoptotic impact, particularly in neuronal tissues, via caspase-3 and JNK (c-Jun N-terminal kinase) pathways.

Though their study was neurocentric, the molecular parallels extend to hematopoietic cells, especially under chronic toxicological stress (Dey and Singh, 2022).

2.5.3. Caspase-1: Structure, Function, and Expression in Anaemia

Caspase-1, also known as interleukin-1β converting enzyme, is a key inflammatory caspase responsible for the cleavage and activation of pro-inflammatory cytokines such as IL-1β and IL-18. It plays a pivotal role not only in canonical inflammasome activation and innate immunity but also in pathological processes like anaemia, especially via pyroptosis and inflammation.

Structurally, caspase-1 comprises a prodomain with a caspase activation and recruitment domain (CARD), a large catalytic domain (p20), and a small subunit (p10). Upon activation, caspase-1 forms tetramers that cleave downstream targets including gasdermin D, leading to cell membrane pore formation and pyroptotic cell death (Molla *et al.,* 2020).

Functionally, caspase-1 is activated by inflammasome complexes such as NLRP3, often in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). This process is tightly regulated and often dysregulated in chronic diseases including anaemia. According to Tyrkalska *et al*. (2019), caspase-1 directly influences hematopoiesis by modulating the stability of GATA-1, a master erythroid transcription factor, thereby contributing to disrupted red blood cell development under inflammatory stress.

Recent work by Lezhenko *et al*. (2019) found elevated levels of caspase-1 in children suffering from anaemia of inflammation, suggesting that excessive inflammasome activation contributes to anaemia by inducing erythroid cell death and systemic inflammation. This aligns with findings from Bolivar *et al*. (2021), who described caspase-1's activation in

response to heme overload, linking hemolytic stress and chronic anaemia with caspase-mediated cell injury.

2.5.4. Caspase-1 in Inflammatory and Hematopoietic Disorders

Caspase-1 plays a pivotal role at the intersection of inflammation and hematopoietic regulation. As the canonical effector of inflammasome activation, caspase-1 mediates the cleavage and secretion of interleukin-1β (IL-1β) and interleukin-18 (IL-18), driving inflammatory cascades in both acute and chronic immune responses. In the context of hematopoietic disorders, this pro-inflammatory signaling has been implicated in the disruption of erythropoiesis and bone marrow homeostasis. One of the primary mechanisms through which caspase-1 contributes to hematopoietic dysfunction is pyroptosis, a form of programmed inflammatory cell death. In bone marrow, overactivation of caspase-1 leads to the destruction of erythroid progenitor cells, resulting in Anaemia of inflammation (also termed Anaemia of chronic disease). This pathway is potentiated under conditions of oxidative stress and systemic infection, where high levels of IL-1β impair erythropoietin signaling and iron metabolism, thereby hindering red blood cell production.

Recent research has shown that persistent caspase-1 activation disturbs the balance between myeloid and erythroid lineages by modifying the microenvironment of the hematopoietic stem cell (HSC) niche. Inflammatory cytokines released due to caspase-1 activity can suppress the expression of GATA-1, a transcription factor critical for erythroid maturation, as demonstrated by Tyrkalska *et al*. (2019). Furthermore, caspase-1 overactivity has been linked to hematologic malignancies and bone marrow failure syndromes. The excessive inflammasome signaling seen in conditions like myelodysplastic syndromes (MDS) supports the hypothesis that chronic low-grade inflammation, mediated by caspase-1, contributes to ineffective hematopoiesis and increased cell death.

2.5.5. CYP11A1 and Steroidogenesis in Blood Cell Development

CYP11A1, also known as cytochrome P450scc (side-chain cleavage enzyme), encodes the enzyme responsible for the first and rate-limiting step in steroid hormone biosynthesis: the conversion of cholesterol into pregnenolone. This step is essential for the production of all classes of steroid hormones, including glucocorticoids, mineralocorticoids, and sex steroids. Recent studies indicate that CYP11A1 also plays a regulatory role in hematopoiesis and blood cell development, especially through its influence on the bone marrow microenvironment and erythropoietic signaling. Steroid hormones derived from CYP11A1 activity such as corticosterone and estrogens are known to modulate erythropoiesis and leukopoiesis (Obazelu and Ezeonyebuchi, 2025). Oguro (2019) demonstrated that cholesterol metabolism and its steroid derivatives influence both normal and malignant hematopoiesis, with a specific role for CYP11A1 in defining hematopoietic stem cell (HSC) behavior through metabolic signaling cascades (Oguro, 2019). Furthermore, steroidogenic regulation at the transcriptional level involves Steroidogenic Factor 1 (SF-1), which binds the promoter regions of CYP11A1 and is critical for tissue-specific expression. LaVoie and King (2009) showed that SF-1, together with other co-factors, modulates CYP11A1 transcription in endocrine and steroidogenic tissues, indirectly affecting steroid availability for hematopoietic regulation (LaVoie and King, 2009). In a zebra fish model, Wang *et al*. (2022) identified that CYP11A2, the ortholog of mammalian CYP11A1, is essential for the development of spermatogonial stem cells and oocytes further suggesting the gene's relevance beyond endocrine tissue, including its impact on germline and somatic lineage development (Wang *et al.,* 2022).

2.5.6. Impact of Aluminium on Caspase and CYP Expression

Aluminium exposure has been increasingly linked to the dysregulation of genes involved in oxidative stress, apoptosis, and metabolic detoxification, with two critical molecular targets being caspases and cytochrome P450 (CYP) enzymes. Aluminium-induced toxicity disrupts redox balance, which subsequently leads to the overexpression of apoptotic mediators (like caspase-3) and alteration in CYP gene expression, particularly in hepatic and hematopoietic tissues. In cancer, caspase-3 is often down-regulated or inactivated, contributing to the resistance of cancer cells to apoptosis and promoting tumor growth and survival (Obazelu and Osazee 2024). Recent studies have established that aluminium oxide nanoparticles significantly affect the HO-1/MT-1/CYP450 signaling pathway and activate oxidative stress-related mechanisms. Karami *et al*. (2023) found that exposure to aluminium stimulated CYP450 expression and modulated antioxidant defenses, suggesting a compensatory response to metal-induced ROS overproduction (Karami *et al.,* 2023). In a related context, Feng *et al*. (2021) reported that aluminium potassium sulfate altered gene expression in insect cells, including CYP family genes and apoptotic regulators like caspase-3, implying that aluminium might cause mitochondrial dysfunction and cell cycle disturbances via the uncoupling of CYP enzymatic pathways (Feng *et al.,* 2021). Sedik *et al*. (2023) demonstrated that aluminium chloride exposure induced liver and kidney injury in rats by increasing expression of TNF-α, caspase-3, and modulating Crat, Car3, and Nrf2 pathways. This suggests that aluminium toxicity promotes apoptotic signaling and impairs redox-regulated transcription factors (Sedik *et al.,* 2023). Furthermore, Abd *et al*. (2019) observed severe chromosomal aberrations and altered CYP gene expression following exposure to aluminium silicate. CYP upregulation was interpreted as a detoxification response, although it may also enhance bioactivation of xenobiotics, exacerbating tissue damage (Abd *et al.,* 2019)

2.6. Cytochrome P450 and Its Role in Haematopoiesis

2.6.1. Overview of Cytochrome P450 Enzyme Superfamily

The cytochrome P450 (CYP450) enzyme superfamily comprises a diverse group of heme-thiolate proteins involved in the oxidative metabolism of both endogenous substrates (e.g., steroids, fatty acids) and xenobiotics (drugs and environmental toxins). These enzymes are categorized into multiple families and subfamilies based on sequence homology, with at least 57 functional genes identified in humans (Esteves *et al.,* 2021). Functionally, CYP450 enzymes catalyze phase I metabolic reactions, including hydroxylation, epoxidation, and dealkylation, thereby increasing substrate solubility and preparing compounds for further conjugation during phase II metabolism (Sandoval *et al.,* 2023). The majority of CYP450 enzymes are expressed in the liver, but their activity is not confined to hepatic tissues. Notably, these enzymes are also found in hematopoietic and immune-related cells, where they play non-canonical roles in immune modulation and hematopoietic regulation. Recent studies underscore the importance of CYP450 activity in shaping the hematopoietic microenvironment, especially in the context of disease. In acute myeloid leukemia (AML), aberrant expression of CYP450 enzymes has been implicated in altered drug metabolism and resistance, as well as modification of inflammatory signaling in the bone marrow niche (Sandoval *et al.,* 2023). This expands the traditional view of CYP enzymes as purely metabolic regulators to key players in hematopoietic homeostasis and pathology.

2.6.2. CYP11A1: Role in Steroid Hormone Biosynthesis

CYP11A1, also known as cytochrome P450scc (side-chain cleavage), is a mitochondrial enzyme that catalyzes the conversion of cholesterol to pregnenolone, the first and rate-limiting step in the biosynthesis of all steroid hormones. This process is essential for the

generation of glucocorticoids, mineralocorticoids, and sex steroids hormones that regulate a broad range of physiological functions including metabolism, immune modulation, and hematopoiesis.

CYP11A1 is predominantly expressed in classical steroidogenic tissues such as the adrenal cortex, testes, and ovaries, but also shows inducible expression in extra-adrenal tissues where local steroid production supports cell differentiation and immune functions (LaVoie and King, 2009). The transcription of the CYP11A1 gene is tightly regulated by Steroidogenic Factor 1 (SF-1) and other nuclear co-regulators in a tissue-specific manner. In the context of hematopoiesis, recent evidence has highlighted the relevance of cholesterol-derived steroidogenesis in the regulation of hematopoietic stem cells (HSCs). Oguro (2019) demonstrated that cholesterol metabolism and the steroidogenic enzymes CYP11A1 and CYP11B1 play critical roles in determining the fate of HSCs during both normal and malignant hematopoiesis. This is achieved through modulation of local hormonal signals that influence lineage commitment and immune response (Oguro, 2019).

2.6.3. Modulation of Hematopoietic Stem Cell Activity by CYP11A1

The modulation of hematopoietic stem cell (HSC) activity by CYP11A1 centers on its pivotal role in steroidogenesis, particularly the synthesis of pregnenolone the precursor of all steroid hormones. CYP11A1 catalyzes the conversion of cholesterol into pregnenolone within mitochondria, a rate-limiting step in steroid hormone biosynthesis that influences diverse physiological pathways, including hematopoiesis (LaVoie and King, 2009). Emerging evidence from animal and stem cell studies indicates that CYP11A1 activity indirectly regulates HSC behavior through local steroid hormone signaling. For example, Wang *et al.* (2022) demonstrated that Cyp11a2 (the zebrafish ortholog of mammalian CYP11A1) is essential for the differentiation of spermatogonial stem cells, highlighting a conserved role in

stem cell maintenance and lineage determination (Wang *et al.,* 2022). Although focused on germline development, these findings are analogous to mechanisms in hematopoietic tissues where steroid hormones modulate self-renewal, proliferation, and differentiation of HSCs. Additionally, CYP11A1 expression in the hematopoietic niche has been shown to affect immune cell lineage specification. Cai *et al*. (2024) found that retinoic acid enhanced ovarian steroidogenesis by modulating the MESP2/STAR/CYP11A1 axis, suggesting a broader transcriptional and hormonal regulatory network involving CYP11A1 across stem cell populations (Cai *et al.,* 2024). This modulation is particularly relevant in inflammatory and anemic conditions where HSC function is tightly regulated by systemic and local steroid levels. In fact, LaVoie and King (2009) emphasized that transcriptional regulation of CYP11A1 in extra-adrenal tissues, such as bone marrow stromal cells, may provide a mechanism for paracrine control of hematopoietic output during stress hematopoiesis.

2.6.4. Steroid Hormones in Regulation of Immune and Blood Systems

Steroid hormones, including glucocorticoids, mineralocorticoids, and sex steroids, play a vital regulatory role in both the immune system and hematopoiesis. These hormones exert their effects through nuclear hormone receptors, modulating gene transcription involved in cell differentiation, proliferation, and apoptosis of immune and blood cells. Glucocorticoids, such as cortisol, are central in modulating immune responses. They suppress pro-inflammatory cytokines and promote the survival of hematopoietic stem cells (HSCs) under stress, supporting their self-renewal and differentiation. This dual immunosuppressive and hematopoietic-supportive effect makes glucocorticoids clinically valuable in treating immune-mediated cytopenias and leukemias (Quatrini *et al.,* 2021). Sex hormones, including estrogen and testosterone, also influence hematopoietic lineage development. Estrogen enhances erythropoiesis and promotes immune cell regeneration by stimulating stromal cells

in the bone marrow microenvironment (Heo *et al.,* 2015). Notably, sex steroid ablation has been shown to rejuvenate the HSC pool and enhance immune recovery post-chemotherapy, further highlighting their role in immune regulation (Khong *et al.,* 2015). On the molecular level, Chakraborty *et al*. (2021) noted the presence of an endogenous steroid-regulatory circuit within immune cells, where local steroidogenesis directly modulates T-cell activation and macrophage function. This circuit is also responsive to environmental stressors, suggesting dynamic crosstalk between hormonal and immune signaling pathways (Chakraborty *et al.,* 2021). Furthermore, studies in *Drosophila* and vertebrates confirm the evolutionary conservation of steroid hormone pathways in immune function, demonstrating their effect on innate immune signaling and hematopoietic progenitor expansion (Keith, 2023).

2.7. Haematological Parameters in Toxicity Studies

2.7.1. Red Blood Cell (RBC) Count and Anaemia Detection

Red blood cell (RBC) count is a fundamental haematological parameter widely used in toxicity studies to evaluate the integrity of erythropoiesis and detect early signs of anaemia, particularly in experimental models exposed to toxicants such as heavy metals and synthetic or natural therapeutic agents. A reduction in RBC count often signals bone marrow suppression, oxidative damage to erythrocytes, or impaired erythropoietin production. In toxicological contexts, heavy metals like aluminium, cadmium, and lead have been shown to induce anaemia by generating oxidative stress and interfering with the lifespan and maturation of red blood cells (Nna *et al.,* 2019). Aluminium chloride, for example, leads to hemolysis and suppresses erythropoietin, resulting in microcytic or normocytic anaemia. Conversely, phytotherapeutic agents such as *Icacina trichantha* and Tetracarpidium conophorum are studied for their potential to ameliorate hematological alterations. These

extracts are rich in antioxidants, which protect red cells from lipid peroxidation and restore normal erythropoiesis (Ezeja *et al.,* 2021; Okafor *et al.,* 2022). The RBC count, in combination with haemoglobin concentration and haematocrit values, provides a robust index for monitoring haematotoxicity and the efficacy of protective interventions.

2.7.2. Haemoglobin Concentration and Oxygen Transport

Haemoglobin (Hb) is the principal oxygen-carrying protein in red blood cells, and its concentration is a direct marker of the blood's capacity to deliver oxygen to tissues. In toxicological studies, particularly those involving heavy metals or phytochemical agents, Hb concentration is a critical parameter for assessing the degree of anaemia and evaluating the efficacy of therapeutic interventions. Exposure to heavy metals like aluminium, lead, and cadmium has been widely reported to reduce Hb levels. This is primarily due to the metals' interference with heme biosynthesis, increased erythrocyte fragility, or oxidative degradation of Hb molecules (Nna *et al.,* 2019). For instance, aluminium chloride can disrupt iron metabolism and inhibit ferrochelatase activity, leading to microcytic hypochromic anaemia and poor oxygen transport. In contrast, plant-based therapeutics especially extracts with antioxidant and hematinic properties such as *Icacina trichantha* have shown promise in restoring haemoglobin levels.

2.7.3. Packed Cell Volume (PCV) and Blood Viscosity

In toxicological studies involving heavy metals, such as aluminium and lead, a consistent decline in PCV is often observed, reflecting red cell destruction or suppression of erythropoiesis. For example, Joseph *et al*. (2024) demonstrated a significant decrease in PCV in Wistar rats treated with a toxic dose of *Pterocarpus santalinus* extract, indicating potential hematotoxic effects (Joseph *et al.,* 2024). On the other hand, therapeutic interventions with

plant-based remedies can restore PCV to near-normal levels. A study by Sinoriya and Singh (2024) observed normalization of PCV in rats treated with polyherbal formulations following CCl_4-induced hepatotoxicity, highlighting the role of plant antioxidants in promoting hematological recovery (Sinoriya and Singh, 2024). Furthermore, changes in PCV also affect blood viscosity, which is important in cardiovascular homeostasis. Ofem *et al*. (2009) found that *Viscum album* (mistletoe) extract helped stabilize PCV and reduce erythrocyte sedimentation rate (ESR), suggesting an improvement in rheological properties and reduced inflammation (Ofem *et al.,* 2009).

2.7.4. White Blood Cell (WBC) Count in Immune Response

White blood cell (WBC) count is a fundamental haematological parameter for assessing the status of the immune system, particularly in toxicological studies involving heavy metals and phytotherapeutic agents. Changes in WBC count can indicate immune suppression, activation, or dysregulation depending on the nature of the toxicant or treatment applied. In models of toxicity, especially those involving aluminium, lead, or cadmium, a significant reduction in WBC count (leukopenia) often reflects bone marrow suppression and impaired leukopoiesis. For example, Mahassni and Alsahafi (2022) demonstrated that exposure to Leptadenia pyrotechnica extract modulated immune cell counts in rats, showing that both stimulatory and suppressive effects can be induced depending on the phytochemical composition and dose administered (Mahassni and Alsahafi, 2022). Similarly, Ghazalee *et al*. (2019) reported that Zingiber zerumbet extract significantly suppressed innate immune responses in Wistar rats by reducing WBC and neutrophil counts, highlighting the potential immunotoxic effects of some herbal preparations at certain concentrations (Ghazalee *et al.,* 2019). On the therapeutic side, herbal compounds have been observed to restore or enhance WBC counts in conditions of immunosuppression. Shirani *et al*. (2015) reviewed over 50 plant-based interventions for

cyclophosphamide-induced immunotoxicity, many of which normalized WBC levels and boosted immune resilience (Shirani *et al.,* 2015). Similarly, Zhuang *et al*. (2009) demonstrated that a traditional Chinese herb complex improved cellular immunity in cancer patients undergoing chemotherapy, reflected by stabilized WBC profiles (Zhuang *et al.,* 2009).

2.7.5. Platelets and Inflammatory Responses in Toxic Conditions

Platelets, primarily known for their role in hemostasis and thrombosis, are increasingly recognized as key players in inflammatory responses, particularly in the context of toxicological exposure to heavy metals and therapeutic interventions using plant extracts. Alterations in platelet count and function serve as important indicators of systemic inflammation, vascular dysfunction, and immune modulation in toxicity studies. Heavy metals such as lead, cadmium, and mercury can provoke oxidative stress, endothelial damage, and promote platelet aggregation. Notariale *et al*. (2021) highlight that erythrocytes and platelets can act as models for studying vascular toxicity induced by heavy metals, with dietary antioxidants offering a potential protective effect by reducing platelet hyperactivity (Notariale *et al.,* 2021). Therapeutically, plant-derived compounds have shown promising effects in modulating platelet activity and inflammation. For example, Hassan *et al*. (2022) demonstrated that a novel metal-targeted compound, CDPDP, significantly reduced inflammatory cytokines and normalized platelet levels in mice exposed to inflammatory insults (Hassan *et al.,* 2022). Similarly, Ceramella *et al*. (2024) reviewed phytochemicals that mitigate silent toxicity caused by metals and found that many promote platelet normalization and reduce systemic oxidative stress (Ceramella *et al.,* 2024). Additionally, El-Boshy *et al.* (2019) observed significant improvements in platelet count and coagulation profiles in rats

treated with *Thymus vulgaris* extract after lead-induced toxicity, affirming the anti-inflammatory and hematoprotective properties of botanical agents (El-Boshy *et al.,* 2019).

2.8. Antioxidant Role of Medicinal Plants in Heavy Metal Detoxification

Medicinal plants play a pivotal role in mitigating the toxic effects of heavy metals through their antioxidant mechanisms, which include free radical scavenging, metal chelation, and restoration of oxidative homeostasis. Heavy metal exposure such as aluminium, cadmium, or lead induces oxidative stress by generating reactive oxygen species (ROS) that damage cellular macromolecules, impair mitochondrial function, and dysregulate antioxidant defense systems. Phytochemicals such as flavonoids, alkaloids, saponins, tannins, and polyphenols, found in various medicinal plants, act as potent antioxidants. These compounds interrupt the oxidative cascade by donating electrons to neutralize free radicals or chelating metal ions to prevent Fenton-like reactions. For instance, flavonoids inhibit lipid peroxidation and preserve glutathione (GSH) levels, while tannins can directly bind heavy metals and reduce their bioavailability. Recent studies have confirmed these protective effects in preclinical models. El-Boshy *et al*. (2019) reported that *Thymus vulgaris* extract significantly reduced lead-induced oxidative stress and hematological damage by enhancing superoxide dismutase (SOD) and catalase (CAT) activity. Similarly, Okafor *et al*. (2022) demonstrated that *Icacina trichantha* leaves restored antioxidant markers and reversed cadmium-induced liver and blood toxicity, confirming its phytoprotective efficacy.

2.8.1. Comparative Efficacy of Other Plants vs. *Icacina trichantha*

The detoxifying and antioxidant efficacy of medicinal plants in mitigating heavy metal-induced oxidative stress has been extensively studied across various models. Among these, *Icacina trichantha* stands out for its promising hematoprotective and antioxidative potential,

particularly in reversing aluminium- and cadmium-induced haematological alterations. However, its efficacy can be better contextualized by comparing it with other well-established plant agents such as *Moringa oleifera*, *Thymus vulgaris*, and *Zingiber officinale* (ginger).

Studies have shown that *Moringa oleifera* leaves, rich in polyphenols and flavonoids, possess strong metal-chelating and reactive oxygen species (ROS)-scavenging activity, contributing to restoration of antioxidant enzyme systems such as superoxide dismutase (SOD) and catalase (CAT). This aligns closely with the antioxidant behavior of *Icacina trichantha* , which also elevates endogenous antioxidants and maintains erythrocyte stability under metal-induced oxidative insult. *Thymus vulgaris*, commonly used in ethnomedicine, has demonstrated strong antioxidant and anti-inflammatory effects in response to lead and cadmium toxicity. As reported by Mirkov *et al*. (2020), ethanolic extracts of *T. vulgaris* and *Rosmarinus officinalis* substantially reduced oxidative stress biomarkers and protected against hepatic and renal oxidative damage in animal models, with effects comparable to synthetic chelators (Mirkov *et al.,* 2020). Similarly, *Zingiber officinale* (ginger) has been widely studied for its bioactive phytochemicals, such as gingerol and shogaol, which act not only as free radical scavengers but also exhibit anti-inflammatory and immunomodulatory properties. Research has shown that lemongrass contains bioactive compounds like citral, which exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Obazelu and Abadaike-Elvis, 2024). As noted by Tiwari *et al*. (2018), *Z. officinale* extract showed strong antioxidative activity, significantly comparable to doxorubicin in modulating immune markers and reducing oxidative parameters (Tiwari *et al.,* 2018). In contrast, *Icacina trichantha* , though less commercially known, holds comparable potential, particularly in gene-level regulation of antioxidant responses and restoration of erythropoiesis, making it a strong candidate for further phytopharmacological development. While mainstream herbs like

M. oleifera and *T. vulgaris* benefit from broader scientific validation, *I. trichantha* is gaining recognition for its nuanced, systemic hematological protection in anaemia-related toxicological models.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Reagents

Reagents used in this study were of analytical grade.

3.2. Study Population

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 then identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City by Dr. A.O. Akinnibosun. This was done 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 a 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. Finally, 1500grams of the leave were precisely weighed for subsequent usage (Obazelu and Ogiza, 2024).

3.4. Preparation of Plants Extract

1500grams of ground powder were mixed with 15litres of distilled water. Subsequently, the mixture was left to soak for a duration of 24 hours under constant stirring .After the specified duration, the mixture underwent filtration using Whatman's (Nitro cellulose 45; 0.45µm pore size) filter paper, with the residue being discarded. Following filtration, the resulting filtrate was subjected to concentration in a Water bath maintained at 37°C.. The concentrated solution was put in an airtight container and refrigerated.

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 .

3.5.1. Inclusion Criteria

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

3.5.2. Exclusion Criteria

- Sick Rats
- Rats weighing less than 150g (<150g)

- Rats with reduced appetite
- Rats with excessive breathing

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/0618073 issued on 18th, June, 2025).

3.7. Preparation of Aluminium Chloride Solution and Ferrous Sulphate Drug Solution

3.7.1. Aluminium Chloride Solution

A solution of aluminum chloride was prepared by dissolving 0.1 grams of aluminum chloride powder (supplied by Guangdong Guanghua Sci-Tech Co., Ltd., Batch No: T/CSTM 00071-2019) in 100 milliliters of distilled water. Following this, 0.1 milliliter of the aluminum chloride solution was administered to each animal in the test groups, all of which had an average body weight of 150 grams.

3.7.2. Ferrous Sulphate Drug Solution

A ferrous sulphate solution was prepared by dissolving 1000 mg of the powdered drug in 50 ml of distilled water. Each animal in group C, with an average body weight of 150 g, received 0.3 ml of this solution orally every 48 hours over a period of 28 days (Obazelu and Faluyi, 2023).

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 Aluminium Chloride intraperitoneally.

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

Group D: The animals in this group were given aluminium chloride solution intraperitoneally and orally treated with a low dose of *Icacina trichantha* leaf extract.

Group E: The animals in this group received aluminium chloride solution intraperitoneally and were orally treated with *Icacina trichantha* leaf extract.

Group F: The animals in this group were given aluminium chloride solution intraperitoneally and orally administered the highest dose of *Icacina trichantha* leaf extract.

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

3.8.2. Extract Dosing

The dosage given to each group was calculated by;

Weight of the animal = g/kg

Dose of extract = mg/kg

Stock of extract = mg/ml

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

Concentration of extract = $\frac{40000\text{mg}}{400\text{ml}}$ = 100mg/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 (Obazelu and Evwaire, 2024);

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

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

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

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 trichatha* Extract

Group A (control) was provided only with standardized feed and unrestricted access to clean water.

Group B (Aluminium Chloride-treated) received 0.1ml of Aluminium Chloride solution intraperitoneally every 48 hours for 28 days.

Group C (ferrous sulphate-treated) was administered 0.1 ml of Aluminium Chloride solution intraperitoneally every 48 hours for 28 days, followed by 0.3 ml of ferrous sulphate solution (6 mg/ml) every 48 hours for the same period.

Group D received 0.1ml of Aluminium Chloride solution intraperitoneally every 48 hours for 28 days and was orally treated with 0.15 ml of a 100 mg/kg body weight *Icacina trichatha* leaf extract via gavage daily for 28 days.

Group E received the same Aluminium Chloride treatment and was orally administered 0.3 ml of a 200 mg/kg body weight of *Icacina trichatha* Extract daily for 28 days via gavage tube

Group F also received Aluminium Chloride treatment and was given 0.6 ml of a 400 mg/kg body weight of *Icacina trichatha* Extract daily for 28 days via gavage tube (Obazelu and Anyafulu, 2025).

3.9. Physical Examination of Animals

The animals were weighed periodically to monitor changes in body weight throughout the duration of 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 experiment, the animals were examined for general physical characteristics. After anesthesia with chloroform and cervical dislocation, a midline incision was made along the ventral wall of each rat. Using a sterile syringe, 5 ml of blood was drawn from each animal and transferred into Ethylene Diamine Tetra-acetic Acid (EDTA) containers for full blood count analysis. Bone marrow samples were collected by longitudinally opening the femur to expose the marrow cavity. The marrow was carefully removed with sterile forceps and placed in Eppendorf tubes 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-525 (Diamond Diagnostic; Holliston, USA). The equipment was calibrated and standardized, and the samples were processed and analyzed strictly in accordance with the manufacturer's instructions

3.11.1.1. Detection Principle of Haematology Autoanalyzer

The instrument counts and sizes blood cells by detecting and measuring changes in electrical resistance as each cell passes through a gem aperture sensor. The sample is diluted in a conductive liquid, and because blood cells are poor conductors, their passage through the aperture causes a momentary increase in electrical resistance. This resistance change is directly proportional to cell volume.

According to Ohm's law (U = RI; where U = voltage, I = current, R = resistance), if the current (I) remains constant, the voltage (U) increases with increasing cell size. This voltage

signal is then amplified by a magnifying circuit, background noise is removed, and the signal is sent for analysis.

White blood cells (WBCs) and red blood cells/platelets (RBCs/PLTs) are analyzed through separate circuits. The microprocessor unit (MPU) counts and calculates cell parameters, generating histograms. For platelet counting, the system uses an advanced combination of liquid, electronic, and software processing to minimize repeated counting of the same cell. If an RBC enters the platelet analysis area, it can generate a pulse similar to that of a platelet, and the system is designed to address this overlap.

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

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.

3.12. High Mobility Group Box-1 (HMGB-1) Gene Assay

3.12.1. Isolation of Total RNA

Total RNA was isolated from whole rat 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 (Elekofehinti *et al.,* 2020).

3.12.3. PCR amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of Caspase 1 and CYP11A1 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 95ºC for 5 min, followed by 30 cycles of amplification (denaturation at 95ºC for 30 s, annealing for 30 s and extension at 72ºC for 60 s) and ending with final extension at 72ºC 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

HMGB1

Forward primer: TGATTAATGAATGAGTTCGGGC

Reverse primer: TGCTCAGGAAACTTGACTGTTT

3.13. Statistical Analysis

Data generated from this study were analyzed using GraphPad Prism version 8.0 (California, USA). Differences among treatment groups for continuous variables were assessed using Analysis of Variance (ANOVA). Where ANOVA indicated statistical significance, Tukey's Honestly Significant Difference (HSD) post hoc test was performed. mRNA gene expression profiles were illustrated using bar charts. A p value of ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1. Results

Table 4.1 presents a detailed comparison of white blood cell (WBC) parameters expressed as Mean ± SEM across six experimental groups: Group A (Control), Group B (Aluminum chloride-induced anaemia), Group C ($AlCl_3$ + ferrous sulphate drug solution), and Groups D, E, and F ($AlCl_3$ + 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Icacina trichantha* extract, respectively). The total WBC count was numerically highest in Group B (7.5 ± 0.91), indicating a possible inflammatory response to $AlCl_3$-induced anaemia, and lowest in Group C (4.92 ± 0.51), which may reflect leukocyte suppression by the standard drug. The extract-treated groups (D, E, and F) showed intermediate WBC counts (6.28 ± 0.46, 5.88 ± 1.17, and 5.98 ± 0.57, respectively), suggesting a mild modulatory effect. However, statistical analysis using one-way ANOVA revealed no significant difference in total WBC counts among the groups ($F = 1.308$, $p = 0.2952$).

Lymphocyte percentages were generally high across all groups, with the lowest in Group B (89.2 ± 2.15) and highest in Group E (92.92 ± 1.06). This indicates that aluminum chloride may have caused a slight reduction in lymphocyte levels, which appeared to normalize with *Icacina trichantha* extract treatment. Nevertheless, the changes were not statistically significant ($F = 0.9954$, $p = 0.4423$). The MID cell percentages, which include monocytes, eosinophils, and basophils, were slightly elevated in Group B (8.52 ± 1.68) compared to other groups, particularly Group E (5.42 ± 0.72), but again the variation did not reach statistical significance ($F = 1.013$, $p = 0.4326$)..

Granulocyte percentages also showed no significant alterations across groups (F = 0.9438, p = 0.4717). Group C recorded the highest granulocyte percentage (2.42 ± 0.39), while Group D had the lowest (1.64 ± 0.17). The minor fluctuations observed across all WBC parameters indicate that while aluminum chloride may induce subtle immune changes and *Icacina trichantha* may exhibit mild regulatory effects, these were not strong enough to produce statistically significant hematological differences at the tested doses. Overall, this suggests that WBC profiles were relatively stable across experimental conditions, and the aqueous leaf extract of *Icacina trichantha* did not significantly affect systemic white blood cell response in aluminum-induced anaemia in Wistar rats.

Table 4.1. Mean Comparison of White Blood Cell Indices of Studied Groups

Parameters	Group A (control) n=10	Group B (**aluminium chloride only)** **n=10**	Group C ($AlCl_3$ + 40mg/kg ferrous sulphate drug solution) n=10	Group D (100 mg/kg dual blend of *Icacina trichantha*) n=10	Group E (200 mg/kg dual blend of *Icacina trichantha*) n=10	Group F (400 mg/kg dual blend of *Icacina trichantha*) 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. $p<0.05$ was considered significant

Figure 4.1 shows the body weight of groups A, B, C, D, E and F, representing control, aluminium chloride group, ferrous sulphate group, aluminium chloride + 100mg/kg *Icacina trichantha* leaf extract, aluminium chloride + 200mg/kg *Icacina trichantha* leaf extract, and aluminium chloride + 400mg/kg *Icacina trichantha* leaf extract respectively. While all groups exhibited weight gain, Group A demonstrated significantly higher weights compared to the other groups at Day 28 ($P<0.05$).

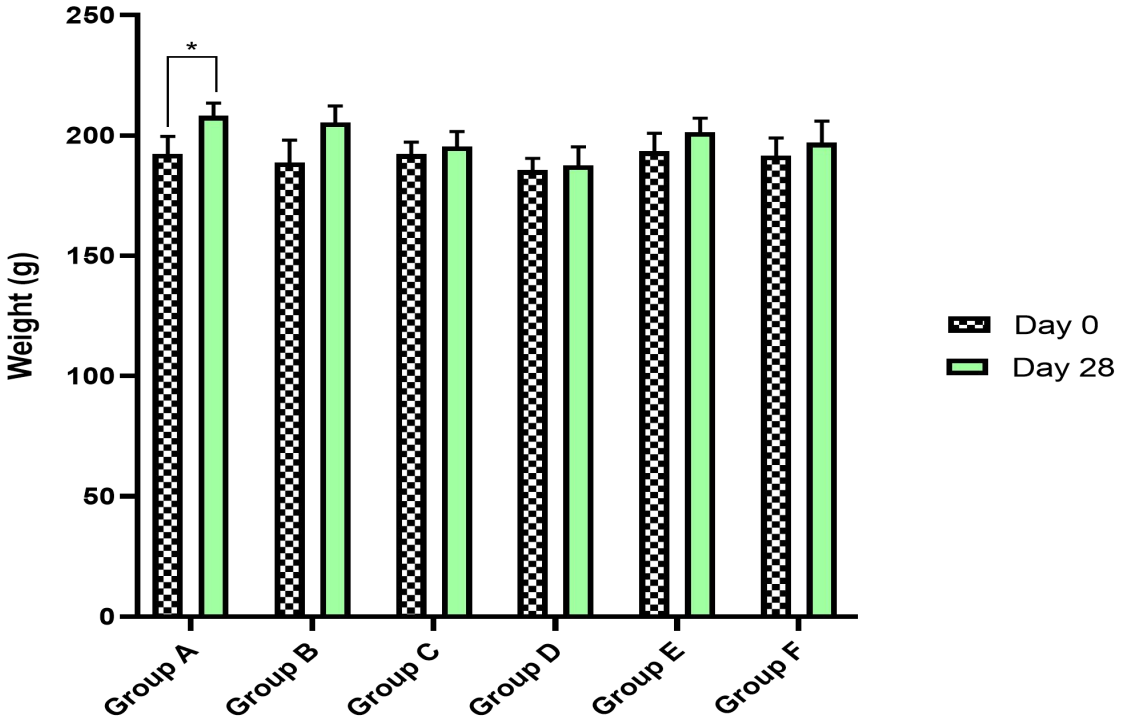


Figure 4.1: Body weights of Groups A, B, C, D, , E and F measured at Day 0and Day 28.

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

Table 4.2 shows the blood morphology of the studied groups namely; representing Group A (Control), Group B (Aluminum chloride-induced anaemia), Group C ($AlCl_3$ + ferrous sulphate drug solution), and Groups D, E, and F ($AlCl_3$ + 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Icacina trichantha* extract, respectively) measured at day 0 and 28. All groups showed the presence of both small and large (atypical) lymphocytes. Small lymphocytes were highly present in group A while it was moderately present in the other groups. Atypical

lymphocytes were highly present in the aluminum chloride induced group, moderately present in groups A, C and D and mildly present in groups E and F. Eosinophils was mildly present in group B, monocytes in groups C and F while basophils in group C only. All groups showed the presence or normocytic and normochromic red blood cells although they were only mildly present in the aluminum chloride induced group compared to the other groups which showed moderate and high presence of these red blood cells. Polychromatic and crenated red blood cells were mildly present in group B and C. Platelets were highly present in all groups.

Table 4.2. Blood Morphology of the Studied Groups

	LYMPH% (Small)	LYMPH% (Large)	EOS%	MON%	BAS%	NORMOCT CELLS %	NORMOCM CELLS %	POLYCMT CELLS %	CRENATED CELLS %	PLT % Normal
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 4.2 shows the expression of genes as represented by gel electrophoresis picture and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GADPH}) of mRNA expression of HMGB1 of groups A, B, C, D, E and F, representing Group A (Control), Group B (Aluminum chloride-induced anaemia), Group C ($AlCl_3$ + ferrous sulphate drug solution), and Groups D, E, and F ($AlCl_3$ + 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Icacina trichantha* extract, respectively) represented on different bars on the bar chart. There was a significant increase in the mRNA expression of HMGB1 of group C, D and E when compared to group A and B ($p<0.05$).

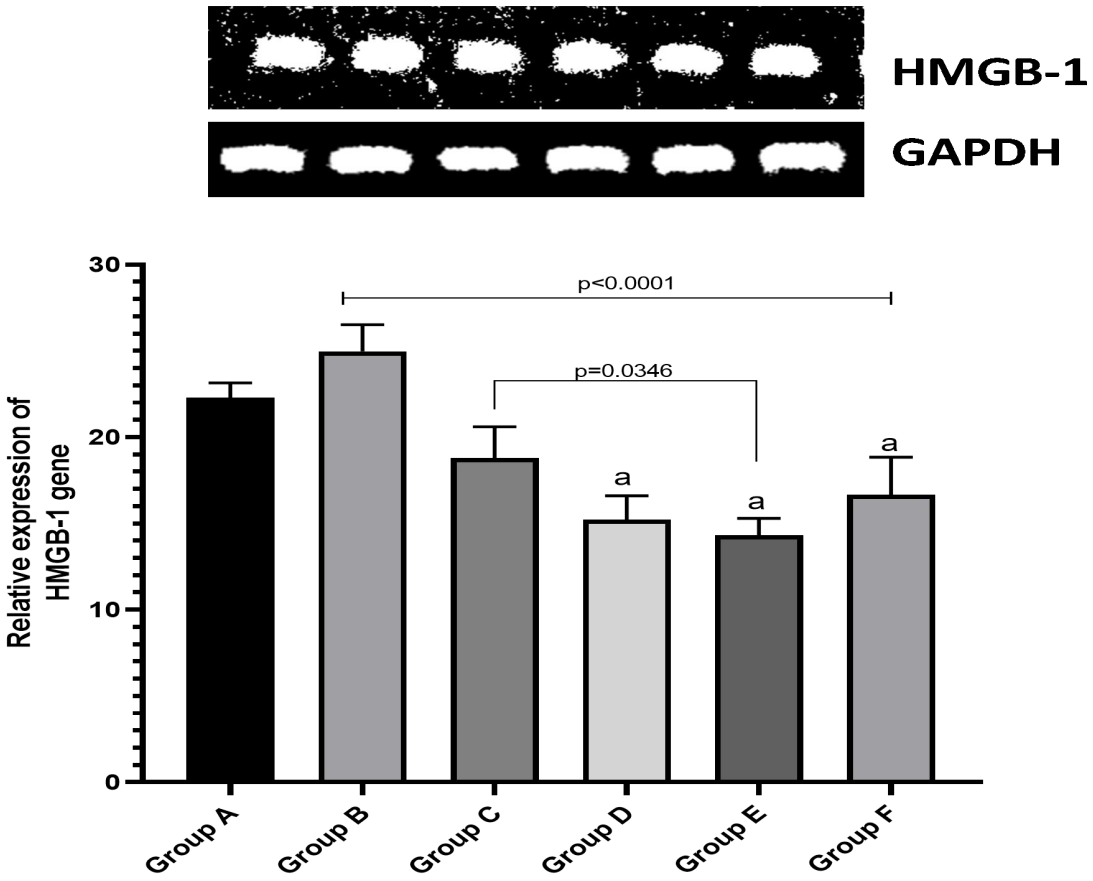


Figure 4.2: mRNA Expression of HMGB1of the Studied Groups.

(Figure shows mean±SEM. Error bar represents triplicate of each groups. $p<0.05$ was considered significant. a represents significance with group A)

Key: GADPH=Glyceraldehyde-3-Phosphate Dehydrogenase, HMGB-1=High Mobility Group Box-1

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Aluminum chloride ($AlCl_3$), a widely used industrial compound and neurotoxicant, has garnered significant attention for its detrimental effects on various organ systems, particularly the hematopoietic and immune systems (Yousef *et al.,* 2021). Prolonged exposure to aluminum salts has been linked to oxidative stress, Anaemia, and immunological disruptions, including alterations in leukocyte profiles and pro-inflammatory gene expression (Abubakar *et al.,* 2022). One such gene, high-mobility group box 1 (HMGB1), functions as a key mediator of inflammation and cellular stress responses and is increasingly recognized as a biomarker for tissue damage and systemic immune activation (Kang *et al.,* 2023). In recent year, research has focused on the therapeutic potential of plant-derived compounds. *Icacina trichantha* , a medicinal plant traditionally used in West Africa for its hematinic and immunomodulatory properties, has emerged as a promising candidate. This study assessed the hematological and immunological impacts of *Icacina trichantha* aqueous leaf extract on aluminum chloride ($AlCl_3$)-induced anaemia in Wistar rats, with emphasis on white blood cell indices, blood morphology, body weight changes, and HMGB1 gene expression. The results are discussed in detail below in relation to previous findings.

The total white blood cell (WBC) count was highest in the $AlCl_3$-only group (7.5 ± 0.91 $\times 10^9$/L), suggesting that aluminum exposure triggered an immune or inflammatory response. Conversely, the ferrous sulphate–treated group recorded the lowest WBC count (4.92 ± 0.51 $\times 10^9$/L), indicating potential drug-associated leukocyte suppression. Treatment with *Icacina trichantha* extract yielded intermediate values, suggesting mild immunomodulation, though

ANOVA revealed no statistically significant differences. These findings align with earlier reports that aluminum toxicity alters hematological parameters by inducing oxidative stress and inflammation. Osman *et al*. demonstrated that $AlCl_3$ exposure reduced red cell indices while also altering leukocyte levels in rats; *Moringa oleifera* supplementation partly restored these indices (Osman *et al.,* 2012). Similarly, Ameh and Alafi reported protective effects of *Moringa oleifera* ethanol extract on leukocyte counts in aluminum-induced *anaemia* models (Ameh & Alafi, 2018). The milder response in our study suggests that *Icacina trichantha* possesses less potent hematoprotective effects compared with these established medicinal plants. Lymphocyte percentages were slightly reduced in the $AlCl_3$ group but normalized in extract-treated groups, which implies an immune recovery effect of *Icacina trichantha* . This is consistent with Oyeniyi's findings that *Parquetina nigrescens* extract improved lymphocyte distribution in aluminum-induced anaemia, reinforcing the role of phytochemicals in immune regulation

Microscopic examination revealed the presence of atypical lymphocytes predominantly in the $AlCl_3$ group, while their presence was reduced in extract-treated groups. This supports the hypothesis that aluminum induces morphological abnormalities in immune cells through genotoxic and oxidative mechanisms. Kozima *et al*. demonstrated that aluminum exposure causes oxidative stress–mediated inflammation and atypical leukocyte formation in animal models (Kozima *et al.,* 2020). In this study, normocytic and normochromic red cells remained moderately or highly present across most groups, except in the $AlCl_3$ group where they were reduced, suggesting the onset of *anaemia*. This pattern is similar to reports by Osman *et al*. (2012), who observed reduced normocytic cells in aluminum-treated rats. The presence of polychromatic and crenated red blood cells in $AlCl_3$-only group and ferrous sulphate–treated group also indicates early signs of oxidative damage and red cell instability, consistent with oxidative stress–related pathology.

Although all groups exhibited weight gain over 28 days, the $AlCl_3$-treated group had significantly lower body weight compared to controls. Aluminum toxicity is known to impair appetite and metabolism, thereby reducing weight gain. Ameh and Alafi (2018) reported similar suppression of growth in aluminum-exposed rats, which was ameliorated by *Moringa oleifera* extract supplementation. The extract-treated groups in this study demonstrated partial improvement in weight gain, indicating some nutritional or metabolic support. However, the improvement was not as pronounced as in studies using *Moringa oleifera* or *Parquetina nigrescens*, suggesting that *Icacina trichantha* may have weaker bioactive compounds in relation to growth promotion.

One of the novel findings of this study is the significant increase in HMGB1 expression in ferrous sulphate–treated group, 100 mg/kg of *Icacina trichantha* group, and 200 mg/kg of *Icacina trichantha* group compared with control and $AlCl_3$-only groups. HMGB1 is a nuclear protein that acts as a pro-inflammatory mediator when released extracellularly, often serving as a biomarker of oxidative stress and tissue damage. Its upregulation in extract-treated groups could signify an adaptive immune priming mechanism rather than a direct suppression of inflammation. This result contrasts with reports where plant extracts generally attenuated pro-inflammatory cytokine expression in aluminum-induced stress models, Oyeniyi (2021) observed downregulation of inflammatory pathways with *Parquetina nigrescens*. The discrepancy suggests that *Icacina trichantha* may exert its protective effects via unique immunostimulatory pathways, potentially enhancing resilience against further oxidative insults rather than merely suppressing inflammation.

The overall findings suggest that while *Icacina trichantha* has modest hematoprotective and immunomodulatory effects, it is less effective compared with other medicinal plants such as *Moringa oleifera* and *Parquetina nigrescens*. Nevertheless, its ability to normalize

lymphocyte distribution, improve blood morphology, and alter HMGB1 expression indicates that the plant possesses bioactive compounds with potential therapeutic applications. Further studies are required to isolate these phytochemicals and elucidate the molecular pathways underlying the observed effects.

5.2 Conclusion

The present study evaluated the protective and modulatory effects of *Icacina trichantha* aqueous leaf extract on aluminum chloride–induced anaemia in Wistar rats. The findings demonstrated that aluminum chloride disrupted hematological parameters, induced atypical lymphocyte morphology, suppressed weight gain, and altered HMGB1 expression. Administration of *Icacina trichantha* extract produced modest improvements in white blood cell distribution, normalization of lymphocyte morphology, and partial recovery of body weight. Moreover, the significant upregulation of HMGB1 expression in extract-treated groups suggests that the plant may mediate its protective action through immune priming rather than direct suppression of inflammation.

5.3 Recommendations

From the findings of this study, several important recommendations can be made. First, further phytochemical investigations of *Icacina trichantha* are strongly encouraged in order to isolate, identify, and characterize the active compounds responsible for its observed hematological and immunomodulatory effects. Understanding its phytochemical profile will provide clearer insight into the bioactive molecules that contribute to its therapeutic potential. In addition, more detailed mechanistic studies should be carried out at the molecular and cellular level to establish how the extract influences immune responses and HMGB1

expression. Such investigations will help clarify whether its action is primarily through antioxidant activity, anti-inflammatory pathways, or immune-priming mechanisms.

REFERENCES

Abd, E. A. Y. M., Abu-Almaaty, A. H., Omar, N. A., and Abdeen, A. M. (2019). Evaluation of protective effects of propolis against aluminium silicate toxicity in rats. *Genetika,* 51(1):299–312.

Abu, O. D., Umar, A. B., and Eiremiokhae, C. O. (2023). Investigation of the cardio-protective capacity of aqueous extract of *Icacina trichantha* leaves in rats exposed to CCl_4. *Journal of Genetics and Cell Biology,* 6(1): 322-328

Adaka, I. C., Uzor, P. F., and Nwodo, N. J. (2021). Bioactivity-guided fractionation of *Icacina trichantha* Oliv. (Icacinaceae) tuber for antimalarial activity against *Plasmodium berghei* infected mice and GC-MS analysis. *Indian Journal of Traditional Knowledge,* 20(3):592–598.

Adedosu, O. T., Adeleke, G. E., Alao, T. A., and Ojugo, N. E. (2018). Effects of vitamin E administration on certain biochemical and antioxidants indices in some rat tissues treated with aluminium chloride. *Annual Research* and*Review in Biology*, 28(6):1–9.

Adelakun, T. A., Adebayo, A. H., and Salawu, S. O. (2020). Anti-inflammatory and antioxidant potential of *Icacina trichantha* leaf extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 17(3):14–22.

Adesipo, A. A., Akinbiola, S., Awotoye, O. O., and Salami, A. T. (2020). Impact of mining on the floristic association of gold mined sites in Southwest Nigeria. *BMC Ecology and Evolution,* 20(1):12.

Ahmad, A., Afzaal, M., Saeed, F., Ali, S. W., Imran, A., Zaidi, S. Y. R., … Al Jbawi, E. (2023). A comprehensive review of the therapeutic potential of citrus bioflavonoid hesperidin against lifestyle-related disorders. Cogent Food and Agriculture, 9(1).

Akinola, R., Pereira, L. M., Mabhaudhi, T., and De Bruin, F. M. (2020). A review of indigenous food crops in Africa and the implications for more sustainable and healthy food systems. *Sustainability, 12*(8):3493.

Akintunde, A., ndubuisi-ogbonna, L., Oyekale, O. O., Shobo, B., nimashaun, O. R., and Adewumi, G. A. (2024). Nutritional composition of *Parquetina nigrescens* leaf and effects of its aqueous extract in aluminium chloride-induced anaemic rats. *Asian Journal of Agriculture*, 8(2):95-103

Akinwumi, K. A., Odunola, O. A., andOlashore, H. O. (2011). The toxicity of methanolic extract of *Icacina trichantha* leaves in Wistar rats. *Journal of Food, Agriculture* and*Environment,* 9 (2): 145-147.

Akoh, O. U., and Mac-Kalunta, O. M. (2021). Phytochemical screening and identification of bioactive constituents of the chloroform extract of *Icacina trichantha* tuber peel Oliv. *Journal of Applied Sciences and Environmental Management,* 25(4):617–624.

Akpanyung, E. O., Bassey, U. E., Ndem, J. I., and Ekpo, D. E. (2020). *Pterocarpus mildbraedii* ameliorates aluminium chloride-induced alterations in renal and haematological indices in Wistar rats. *Journal of Pharmacognosy and Phytotherapy*, 12(2):31–40.

Alawode, T. T. (2024). Phytochemical, proximate and mineral analysis of leaves and tubers of *Icacina trichantha* . *Journal of Chemical Society of Nigeria, 49*(1):189–198.

Algridi, M. A., and Azab, A. E. (2021). Ameliorating effects of fenugreek seeds powder against hematotoxicity induced by aluminum chloride in male rabbits. *Biotechnology and Bioprocessing* 2(4):01-09

Alsawaf, S., Alnuaimi, F., Afzal, S., and Thomas, R. M. (2022). Plant flavonoids on oxidative stress-mediated kidney inflammation. *Biology*, 11(12):1717.

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. *Journal of Biotechnology and Biochemistry,* 4(6)34-52.

Anka, A. U., Usman, A. B., and Kaoje, A. N. (2022). Potential mechanisms of some selected heavy metals in the induction of inflammation and autoimmunity. *Cardiovascular Journal of Africa,* 33(3):122–128.

Behrouz, S., Saadat, S., Memarzia, A., and Sarir, H. (2022). The antioxidant, anti-inflammatory and immunomodulatory effects of camel milk. *Frontiers in Immunology*, 13(5): 331-342.

Bojanić, N., Milenković, J., and Stojanović, D. (2020). Pathophysiological mechanisms of aluminium toxicity. *Acta Medica Medianae,* 59(1):100-109.

Bolivar, B. E., Brown-Suedel, A. N., andRohrman, B. A. (2021). Noncanonical roles of caspase-4 and caspase-5 in heme-driven IL-1β release and cell death. *The Journal of Immunology, 206*(8), 1878–1886.

Bouasla, I., Bouasla, A., and Boumendjel, A. (2014). *Nigella sativa* oil reduces aluminium chloride-induced oxidative injury in liver and erythrocytes of rats. *Biological Trace Element Research*, 159(1–3):315–323.

Cai, S., Chen, M., Xue, B., Zhu, Z., Wang, X., and Li, J. (2024). Retinoic acid enhances ovarian steroidogenesis by regulating granulosa cell proliferation and MESP2/STAR/CYP11A1 pathway. *Animal Nutrition,* 10(1):112–120.

Ceramella, J., De Maio, A. C., Basile, G., Facente, A., and Scali, E. (2024). Phytochemicals involved in mitigating silent toxicity induced by heavy metals. *Foods,* 13(7):978.

Chakraborty, S., Pramanik, J., and Mahata, B. (2021). Revisiting steroidogenesis and its role in immune regulation with the advanced tools and technologies. *Experimental Hematology* and *Oncology,* 10(1):1–10.

Chan, Y., Allam, V. S. R. R., and Paudel, K. R. (2023). Nutraceuticals: Unlocking newer paradigms in the mitigation of inflammatory lung diseases. *Critical Reviews in Food Science and Nutrition*, 63(1):1–18.

Cheng, W., Chen, G., Zhang, X., and Chen, W. (2020). HMGB1 expression and oxidative stress in lung injury. *International Journal of Molecular Sciences*, 21(10):3790.

Chowra, U., Yanase, E., Koyama, H., and Panda, S. K. (2017). Aluminium-induced excessive ROS causes cellular damage and metabolic shifts in black gram (*Vigna mungo*). *Protoplasma,* 254(6):2215–2225.

Dey, M., and Singh, R. K. (2022). Neurotoxic effects of aluminium exposure as a potential risk factor for Alzheimer's disease. *Neurological Sciences and Neurosurgery,* 41(1)*:*105353.

Dinarello, C. A. (2018). *Overview of the IL-1 family in innate inflammation and acquired immunity*. *Immunological Reviews*, 281(1):8–27.

El-Boshy, M. E., Refaat, B., Qasem, A. H., and Khan, A. (2019). The remedial effect of *Thymus vulgaris* extract against lead toxicity-induced oxidative stress, hepatorenal damage, immunosuppression, and hematological disorders. *Environmental Science and Pollution Research, 26*(19):19868–19878.

Elekofehinti, O.O., Lawal, A.O., Ejelonu, O.C. Molehin O.R., 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. *J Diabetes Metab Disord,* 19(1):197-204

El-Gendy, A. M. (2011). Amelioration of Aluminium-intake oxidative stress by some antioxidants in male albino rats. *The Egyptian Journal of Hospital Medicine, 43*(1):494–504.

Esteves, F., Rueff, J., and Kranendonk, M. (2021). The central role of cytochrome P450 in xenobiotic metabolism a brief review on a fascinating enzyme family. *Journal of Xenobiotics, 11*(3):7.

Ezeala, I. C., Uzor, P. F., Obiora, C. U., and Enendu, C. M. (2023). Antimicrobial and antitrypanosomal activities of methanol root extract and fractions of *Brenania brieyi* (De Wild) Petit (Rubiaceae). *African Journal of Pharmaceutical Research* and*Development,* 15(2):34–41.

Ezeja, M. I., Anaga, A. O., and Asuzu, I. U. (2021). Antioxidant and hematopoietic activities of methanolic leaf extract of *Icacina trichantha* . *Journal of Ethnopharmacology,* 267(11):113505.

Feng, R., Chen, L., and Chen, K. (2021). Cytotoxicity and changes in gene expression under aluminium potassium sulfate on *Spodoptera frugiperda* 9 cells. *Ecotoxicology and Environmental Safety,* 208(9)*:*12594.

Fernandes, R. M., Eiró, L. G., and dos Santos Chemelo, V. (2021). Aluminum toxicity and oxidative stress. In *Environmental Contaminants and Neurological Disorders* pp.127-135

Ghazalee, N. S., Jantan, I., and Arshad, L. (2019). Immunosuppressive effects of the standardized extract of *Zingiber zerumbet* on innate immune responses in Wistar rats. *Phytotherapy Research,* 33(5):1257–1266.

Hassan, S. S., Abbas, S. Q., Muhammad, I., and Wu, J. J. (2022). Metals-triggered compound CDPDP exhibits anti-arthritic behavior by downregulating inflammatory cytokines and modulating oxidative storm in mice models. *Frontiers in Pharmacology,* 13(9):1053744.

Hazarika, S., Borah, P., Deb, P. K., Venugopala, K. N., and Hemalatha, S. (2023). Icacinaceae plant family: A recapitulation of the ethnobotanical, phytochemical, pharmacological, and biotechnological aspects. *Current Pharmaceutical Design,* 29(15):1154–1168.

Heo, H. R., Chen, L., An, B., Kim, K. S., and Ji, J. (2015). Hormonal regulation of hematopoietic stem cells and their niche: a focus on estrogen, *Int J Stem Cells.* 8(1):18–23.

Igbokwe, I. O., Igwenagu, E., and Igbokwe, N. A. (2019). Aluminium toxicosis: a review of toxic actions and effects. *Interdisciplinary Toxicology,* 12(1):45–51.

Ige, S. F., and Aiyeola, A. (2017). Effect of aqueous *Allium cepa* extract from red onion on aluminum chloride-induced Anaemia in female rats. *Journal of Applied Sciences and Environmental Management*, 21(1):56–60.

Isaac, I. O., Etesin, U. M., and Nya, E. J. (2022). *Ethnobotanical study, phytochemical composition and in vitro antioxidant activity of the methanol extracts of thirty-two medicinal plants from Southern Nigeria*. *Journal of Medicinal Plants Research,* 16(10):288-299

Joseph, O. S., Joseph, O. T., and El-Gazzar, A. M. (2024). Toxicological evaluation of ethanol leaf extract of *Pterocarpus santalinus* on lungs, stomach, brain and hematological parameters of Wistar rats. *Cogent Biology,* 10(1):2303828.

Karami, E., Goodarzi, Z., Shahtaheri, S. J., and Kiani, M. (2023). Extract of *Artemisia absinthium* L. stimulates HO-1/MT-1/CYP450 signaling pathway via oxidative stress regulation induced by aluminium oxide nanoparticles. *BMC Complementary Medicine and Therapies,* 23(1):310.

Kassa, S. T., Oyelowo, M. A., and Ezenwali, M. O. (2023). Modulatory effect of medicinal plants on oxidative stress and inflammatory gene expression in rats. *Journal of Ethnopharmacology*, 309(23):116311.

Keith, S. A. (2023). Steroid hormone regulation of innate immunity in *Drosophila melanogaster*. *PLoS Genetics,* 19(5):1010782.

Khong, D. M., Dudakov, J. A., Hammett, M. V., and Jurblum, M. I. (2015). Enhanced hematopoietic stem cell function mediates immune regeneration following sex steroid blockade. *Stem Cell Reports,* 4(4):707–720.

Kozima, E. T., Souza, A. B. F., & Castro, T. F. (2020). Aluminum hydroxide nebulization-induced redox imbalance and acute lung inflammation in mice. *Toxicology and Industrial Health,* 36(3):161–172.

Kumar, V., and Gill, K. D. (2014). Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *NeuroToxicology,* 41(23)154–166.

LaVoie, H. A., and King, S. R. (2009). Transcriptional regulation of steroidogenic genes: STARD1, CYP11A1 and HSD3B. *Experimental Biology and Medicine,* 234(8):880–907.

Lezhenko, H. O., Abramov, A. V., and Pohribna, A. O. (2019). The content of apoptosis mediators in children with Anaemia of inflammation acquired on the background of acute bacterial diseases of respiratory organs. *Scientific and Practical Journal of Medical Institute*, (5):25–30.

Mahassni, S. H., and Alsahafi, B. S. (2022). Aqueous extract of *Leptadenia pyrotechnica* modulates the immune system affecting white and red blood cell counts. *Jordan Journal of Biological Sciences,* 15(3):449–456.

Mahieu, S., Contini, M. C., Gonzalez, M., and Millen, N. (2000). Aluminum toxicity: Hematological effects. *Toxicology Letters,* 117(1):77–84.

Mello, F. V., Land, M. G. P., Costa, E. S., and Teodósio, C. (2019). Maturation-associated gene expression profiles during normal human bone marrow erythropoiesis. *Cell Death Discovery,* 5(1):14.

Mirkov, I., Stojković, D., and Aleksandrov, A. P. (2020). Plant extracts and isolated compounds reduce parameters of oxidative stress induced by heavy metals: an up-to-date review on animal studies. *Current Pharmaceutical Design,* 26(21):2536–2549.

Mohammed, A. N., and Obaid, A. K. (2025). Immunophysiological comparison for induced hepatocellular carcinoma treated by alcoholic extract and nano-extract of *Opuntia ficus indica*. *International Journal of Experimental Science*, 15(2):141–150.

Nikpour, M., Pellagatti, A., Liu, A., and Karimi, M. (2010). Gene expression profiling of erythroblasts from refractory anaemia with ring sideroblasts (RARS) and effects of G-CSF. *British Journal of Haematology,* 149(5):777–788.

Nna, V. U., Ujah, G. A., and suleiman, J. B. (2019). Aluminum-induced hematotoxicity: The ameliorative role of antioxidants. *Toxicology Reports,* 6(3):146–154.

Notariale, R., Infantino, R., andPalazzo, E. (2021). Erythrocytes as a model for heavy metal-related vascular dysfunction: the protective effect of dietary components. *International Journal of Molecular Sciences,* 22(12):6604.

Novaes, R. D., Mouro, V. G. S., and Gonçalves, R. V. (2018). Aluminum: A potentially toxic metal with dose-dependent effects on cardiac bioaccumulation, mineral distribution, DNA oxidation and microstructural remodeling. *Environmental Pollution,* 243(34):1742–1751.

Obani, I. S., and Anyachor, C. P. (2023). Preclinical and epidemiological musculoskeletal evidence of aluminium toxicity: A systematic review. *International Journal of Public Health,* 3(1):10-24.

Obazelu, P. A. and Abadaike-Elvis, N. O.(2024): Proinflammatory Effect of BiHerbal Formula of Picralima nitida and Cymbopogon citratusAqueous Leaf Extract In Phenyl Hydrazine Induced Anaemia inAlbino Wistar Rats.*BJMLS* 9(1):187194

Obazelu, P. A. and Abadaike-Elvis, N. O. (2024). Proinflammatory Effect Of Bi-Herbal Formula Of Picralima Nitida And Cymbopogon Citratus Aqueous Leaf Extract In Phenyl Hydrazine-Induced Anaemia In Albino Wistar Rats, Bayero Journal of Medical Laboratory Science, 9(1): 187- 194.

Obazelu P.A., and Anyafulu LC. Expression of BCL-2 and BAD Genes in Benzene-Induced Haematotoxicity Bearing Wistar Rats Treated with Bi- Herbal Formula of Picralima Nitida and Cymbopogon Citratus Extract *Afr J Lab Haem Transf Sci* 2025;4(2): 151 – 158.

Obazelu, P. A. and Efosa, G. (2025). Protective Effect Of An Herbal Mixture Extract On Aryl Hydrocarbon Receptor And Nadph Quinone Oxidoreductase-1 Expression In Benzene-Induced Blood Toxicity In Wistar Rats. *African Journal of Health, Safety and Environment,* 6(1):111-119.

Obazelu, P.A., and Evwaire, O. P 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. *Afr J Lab Haem Transf Sci* 2024;3(2): 138-146.

Obazelu PA, and Ezeonyebuchi MC. Expression bearing Wistar rats treated with a biherbal extract of Picralima nitida and of caspase-1 and CYP11A1 genes in benzene-induced hematotoxicity Cymbopogon citratus. *JNat Sci Med* 0;0:0.

Obazelu, P. A., Faluyi, O. (2023). Effect of Launaea TaraxacifoliaAqueous Leaves Extracts on ICAM-1 and VCAM-1 Gene Expressions in Benzene-Induced Haematotoxicity in Albino Wistar Rats. *J. Appl. Sci. Environ. Manage*. 27 (12) 28452852.

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., Ighomereho, S. and Ukatu, C. O. (2025).Expression of P13K And STAT3 Genes in Benzene Bone Marrow-Induced Toxicity in Wistar Rats Treated with A Dual-Blend Formula of Picralima nitida And Cymbopogon citratus Aqueous Leaf Extracts, *J Basic and Appl Med Sci* 2025, 5(1).

Obazelu P.A., and Osarinmwian, I. (2025). Gene Expression of Ifn- Gamma And Icam-1 in BenzeneInduced Haematotoxicity Bearing Wistar Rats Treated with a Bi- Herbal Mixture of Picralima Nitida and Cymbopogon Citratus Extract, Dutse *Journal of Pure and Applied Sciences,* 11 (2a):253-262.

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*, 9(1): 111– 120.

Ofem, O. E., Eno, A. E., Nku, C. O., and Antai, A. B. (2009). *Viscum album* (mistletoe) extract prevents changes in levels of red blood cells, PCV, Hb, serum proteins and ESR in high salt-fed rats. *Journal of Ethnopharmacology,* 123(1):16–22.

Ogunmoyole, T., Okoronkwo, C. A., and Adeyemi, O. S. (2020). Antioxidant and protective properties of *Moringa oleifera* in heavy metal toxicity: A review. *Journal of Pharmacognosy and Phytochemistry,* 9(1):202–209.

Oguro, H. (2019). The roles of cholesterol and its metabolites in normal and malignant hematopoiesis. *Frontiers in Endocrinology,* 10(1):204.

Ojah, E. O. (2020). Phytochemical investigation and antimicrobial activity of hexane, ethyl acetate and methanol fractions from stem bark of *Icacina trichantha* Oliv. (*Icacinaceae*). *Current Botany, 11*(3):65–72.

Ojatula, A. O., and Ogunwande, S. O. (2022). *Physicochemical screening and biosafety evaluation of extract of Oliver seed in Wistar albino rats Icacina trichantha . Educational Research Journal, 10*(3):182–191.

Okafor, P. N., Onwusonye, J. C., and Uche, I. (2022). Protective effects of *Icacina trichantha* leaf extract against cadmium-induced hematological alterations in rats. *Toxicology Mechanisms and Methods, 32*(5):391–398.

Okeke, U. B., Adeboye, O. M., and Adeniyi, F. R. (2023). A review on ethnobotany, phytochemistry, and pharmacology of *Microdesmis keayana* and *Microdesmis puberula* (Pandaceae). *Journal of Applied Pharmaceutical Science,* 13(5):7–13.

Okonkwo, C. J., Nwankwo, E. U., and Nwachukwu, O. (2019). Phytochemical and antioxidant activity of *Icacina trichantha* leaf extract. *Nigerian Journal of Herbal Medicine*, 23(4):112–119.

Okosodo, E. F., Ogundare, A., and Kolawole, O. (2021). Ethno-botanical survey of medicinal plants species used for the treatment of tropical diseases in Federal Polytechnic Ilaro. pp. 302 - 313

Olubomehin, O. O., Odubote, E. O., and Ogunmoye, A. O. (2024). Chemical constituents, antimicrobial and antioxidant activities of essential oils from fresh and air-dried leaves of *Icacina trichantha* (Oliv.). *Scientia Africana,* 23(1):1–14.

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

Onakpa, M. M., Ode, J. O., and Ajagbonna, O. P. (2016). In vivo and in vitro antioxidant effects of *Icacina trichantha* tuber extract. *Nigerian Veterinary Journal,* 37(3):173–178.

Orimisan, O. A., and Olakunle, T. F. (2023). Studies on In-vitro Bioactivity of *Icacina trichantha* Methanol Seed Extract. *International Journal of Science for Global Sustainability,* 9(1):13.

Osman, H. M., Shayoub, M. E., & Babiker, E. M. (2012). Effect of Ethanolic Leaf Extract of Moringa oleifera on Aluminum-induced Anaemia in White Albino Rats, *Jordan Journal of Biological Sciences* 5(4):255 - 260

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

Padonou, E. A., Teka, O., and Bachmann, Y. (2015). Using species distribution models to select species resistant to climate change for ecological restoration of bowé in West Africa. *African Journal of Ecology,* 53(1):103–111.

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

Quatrini, L., Ricci, B., Ciancaglini, C., and Tumino, N. (2021). Regulation of the immune system development by glucocorticoids and sex hormones. *Frontiers in Immunology,* (12): 672853.

Rezaee, N., Fernando, W. B., and Hone, E. (2021). Potential of Sorghum polyphenols to prevent and treat Alzheimer's disease: A review. *Frontiers in Aging Neuroscience*, 13(4):72-99.

Saad, H. M., Hassieb, M. M., Oda, S. S., and Tohamy, H. G. (2018). Histopathologic study on the toxic effect of aluminium chloride on the heart, liver and kidneys of rabbits. *Alexandria Journal of Veterinary Sciences,* 56(1):1–7.

Samir, A. M., and Rashed, L. A. (2018). Effects of occupational exposure to aluminium on some oxidative stress and DNA damage parameters. *Human* and*Experimental Toxicology,* 37(5):518–527.

Sandoval, C., Calle, Y., Godoy, K., and Farías, J. (2023). An updated overview of the role of CYP450 during xenobiotic metabolization in regulating the acute myeloid leukemia microenvironment. *International Journal of Molecular Sciences,* 24(7), 6031.

Sedik, A. A., Hassan, S. A., Shafey, H. I., and Khalil, W. K. B. (2023). Febuxostat attenuates aluminum chloride-induced hepatorenal injury in rats with the impact of Nrf2, Crat, Car3, and MNK-mediated apoptosis. *Environmental Science and Pollution Research,* 30(2):28182.

Shagal, M. H., and Kubmarawa, D. (2013). Antimicrobial and phytochemical screening of *Icacina trichantha* . *International Journal of Modern Botany,* 3(2):15–18.

Sharma, D. R., Sunkaria, A., Wani, W. Y., and Sharma, R. K. (2013). Aluminium induced oxidative stress results in decreased mitochondrial biogenesis via modulation of PGC-1α expression. *Toxicology,* 303(1):9–36.

Shirani, K., Hassani, F. V., and Razavi-Azarkhiavi, K. (2015). Phytotherapy of cyclophosphamide-induced immunosuppression. *Toxicology Letters,* 235(1):30–37.

Sinoriya, S. K., and Singh, K. (2024). Variations in hematological parameters during the treatment of carbon tetrachloride-induced hepatotoxicity with three different polyherbal formulations. *Journal of Stress Physiology* and*Biochemistry,* 20(4):*87-105*

Sofidiya, M. O., Odukoya, O. A., and Afolayan, A. J. (2021). Ethnobotany and pharmacognostic potential of *Icacina trichantha* . *South African Journal of Botany*, 137(12):90–97.

Tiwari, R., Latheef, S. K., Ahmed, I., and Iqbal, H. M. N. (2018). Herbal immunomodulators a remedial panacea for designing and developing effective drugs and medicines: current scenario and future prospects. *Current Drug Metabolism,* 19(2):169–180.

Turgut, S., Bor-Kucukatay, M., Emmungil, G., andAtsak, P. (2006). The effects of low dose aluminum on hemorheological and hematological parameters in rats. *Archives of Toxicology,* 80(12), 790–795.

Tyrkalska, S. D., Pérez-Oliva, A. B., Rodríguez-Ruiz, L., Martínez-Morcillo, F. J., Alcaraz-Pérez, F., and Martínez-Núñez, L. (2019). Inflammasome regulates hematopoiesis through cleavage of the master erythroid transcription factor GATA1. *Immunity,* 51(3):647–661.

Vota, D. M., Crisp, R. L., and Nesse, A. B. (2012). Oxidative stress due to aluminum exposure induces eryptosis which is prevented by erythropoietin. *Journal of Cellular Biochemistry,* 113(8):2736–2745.

Wang, Y., Ye, D., Zhang, F., Zhang, R., Zhu, J., and Wang, H. (2022). Cyp11a2 is essential for oocyte development and spermatogonial stem cell differentiation in zebrafish. *Endocrinology,* 163(2):258.

Yang, H., Wang, H., and Tracey, K. J. (2013). Targeting HMGB1 in inflammation. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1832(12):2485–2490.

Yazawa, T., Umezawa, A., and Miyamoto, K. (2012). Differentiation of pluripotent stem cells into steroidogenic cells: role of SF-1 regulator. In *Nuclear Receptors as Drug Targets* pp. 265–278.

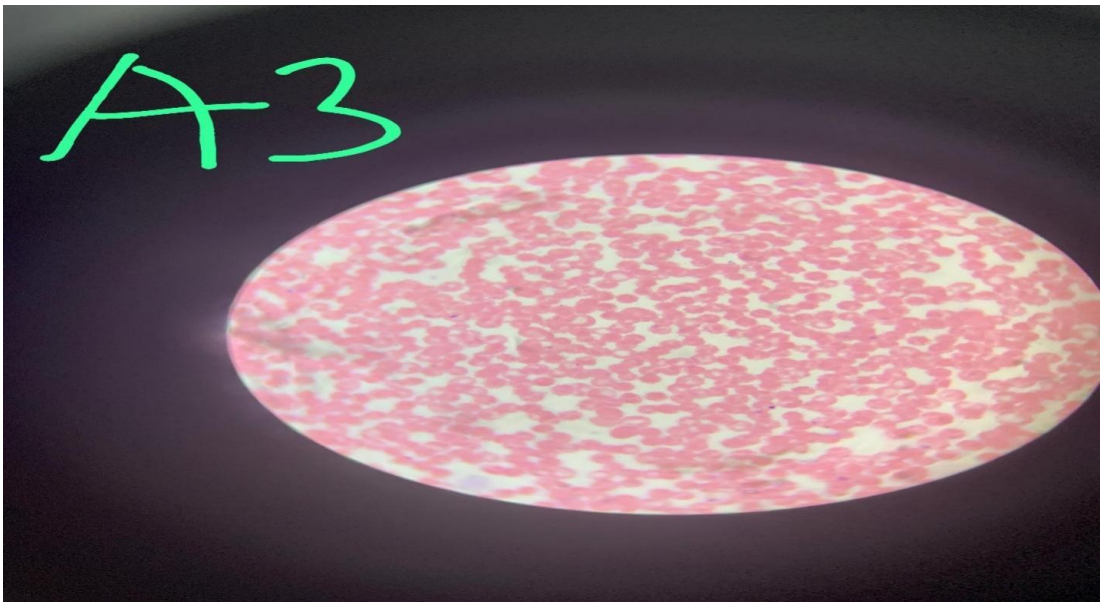
Zhang, Q., Cao, Z., Sun, X., Zuang, C., and Huang, W. (2016). Aluminum trichloride induces hypertension and disturbs the function of erythrocyte membrane in male rats. *Biological Trace Element Research*, 171(2):347–356.

Zhang, S., Sun, L., Zhang, J., Liu, S., and Han, J. (2020). Adverse impact of heavy metals on bone cells and bone metabolism dependently and independently through Anaemia. *Advanced Science,* 7(16):2000383.

Zhuang, S. R., Chen, S. L., and Tsai, J. H. (2009). Effect of citronellol and a Chinese medical herb complex on cellular immunity of cancer patients receiving chemotherapy/radiotherapy. *Phytotherapy Research, 23*(6):785–792.

APPENDIX I

Peripheral Blood Film slide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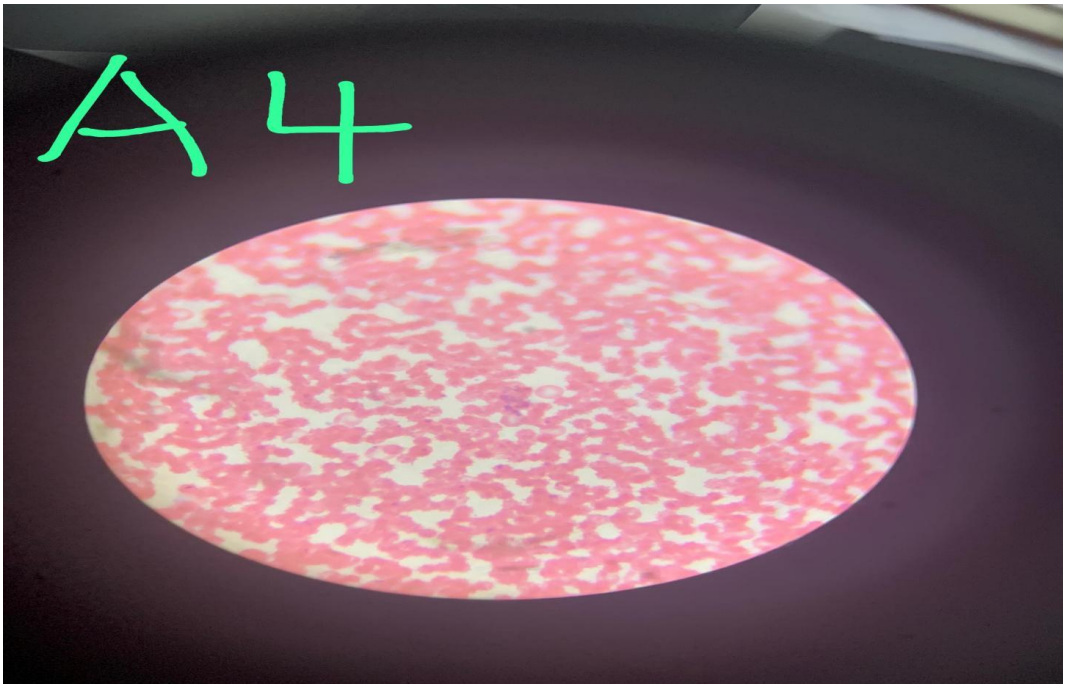
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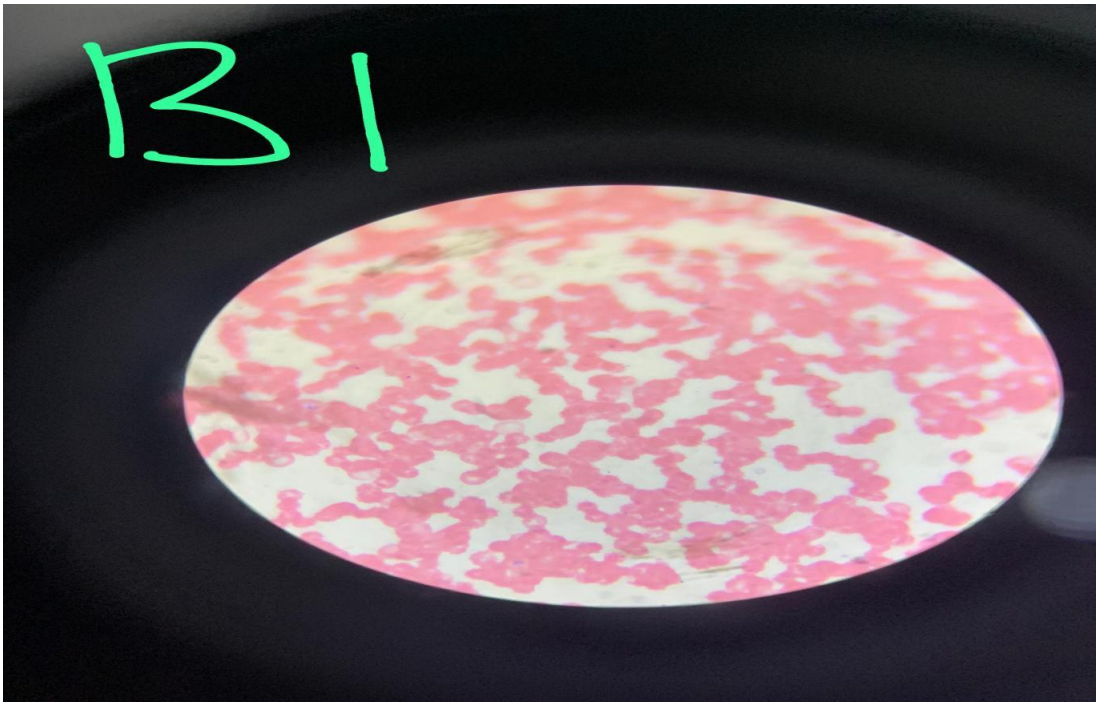
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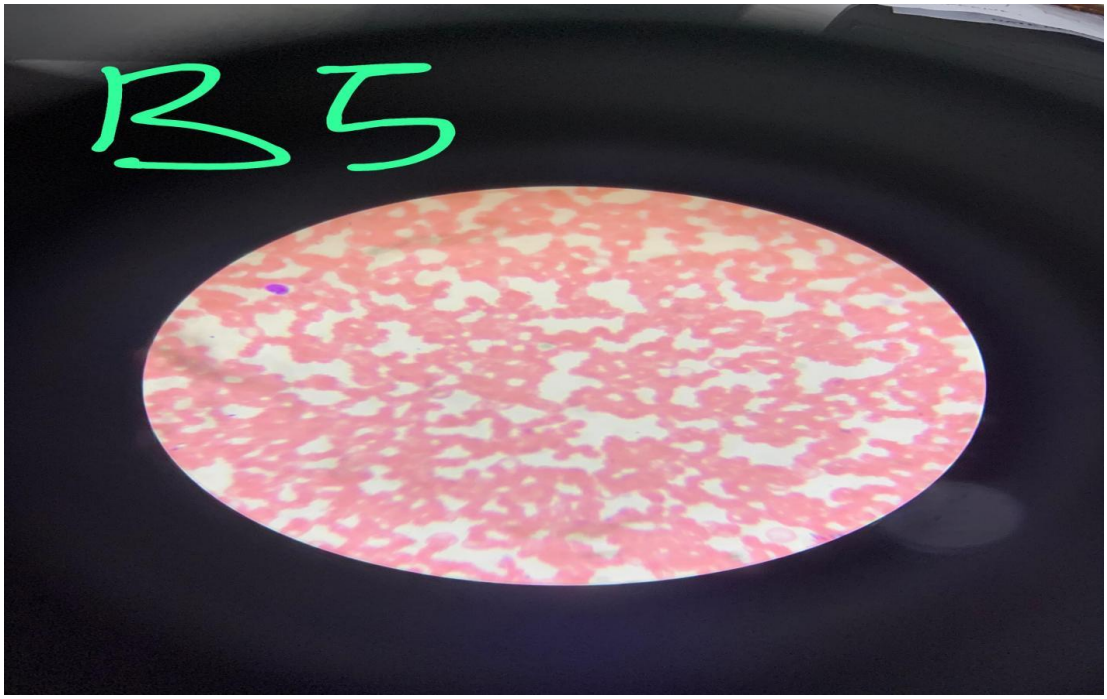
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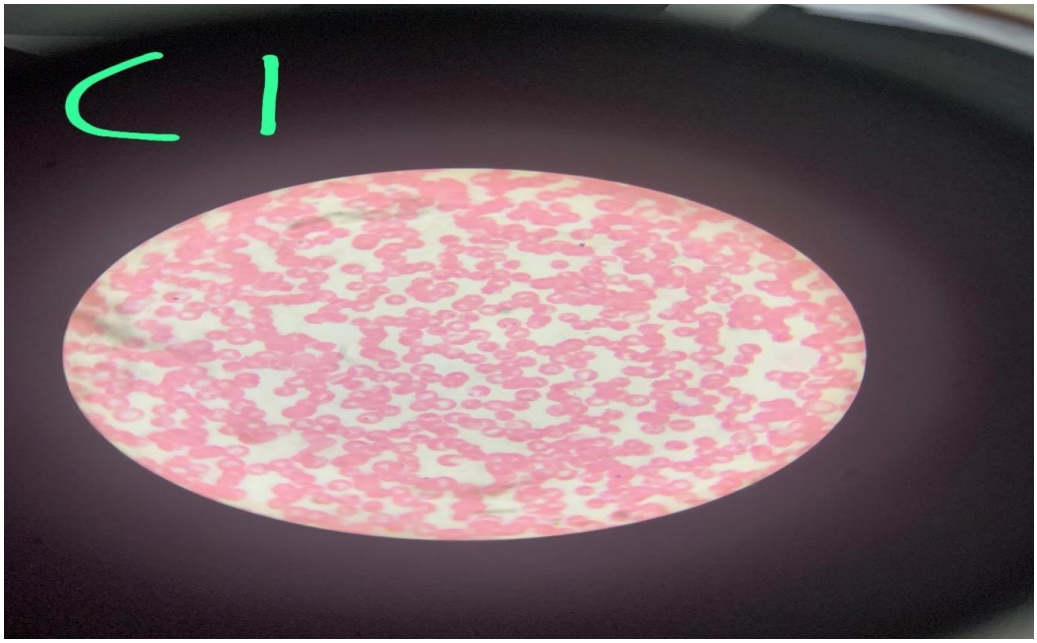
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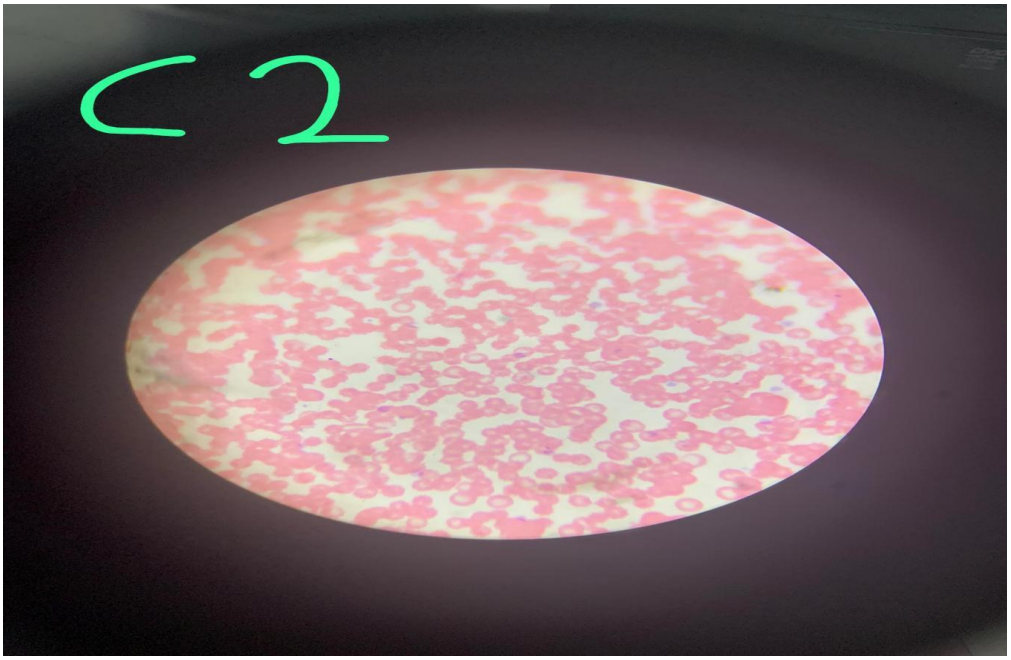
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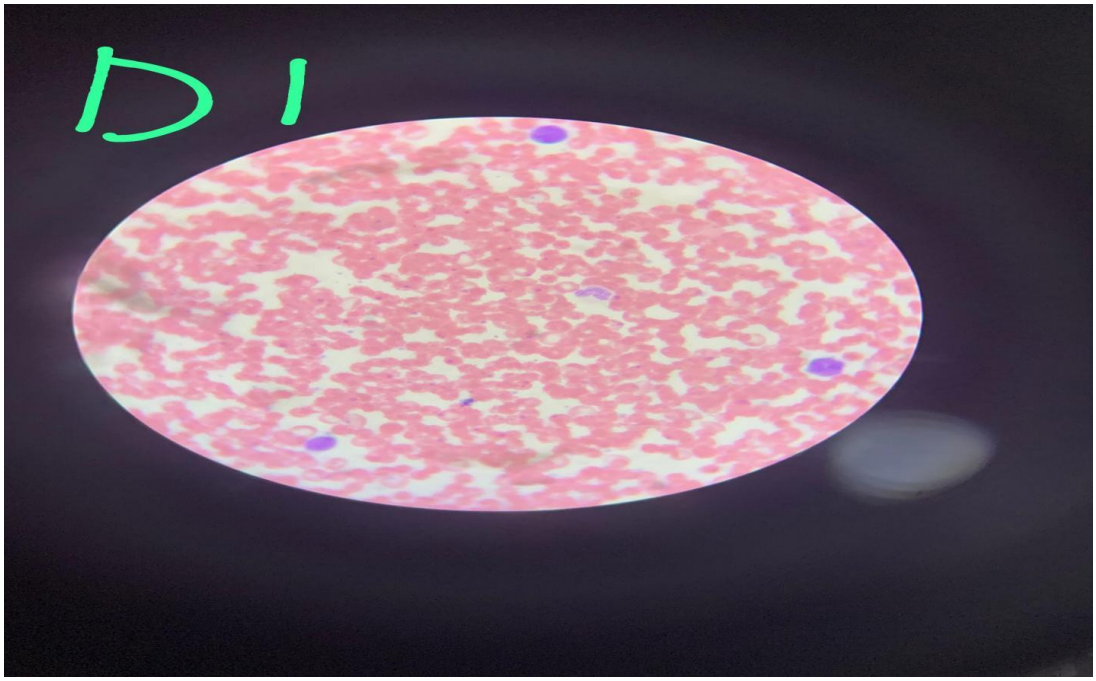
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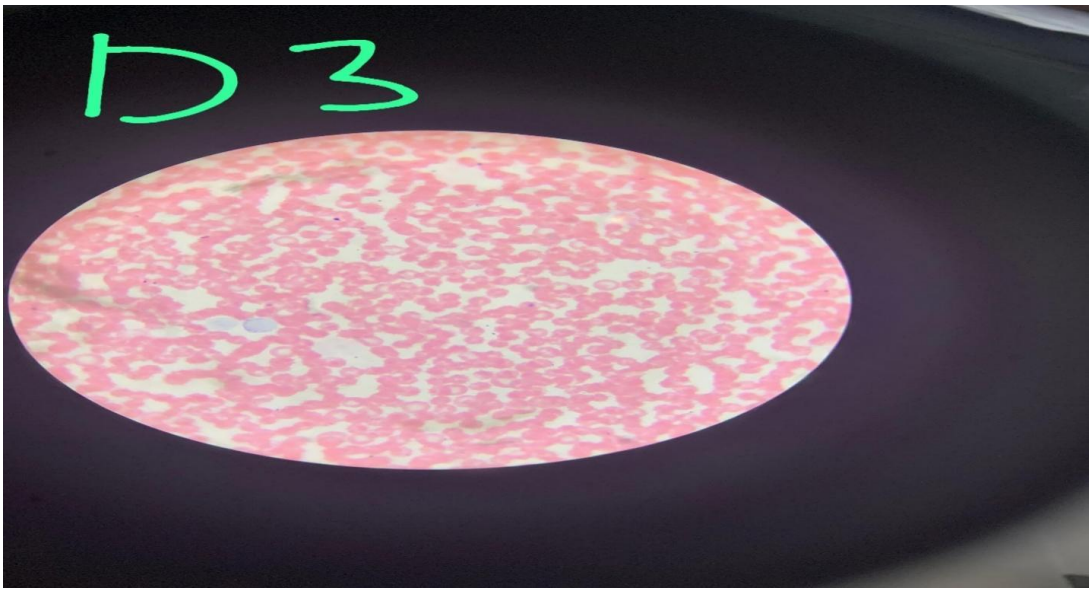
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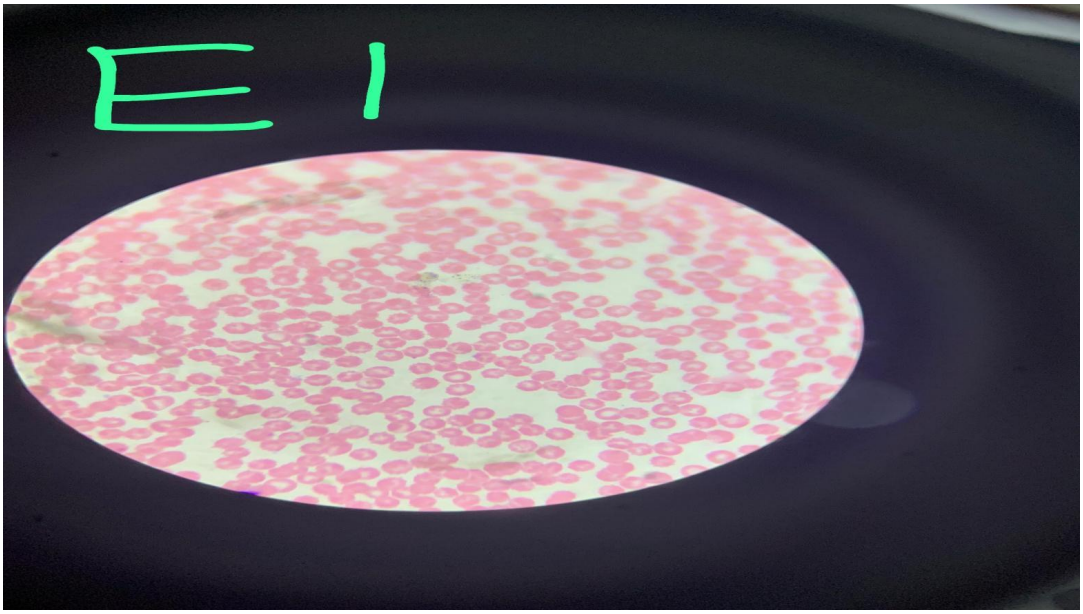
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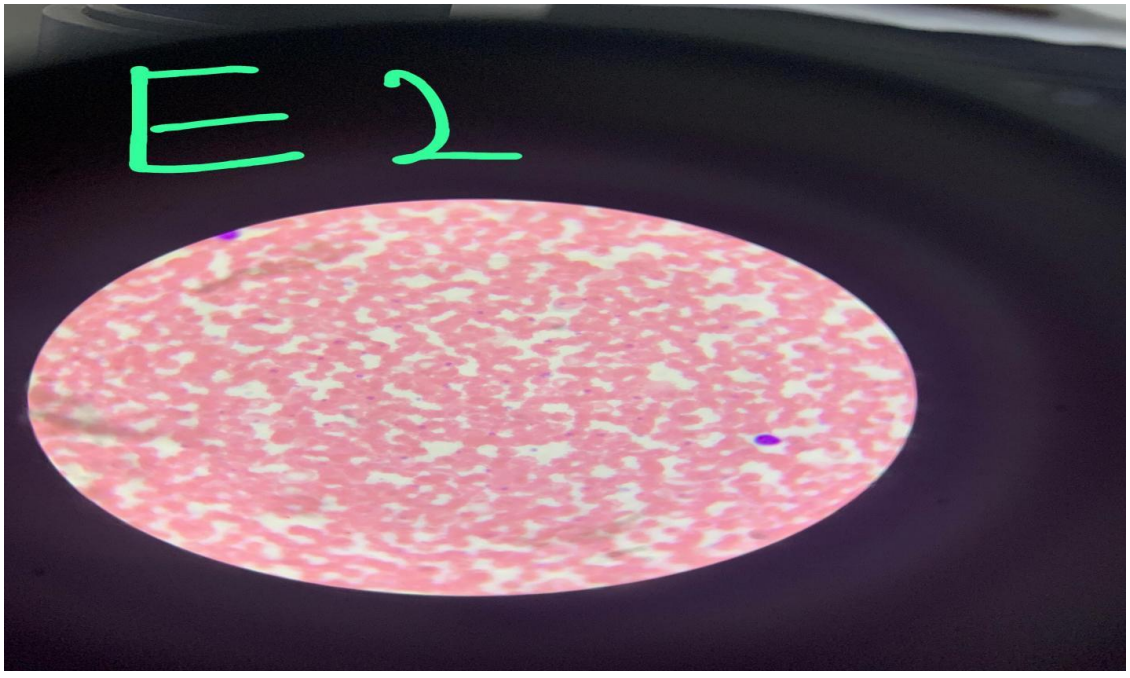
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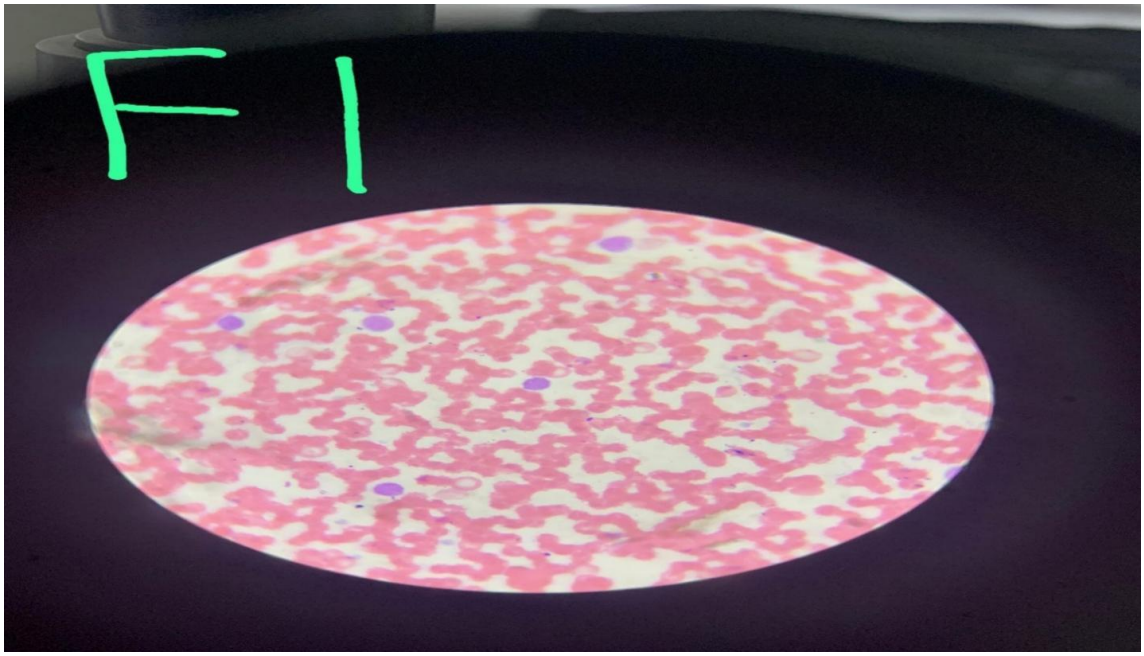
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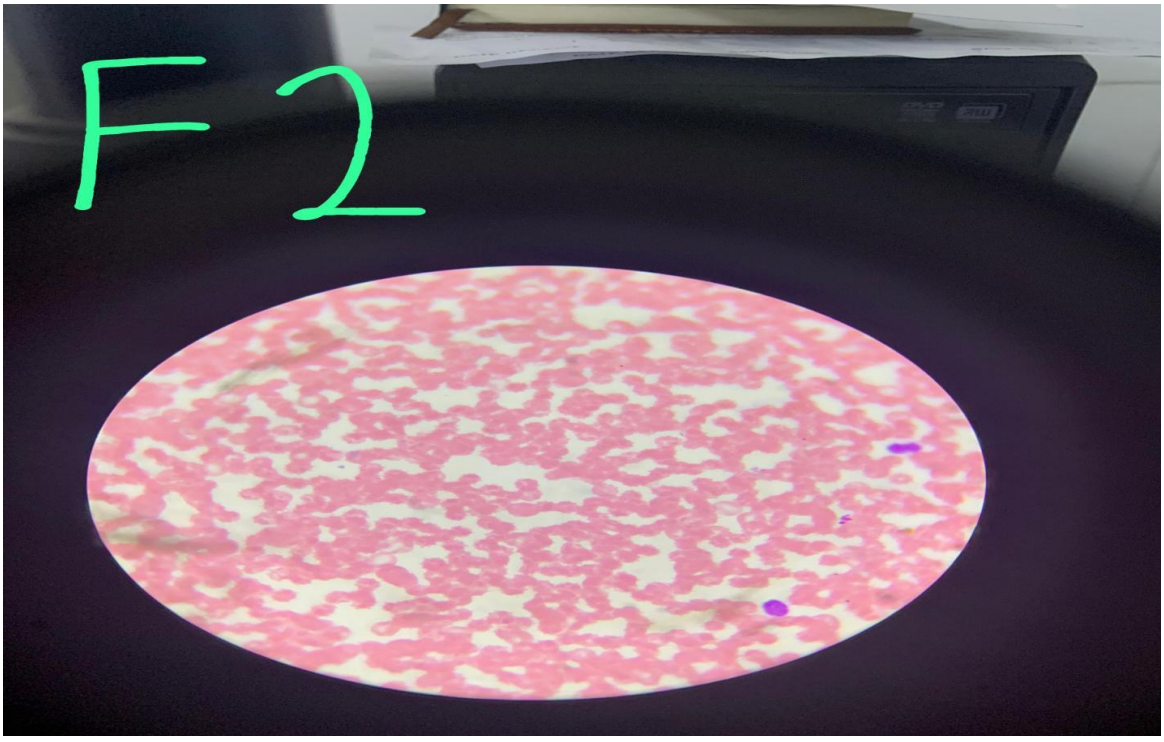
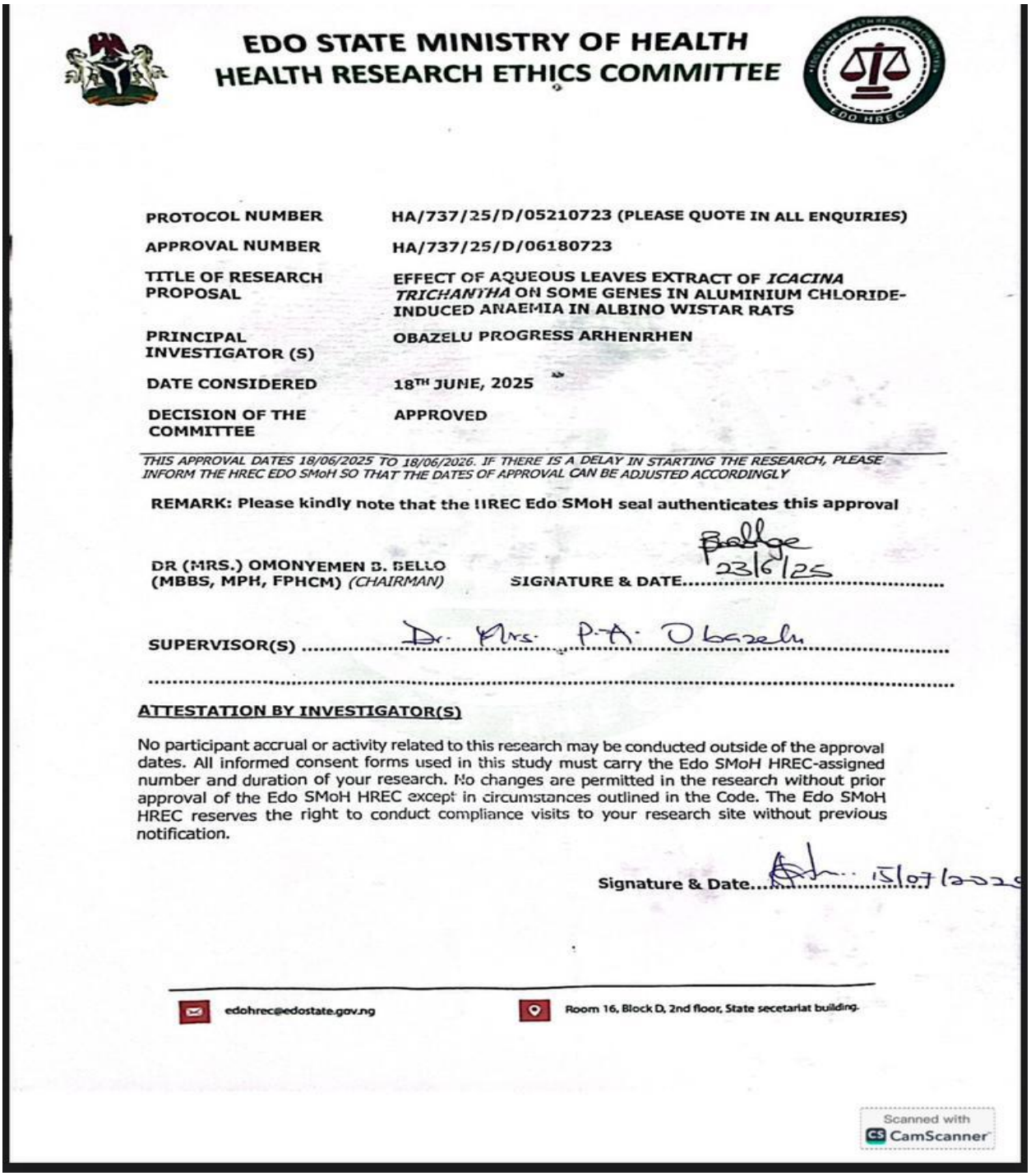

F2

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 secetariat building.

APPENDIX III

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 IV

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