EXPRESSION OF CYCLOOXYGENASE-1 GENE IN ALUMINIUM CHLORIDE-INDUCED ANAEMIA IN ALBINO WISTAR RATS TREATED WITH AQUEOUS LEAVES EXTRACT OF *Icacina trichantha*

BY

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BMS1908769

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN.

OCTOBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE (BMLS) DEGREE IN THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE.

OCTOBER, 2025

CERTIFICATION

This is to certify that this project write up is an authentic work carried out by OLAWOLE, BLESSING FUNMILOLA with the matriculation number BMS1908769 under the supervision of DR. (MRS.) P.A. OBAZELU in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree of the department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo state.

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EXTERNAL EXAMINER

DEDICATION

I dedicate this project work to God almighty for making this a huge success and also to my lovely Family and Friends for their love and support during the course of this study.

ACKNOWLEDGEMENT

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

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ABSTRACT

Icacina trichantha, known for their medicinal use possessing bioactive compounds with anti-inflammatory and hematopoietic properties, offer promises for novel treatments. This study explores the effect of the aqueous leave extract on Cyclooxygenase-1 (COX-1) expression to elucidate the molecular mechanisms driving its therapeutic action. Therefore, the aim of this study is to determine the effect of *Icacina trichantha* aqueous leaves extract on Cyclooxygenase-1 expression in Aluminum Chloride-Induced Anaemia in Albino Wistar Rats. A total of sixty (60) adult male albino Wistar rats were divided into six (6) groups; A, B, C, D, E and F representing control, aluminum chloride group, ferrous sulphate group, aluminum chloride + 100mg/kg leaf extract of *Icacina trichantha*, aluminum chloride + 200mg/kg leaf extract of *Icacina trichantha* and aluminum chloride + 400mg/kg leaf extract of *Icacina trichantha* groups respectively. 5 milliliters (ml) of blood sample was drawn from each rat, and haematological parameters and mRNA of COX-1 were determined using a SRFI Haematology autoanalyzer and polymerase chain reaction respectively. Data obtained was analyzed using the GraphPad prism 8.02 software. The comparison of Haematological parameters amongst the study groups showed that there was no significant difference in Total white blood cell count (TWBC μL) across the groups. Platelet Distribution Width (PDW) was significantly lower in group E (8.96±0.27) when compared to group D (10.6±0.31) ($p<0.05$). There was no significant difference in other platelet parameters across the groups. Group C and D showed significantly lower expressions of COX-1 when compared to group B ($p<0.05$). Group C, D and E had significantly lower expression of COX-1 when compared to group F ($p < 0.05$). Groups E had significantly higher expressions of COX-1 when compared to group A, and significantly lower expression when compared to group B ($p<0.05$). Groups F had significantly higher expressions of COX-1 when compared to group A ($p<0.05$). In conclusion, administration of aluminum chloride resulted in no significant difference in white blood cell counts. There was also no alteration in platelet parameters, except PDW which showed slight difference. Administration of leaf extract of *Icacina trichantha* led to alteration in the gene expression of COX-1.

CHAPTER ONE

IINTRODUCTION

1.1 Background of Study

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times (Gershenzon and Ullah, 2020). Plants have been utilized for their therapeutic properties for centuries, with various cultures harnessing the healing power of nature to treat ailments and promote well-being (Obazelu and Gaius-Igboanugwo, 2024). Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, against parasites and herbivorous mammals. The importance of medicinal plants extends beyond traditional uses. They are natural reservoirs of bioactive compounds that have led to the development of many modern pharmaceuticals (Gershenzon and Ullah, 2020). Medicinal plants contain a wide range of bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which can have therapeutic effects on the human body. Recent studies have demonstrated that these alkaloids can modulate significant enzymatic and receptor-mediated processes, suggesting potential protective mechanisms against oxidative and cytotoxic damage in mammalian systems (Obazelu *et al*., 2025). These compounds can exhibit various pharmacological properties, such as anti-inflammatory, antimicrobial, analgesic, antioxidant, and anticancer activities (Obazelu and Osazee, 2024). They can also influence a wide range of biological processes, such as modulating signaling pathways, Regulating gene expression, and altering protein synthesis and activity (Obazelu and Ezeonyebuchi, 2025).

Icacina trichantha Oliv. (Icacinaceae), a drought-resistant shrub indigenous to West and Central Africa, is a medicinal plant used by the natives such as the indigenous tribes in Nigeria. It is known as “Urumbia” or “Eriagbo” (referring to its emetic effect) among the Igbos of Nigeria, or “Gbegbe” (meaning to cleanse) by the Yoruba of western Nigeria (Asuzu and Abubakar, 1995). Icacina trichantha is a shrub that can grow up to two meters high. The fruit of this plant is a drupe with a soft sweet outer pulp which is edible (Burkill, 1994). In western Nigeria and neighboring areas, I. *trichantha* is used as a common household medicine for emergency and first-aid treatment for food poisoning (Mbatchou, 2012). Tubers and leaves of the plant are allegedly aphrodisiacs (Burkill *et al.,* 1994). The leaves and seeds, when crushed and macerated in local gin, can be used for the treatment of hypertension and asthma (Ajibesin *et al.,* 2008). In vitro screening of the leaf, wood, and root parts of I. *trichantha* exhibited moderate levels of antioxidant activity in the 2,2-diphenyl-picryl-hydrzyl radical assay (Udeh and Nwaehujor, 2011). Antioxidant activity of the leaf was correlated to total phenol contents (Sofidiya *et al.,* 2006) and a hexane extract was also claimed to be active in three almost identical reports (Otun *et al.,* 2015). Phytochemical screening of the leaf extract of I. *trichantha* has shown the presence of alkaloids, tannins, phenols and saponins. Fatty acid components such as stearolic acid, oleic acid and erucic acids were identified (Otun *et al.,* 2015). In the tuber, the presence of alkaloids, tannins, saponins, steroids, carbohydrates and cardiac glycosides has been detected (Edori *et al.,* 2015).

Cyclooxygenase 1 (COX-1), also known as prostaglandin-endoperoxide synthase 1 (HUGO PTGS1), is an enzyme that in humans is encoded by the *PTGS1* gene. COX-1 is one of two main cyclooxygenase isozymes, the other being COX-2, and both play crucial roles in the biosynthesis of prostanoids, including prostaglandins and thromboxanes, from arachidonic acid

(Funk *et al.,* 1991). COX-1 is primarily responsible for maintaining physiological homeostasis. It regulates several critical processes, including Angiogenesis in endothelial cells, cell signaling pathways, and maintenance of tissue homeostasis, such as gastric mucosal protection and platelet aggregation. Unlike COX-2, which is inducible and mainly associated with inflammation, COX-1 is present in nearly all tissues and supports essential housekeeping functions. (Fitzpatrick, 2004).

COX-1 is constitutively expressed and is responsible for the biosynthesis of PGs involved in various housekeeping functions, such as the regulation of renal, gastrointestinal, and platelet function (Smith *et al.,* 1996).

Aluminum (Al) is one of the toxic trace elements, to both humans and animals. Humans are exposed to aluminum through diet (salt, herbs, spices, corn etc.) (Yousef, 2004), drinking water, cosmetics, cookware utensils, containers, antacids, vaccines and tooth paste (Abbasali et al., 2005). There is no known physiological role of aluminum in the living tissues; however, exposure to excess aluminum produces adverse physiological effects. Aluminum is a known environmental toxicant that can cause neurological diseases (Yousef, 2004), reproductive dysfunctions such as, damage of the ovarian structure, ovulation inhibition and testicular dysfunction (Wang et al., 2012; Fu et al., 2014; Ighodaro et al., 2012). It is also known to induce anemia (Mahieu et al., 2000).

Reduction in erythropoietin synthesis and secretion, and inhibition of intestinal absorption of iron has been proposed as mechanisms of aluminum-induced anemia (Kalaiselvi et al., 2015) with increase hemolysis and reduce heme and globulin syntheses reported due to aluminum toxicity. Uremia is usually seen with aluminum-hematotoxicity (Mahieu et al., 2000). Likewise,

cardiovascular diseases, cognitive impairment and reduction in quality of life are associated with anemia in kidney diseases (Wang et al., 2012). Erythropoietin deficiency is the predominant cause of anemia in kidney diseases (Babitt and Lin, 2012) due to the fact that kidney is the main source of erythropoietin. So also, oxidative stress has been implicated in the genesis and progression of kidney diseases (Jha et al., 2016), long-chain polyunsaturated fatty acids present in the kidney enhance the oxidative stress-induced kidney disease.

1.2 Justification of Study

Anaemia remains a significant public health concern, especially in developing regions, where exposure to environmental toxins such as aluminum chloride contributes to its prevalence. Aluminum chloride ($AlCl_3$), a widely used industrial compound, has been implicated in systemic toxicity, including hematotoxic effects. It interferes with haemopoiesis by damaging the bone marrow and impairing red blood cell production, which can lead to anaemia. Furthermore, aluminum toxicity is associated with inflammatory responses and oxidative stress, potentially altering the expression of key regulatory enzymes such as Cyclooxygenase-1 (COX-1). COX-1 plays a vital role in maintaining physiological functions such as platelet aggregation, gastrointestinal protection, and renal blood flow. Changes in its expression during anemia may contribute to or exacerbate disease pathology. *Icacina trichantha*, a medicinal plant widely used in traditional medicine in West Africa, is reported to possess anti-anemic, antioxidant, and anti-inflammatory properties. However, scientific validation of its effects, especially its ability to modulate COX-1 expression during chemically-induced anemia, is lacking, which necessitate this study. This study therefore seeks to elucidate the effect of aluminum chloride on hematological parameters and COX-1 expression and investigate the therapeutic potential of *Icacina trichantha* leaf extract in treating anemia and restoring COX-1 balance.

1.3 Aim of Study

The aim of this study is to investigate the expression levels of the cyclooxygenase-1 (COX-1) in an experimental model of anaemia induced by aluminium chloride in Albino Wistar rats, and to evaluate the therapeutic effects of the aqueous leaf extract of *Icacina trichantha* on this condition.

1.4 Specific Objective

1. To determine the effect of *Icacina trichantha* leaf extracts on Cyclooxygenase-1 (COX-1) gene expression in Aluminum Chloride-induced Anaemia in Albino Wister Rats.
2. To determine the effect of *Icacina trichantha* on haematological parameters in Aluminum Chloride-induced Anaemia in Albino Wistar Rats.
3. To observe the effect of *Icacina trichantha* aqueous leaf extracts on the morphology of blood cells in Aluminum Chloride-induced anaemia in albino Wistar rat.

1.5 Research Questions

1. Does *Icacina trichantha* leaf extract have any effect on Cyclooxygenase-1(COX-1) gene expression in Aluminum Chloride-induced Anaemia in Albino Wistar rats ?
2. Does *Icacina trichantha* leaf extract have any effect on haematological parameters in Aluminum Chloride-induced Anaemia in Albino Wistar rats?
3. Does *Icacina trichantha* aqueous leaves extract have any effect on the morphology of blood cells in aluminium chloride-induced anaemia bearing wistar rats?

1.6 Research Hypothesis

1.6.1 Null Hypothesis (Ho)

1. *Icacina trichantha* aqueous leaf extract does not have any effect on Cyclooxygenase-1 (COX-1) gene expression in aluminum chloride-induced anaemia in Albino wistar rats.

2. *Icacina trichantha* aqueous leaf extract does not have any effect on haematological parameters in aluminum chloride-induced anaemia in albino wistar rats.
3. *Icacina trichantha* aqueous leaf extract does not have any effect on the morphology of blood cells in aluminium chloride-induced anaemia bearing wistar rats.

1.6.2 Alternate Hypothesis (HA)

1. *Icacina trichantha* aqueous leaf extract has an effect on Cyclooxygenase-1 (COX-1) gene expression in Aluminum Chloride-induced Anaemia in Albino Wistar rats.
2. *Icacina trichantha* aqueous leaf extract has *an* effect on haematological parameters in Aluminum Chloride-induced Anaemia in Albino Wistar rats.
3. *Icacina trichanta* aqueous leaf extract has an effect on the morphology of blood cells in aluminium chloride-induced anaemia in albino wistar rats.

1.7 Scope of Study

This study was designed to cover the effect of *Icacina trichantha* on some molecular pro-inflammatory biomarkers and haematological parameters.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of *Icacina trichanta*

Icacina trichantha Oliv., from the Icacinaceae family is a perennial shrub commonly found in field crops, forest regrowth and waste areas in most parts of Nigeria. It can grow up to 2 m with scandent growth above having leaves that are simple, alternate and broadly elliptic while the stem is straggling, semi-wood, round in cross-section, with soft brown hairs and ascends from an underground tuber that also has soft brown hairs (Agyakwa and Akobundu, 1998). The Yoruba people of Nigeria call it 'Gbegbe' while among the Igbo it is called 'Ibugo' (Burkhill, 1985). The plant is widely used in rural areas and regarded as a major handy household medicine for emergency treatment; hence, almost all households have the macerated tuber in ethanol stored in corked bottles, while the Igbo people regard the plant as an aphrodisiac (Burkhill, 1985). The leaves are used by the Yoruba people during the coronation of their chiefs called 'Obas' (Asuzu and Egwu, 1998), and also as a wrapper for processed oil bean seeds known as 'ugba' in Igbo (Asuzu and Abubakar, 1995). The tuber has been widely used traditionally in the treatment of constipation, as a poison antidote, to induce emesis, and to cure malaria (Asuzu and Abubakar, 1995; Timothy and Idu, 2011). The plant is reported to Become a weed of rice-padis in former Bendel state presently Edo and Delta states. The tuber is inflammable and when Burning gives

out so fierce a heat as to be unapproachable. Though the root is edible, a strong presence of alkaloid and Benzophenone has been reported, the leaf contain a trace only. The Kernel appears to be not edible (Burkill, 1985).

False yam, Icacina trichantha tubers analyzed for proximate composition revealed presence of carbohydrate 91.93% and proteins 5.25% (Sunday et al., 2016). The nutritional contents of the seeds include: 13% moisture, 72% carbohydrate, 8 – 10% proteins, 0.1% fat (Fay, 1991). The leaves are thinly pilosed with simple, fascicled hairs beneath while flowers are densely crowded and subsessile with calyx nearly as long as the petals which are usually villous outside; the fruits are tomentose on the surface, ellipsoid to globose in shape measuring up to 2.5 cm in length (Hutchinson and Dalziel, 1958; Akobundu and Agyakwa, 1998). The leaf epidermis properties of *Icacina trichantha* include paracytic and diacytic stomatal types, irregular epidermal cell shape together with angular or curved anticlinal wall patterns (Kadiri et al., 2020).

Figure 2.1: Leaves of *Icacina trichantha* (Wahua *et al.,* 2024).

Table 1: Some Species of *Icacina trichanta* According to International Board for Plant Genetic Resources (IBPGR, 1992).

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

Table 2: Taxonomy of *Icacina trichanta* (Akoegninou *et al.*, 2006).

Domain	Eukaryota
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Sub-class	Magnoliidae
Order	Icacinales
Family	Icacinaceae
Genus	*Icacina*
Species	*Icacina trichantha*

2.1.1. Uses of Some Plant Parts of the *Icacina trichanta*

• **Tubers:** Tubers are used by traditional healers to treat various medical conditions including constipation, poisoning, malaria, rheumatism, toothache, as well as to induce emesis and abortion (Ariwaodo *et al*., 2012). The tuber juice can be used for treating mumps (Ubom, 2010).

• **Root:** The thick yam-like root attains a large size. Some native people say that the root is edible alone, or can be dried and pounded to a white powder, called gbẹ-wutu, which is used in soups, or added to a food known as ìgbãlò made from the roasted seeds of watermelon (Citrullus lanatus). Others treat it as a famine-food eating the flour only after prolonged maceration and repeated washings. Some native people say that the root is edible alone, or can be dried and pounded to a white powder, called gbẹ-wutu, which is used in soups, or added to a food known as ìgbãlò (Burkill, 1994).

• **Leaves:** Icacina trichantha leaves contains bioactive compounds of potentially therapeutic significance and thus could be a promissory candidate for drug development and validates folkloric claim by the traditional healers (Mohammed *et al.,* 2013). It likely contain compounds with membrane-stabilizing activities. These categories of compounds are known to interfere with the early stages of inflammatory reactions and prevent the release of phospholipase that triggers the formation of inflammatory mediators (K.H *et al.,* 1996).

• **Seeds:** The seed of Icacina trichanta contains biochemical compounds which may therefore justify both its antioxidant and antimicrobial agent of natural origin.

2.1.2. Biochemical and Phytochemical Constituents of *Icacina trichantha*

The phytochemical screening results for the extracts shows that hexane, ethyl acetate, and methanol extracts of the leaves and tubers of *Icacina trichantha* revealed a wide array of phytoconstituents. The hexane extract of the tuber tested positive for saponins, flavonoids, and alkaloids. Conversely, the ethyl acetate extract exhibited the presence of tannins, resins, saponins, alkaloids, and terpenoids. These variations are attributable to the different polarities of the solvents used for extraction: hexane is non-polar, ethyl acetate has medium polarity, and methanol is polar. These bioactive compounds function as free radical scavengers or antioxidants and have significant anti-inflammatory, anti-diabetic, anti-aging, antibacterial, anti parasitic, antidepressant, anti-cancer, antioxidant, and wound-healing properties (Obazelu and Osarinmwian, 2025). Consequently, the phytoconstituents are distributed across these extracts according to their polarities. Furthermore, the phytochemical profiles of the leaves differ significantly from those of the tuber. For instance, the hexane extract of the leaves

tested positive for a greater number of phytochemicals compared to the hexane extract of the tuber (Alawode, 2024).

2.1.3. Antimicrobial Activity of *Icacina trichantha*

The phytochemicals present in both ethanol and water extracts of the tested plant part is an indication that the plant is of pharmacological importance. Studies showed the antimicrobial activities of the ethanol and water extracts of the leaves of *Icacina trichantha*. Both extracts were active against all the tested microorganisms. The inhibition of growth of the tested microorganisms by the extracts has justified their use for the as traditional Medicine. The results obtained in this study indicate that the ethanol extract had preferable antimicrobial activity than the corresponding water extract, except for Streptococcus spp in which the water extract is more than the ethanol extract. The ability of the extracts to inhibit the growth of microorganisms shows a broad spectrum activity. The results also showed that Escherichia coli and Staphylococcus aureus were more susceptible to the Ethanol extract than the water extract followed by Klebsiella pneumonia, Streptococcus spp and Candida Albican. This is similar to the report of Timothy *et al* (Mohammed *et al.,* 2013).

2.1.4. Antioxidant and Anti-inflammatory activity of *Icacina trichantha*

Antioxidants are compounds that help protect the body against oxidative stress caused by free radicals (Obazelu and Omoregie, 2024). Antioxidants neutralize free radicals by donating electrons, thereby stabilizing them and preventing further damage to cells (Obazelu and Williams, 2024). A very high correlation was observed between the phenolic contents of the extracts and their antioxidant activities. The large concentration of phenolic compounds in methanol extracts of the leaves and tuber could be responsible for their high antioxidant properties. Synthetic

antioxidants such as Butyl Hydroxyl Anisole (BHA) and Butylhydroxyl Toluene (BHT) have been widely employed to slow down diseases arising from oxidative stress. Reactive oxygen species have been implicated in the development and progression of a large number of diseases including cancer, inflammation, cardiovascular and neurodegenerative diseases. However, concerns have been raised on their toxicities, hence the increasing attention being given to the search for natural antioxidants (Wiseman *et al*., 2002). The methanol extracts of the plants under study could also be sources of potent antioxidants. All the tuber extracts had a membrane stabilizing activity comparable to that of Indomethacin at 0.5 mg/ml but lesser activities at lower concentrations. The ethylacetate leaf extract of *Icacina trichantha*, ICLEE has membrane stabilizing activity of 92.69 ± 3.15 at 0.1 mg/ml and 83.82 ± 1.50 at 0.3 mg/ml. Value of 87.63 ± 2.07 was obtained for the methanol extract of the leaves of *Icacina trichantha* at 0.5 mg/ml. These values were higher than that obtained for Indomethacin at these concentrations (Chagas-Paula *et al.,* 2011). The leaves of *Icacina trichantha* likely contain compounds with membrane-stabilizing activities. These categories of compounds are known to interfere with the early stages of inflammatory reactions and prevent the release of phospholipase that triggers the formation of inflammatory mediators (Baser *et al.,* 1996).

2.2. Inflammation

Inflammation is a systemic response to pathogen challenge and injury. It is characterized by the influx of inflammatory cells (e.g., macrophages and neutrophils), induction of vasoconstriction, edema (swelling), erythema (redness), and sensitivity to pain. The logic of inflammation is to defend against the invading pathogen by conducting chemical warfare while limiting damage to the region of the initial infection. Ultimately, inflammatory lesions resolve, and local wounds

heal. Acute inflammation is a critical element of host defense, and individuals with genetic mutations that disable the inflammatory response are at elevated risk of infection (Seger and Curr, 2011). Inflammation is the body's intricate response to harmful agents, such as pathogens, toxins, or injury (Obazelu and Efosa, 2025). Although acute inflammation is beneficial to the organism (albeit perhaps painful and annoying), chronic inflammation contributes to the etiology of many diseases. There are many mediators generated during an inflammatory response. Some contribute to the toxicological events that kill the invading pathogen, whereas others recruit additional inflammatory cells to the site of the lesion, induce vasoconstriction, or promote resolution and wound healing. Oxidized lipids, particularly those derived from polyunsaturated fatty acids, contribute to all of these events (Serhan and Haeggstrom, 2010).

2.2.1. Process of Inflammation

The inflammatory response is initiated upon tissue damage which triggers activation of endothelial cells present in blood vessel walls. Epithelial cells in the damaged tissue initiate the enzymatic breakdown of phospholipids that are found in the plasma membrane, thereby causing their conversion to arachidonic acid, which is responsible for the formation of pro-inflammatory molecules such as leukotrienes and prostaglandins (Wang *et al.,* 2021).

2.3. Mediators of Inflammation

2.3.1. Prostaglandin

Prostaglandins (PGs) are defined as active lipid compounds, with functions similar to hormones in animal cells. Like leukotrienes, PGs belong to the eicosanoids, compounds formed by twenty carbon atoms. Prostaglandins differ from leukotrienes, as they are formed by the action of the enzyme cyclooxygenase (COX) on arachidonic acid (Miller, 2006). The production of

prostaglandins begins with the release of arachidonic acid from cell membrane phospholipids through the action of phospholipase A2 in the inflammatory site. Arachidonic acid is then converted into an intermediate product called PGH 2 by the action of COX (Wilson *et al.,* 2004). This type of prostaglandin has a few functions in the body, yet serving as a substrate for biosynthesis of PGs with several distinct biological activities. Thus, PGH 2 is converted by prostaglandin synthases, in specific cells, into various derivatives such as PGD2, PGE2, PGF2a, prostacyclin (PGI2), and thromboxanes (TX) (Ricciotti and FitzGerald, 2011). Prostaglandins are involved in several pathophysiological processes such as: homeostasis, thrombosis, angiogenesis, cancer, and ulcers. They are also responsible for tissue inflammation and development of the cardinal signs. Sensations of pain, for example, occur thru PGE2 receptor activation in sensory neurons (Sykes, 2014). PGs are also involved in immunosuppression. In vitro studies have shown that the PGE2/cAMP mediator suppresses differentiation in TH1 cells (Aoki and Narumiya, 2012).

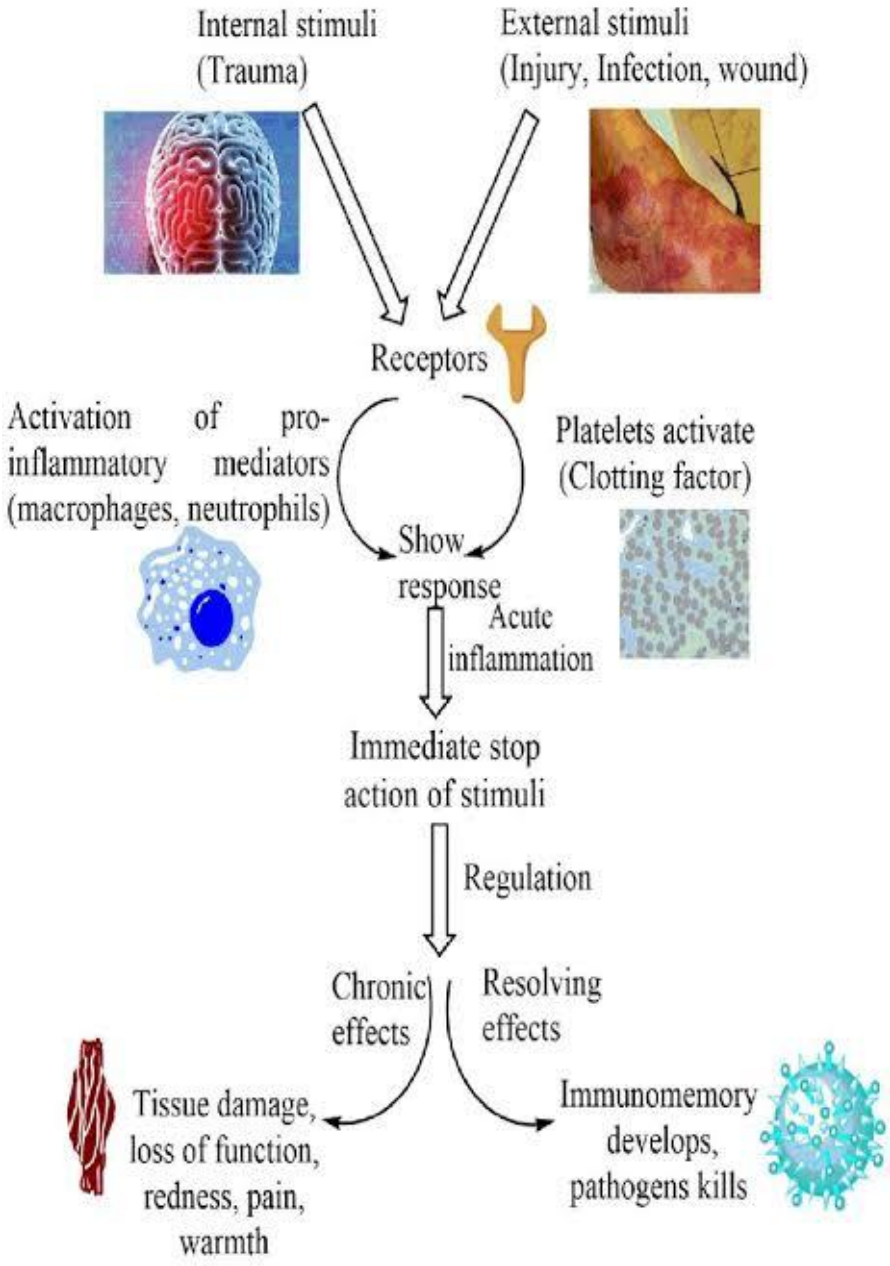

Figure 2.2: Acute and Chronic Inflammation in mammal (Muzamil *et al*., 2021).

2.3. Cyclooxygenase 1

Cyclooxygenases (COXs), also known as prostaglandin H synthases or prostaglandin endoperoxide synthases are fatty-acid oxygenases of the myeloperoxidase superfamily that are most closely related to the pathogen-inducible oxidases and linoleate diol synthases of plants and fungi (Daiyasu and Toh, 2000). The purification of COX-1 (then called simply COX) from sheep (Hemler *et al.,* 1976) and bovine (Miyamoto *et al.,* 1976) seminal vesicles in 1976 led to the cloning of the COX-1 gene in 1988 (DeWitt and Smith, 1988). For many years, it was thought that the constitutively active COX-1 protein was the only cyclooxygenase in eukaryotic cells, but in 1991 a second, inducible enzyme was identified through studies of cell division; this second enzyme is now called COX-2 (Xie *et al*., 1991). All vertebrates investigated, including cartilaginous fishes, Bony fishes, birds, and mammals, have two COX genes: one encoding the constitutive COX-1 and another the inducible COX-2. COX-1 and COX-2 share approximately 60-65% Amino-acid identity with each other; COX-1 orthologs (without the signal peptide) share approximately 70-95% amino-acid identity across vertebrate species and COX-2 Orthologs share 70-90%. Additionally, coral (of the phylum Cnidaria) and sea squirt (ascidian) each have two COX genes, which may have arisen from gene-duplication events independent from those that produced vertebrate COX-1 and COX-2 [9]. It is clear that the vertebrate, coral and ascidian COX genes all descend from a common ancestor.

2.3.1. Structure and location of Cyclooxygenases

COXs are all close to 600 amino acids in size and have a similar primary structure (Graviton *et al*., 2002). The crystal structure of sheep COX-1 (minus the post-translationally cleaved signal peptide), was obtained in 1994 (Picot *et al.,* 1994); human and mouse COXs have since been crystallized and show strikingly similar features (Luong *et al.,* 1996). After the signal peptide,

the amino terminus of the protein contains a single epidermal growth factor (EGF) module with conserved disulfide bonds that functions as a dimerization domain. This is followed by a series of four amphipathic helices that anchors the protein to one leaflet of the membrane. This 'monotopic' type of insertion into a membrane has been found only in this enzyme and a few other proteins such as squalene cyclase and S-mandelate Dehydrogenase (Wendt *et al.,* 1997). The remainder of the protein consists Of the catalytic domain, which has two distinct cyclooxygenase and peroxidase active sites. COXs are highly conserved, and few significant differences are seen in the dimerization, membrane-binding and catalytic domains between COXs from different species. The aminoterminal hydrophobic signal peptides differ significantly in length between species. In the case of two splice variants of Canine COX-1, the signal peptide has been found not to be Cleaved from the enzyme when expressed in insect cells (Chandrasekharan *et al.,* 2002).

COX-1 is ubiquitously and constitutively expressed in mammalian tissues and cells, whereas COX-2 is highly inducible and is generally present in mammalian tissues at very low levels, unless increased by one of many types of stimuli such as cytokines and growth factors. Both COXs are largely located on the lumenal side of the endoplasmic reticulum (ER) membrane and the nuclear envelope, although they have also been detected in some situations in lipid bodies, mitochondria, filamentous structures, vesicles and in the nucleus (Bozza *et al.,* 1997; Liou *et al.,* 2001; Coffey *et al.,* 1997). The lumen of the ER is important for both the structure and function of COXs: Its oxidative potential allows formation of the disulfide bonds of the enzymes, and N-linked glycosylation – which occurs in the ER – appears to be necessary for proper protein folding (Otto *et al.,* 1993). Moreover, the final product of COXs, Prostaglandin H2, is sufficiently non-polar to diffuse through the membrane of the ER to isomerases located on the

cytosolic surface of the ER or in the cytosol. Lipid bodies may provide a similar environment, but the role of COXs in the nucleus is unknown.

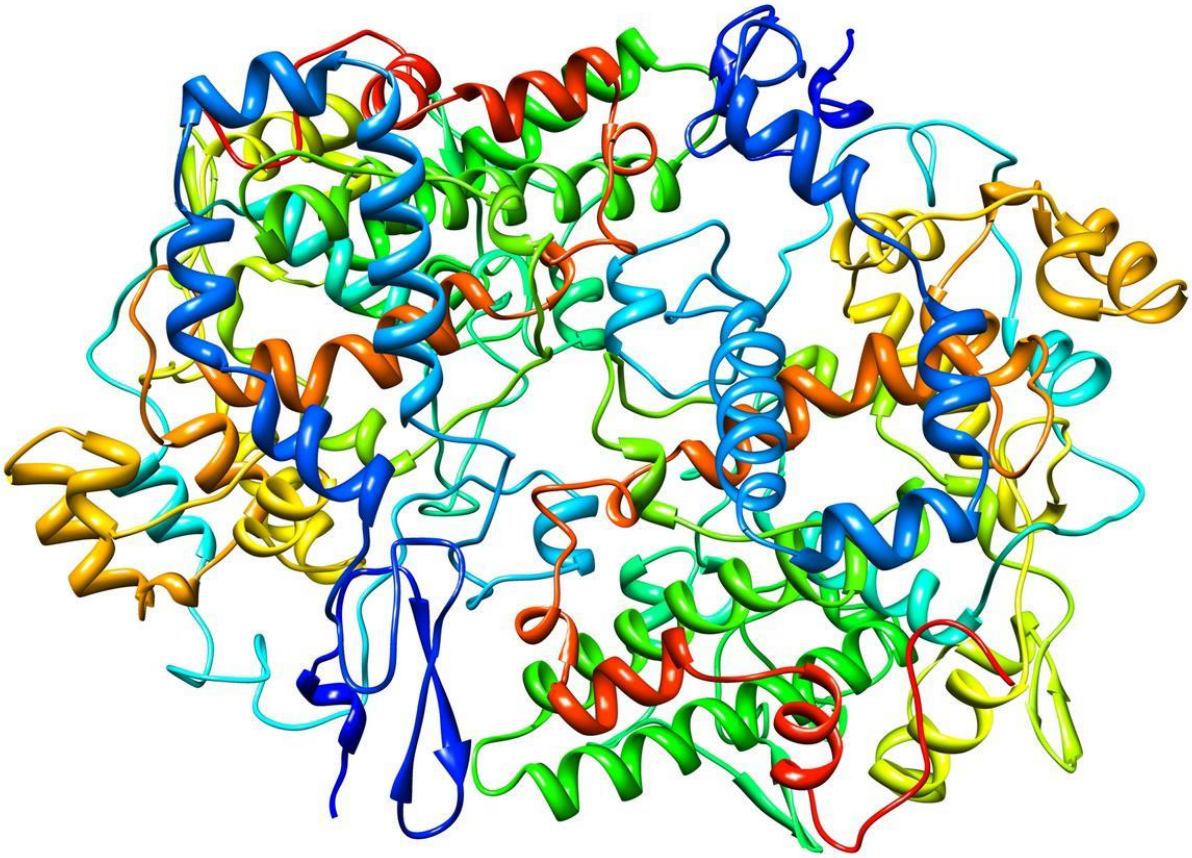

Figure 2.3. Crystallographic structure of prostaglandin H2 synthase-1 complex with flurbiprofen (Picot *et al.,* 1994).

2.3.2. Physiological role of Prostaglandin

Both classes of COX are bifunctional enzymes with two distinct catalytic activities: cyclooxygenase (or bis-dioxygenase) activity and peroxidase activity. The primary products of COXs were first detected in human seminal fluid by clinicians studying uterine contraction (Goldblatt, 1933). Thought to be the product of the prostate gland, these highly potent bioactive compounds were given the name prostaglandins. They are synthesized in virtually all tissues in vertebrates, however, and some organisms that lack prostate glands, such as corals, also synthesize prostaglandins. Thus, in many respects the term prostaglandin is a misnomer. Initially, the enzyme activity that synthesized prostaglandins was frequently called prostaglandin synthetase, but because it does not require ATP it is now called prostaglandin G/H synthase to fit the nomenclature convention. It is more popularly known as cyclooxygenase, a name that only partially describes the enzyme since it refers to only one of its two enzymatic activities (Narumiya *et al.,* 1999).

2.3.2.1 Role of COX-1 in Digestive System

Cyclooxygenase-1 and low levels of COX-2 contribute to gastric mucosal defense, and the isoforms affect different components of this defense. Prostaglandins produced by COX-1 regulate the mucosal blood flow and epithelial secretion of mucus and bicarbonate, while PGs produced by COX-2 affect epithelial proliferation and endothelial–leukocyte adherence (Wallace and Devchand, 2005). When the COX-2 isoform is inhibited, the absence or underdevelopment of lesions in the stomach indicates that COX-1 is the mediator of gastric mucosal defense under normal conditions (Wallace and Devchand, 2005; Martin-Sanz *et al.,* 2006). Surprisingly, it has been reported that gastric lesions do not develop in mice with COX-1 gene knockout; they experience less gastric damage, bleeding, and ulceration than wild-type mice when administered

with an NSAID (Langenbach *et al.,* 1995). In rat intestine, COX-2 expression might increase as a result of the selective inhibition of COX-1. Recent studies have shown that COX-2 plays a role in modulating resistance to lumen irritants when other factors of mucosal defense are genetically or pharmacologically depressed. For example, when nitric oxide (an important mediator of many components of mucosal defense) synthesis is inhibited, selectively inhibiting the COX-2 enzyme leads to stomach damage. The COX-2 isoform increases rapidly when mucosal damage occurs or when the COX-1 isoform is inhibited. In these cases, suppressing the activity of the COX-2 isoform results in increased mucosal damage and delayed repair (Wallace and Devchand, 2005).

2.3.2.2. Role of COX-1 in Kidney

Prostaglandins have important physiological roles in the modulation of glomerular hemodynamics, the reabsorption of sodium/water, and the regulation of renin secretion (Dubois *et al.,* 1998). Both isoforms (but mainly COX-1) are found in the kidney (Claria, 2003). However, the relevant kidney data are not clear, and the COX-1 and -2 distribution differs according to the organism (Mohale *et al.,* 2014). In this context, COX-1 is preferentially expressed in the renal vessels and in the papillary and medullary collecting ducts in monkeys, humans, rabbits, dogs, and rats (Claria, 2003). After mother's use of Nimesulide (selective COX-2 inhibitor NSAID) as tocolytic, the lack of effect of nimesulide on fetal renal and ductal function indicates that fetal prostaglandin synthesis is mainly mediated by the COX-1 isoform.45 However, some studies reported neonatal irreversible end-stage renal failure or severe oligohydramnios after use of nimesulide as a tocolytic (Peruzzi *et al*., 1999; Holmes *et al*., 2000). While the COX-1 protein levels did not change in the kidneys of cirrhotic rats, COX-2 protein expression was increased in the corticomedullary region in these animals. Although urinary PGE2 excretion was equally reduced by both selective COX-1 and COX-2 inhibitors in these animals, urinary sodium

excretion, the glomerular filtration rate, and renal plasma flow significantly decreased, and the diuretic and natriuretic responses to furosemide were markedly impaired only when COX-1 was selectively inhibited. The selective inhibition of both isoforms in cirrhotic rats did not affect renal water metabolism. These results indicate that preservation of renal function in cirrhotic rats is dependent on the COX-1 isoform despite the abundant expression of renal COX-2 protein (Lopez *et al.,* 2002).

2.3.2.3. Role of COX-1 in Heart and Platelets

The use of NSAIDs and celecoxib or rofecoxib increases risk for cardiovascular events in medical conditions such as diabetes mellitus, congestive heart failure, dyslipidemia, and colorectal adenomas. Additionally, at moderate doses, celecoxib was not found to be inferior to ibuprofen or naproxen in cardiovascular safety (Nissen *et al.,* 2016). Metabolites of COX play important roles in the homeostasis in an organism. The COX-1 isoform is involved in vascular homeostasis in platelets and in most tissues (Smith and Dewitt, 1996). The only isoform detectable in platelets is COX-1 (Vane *et al*., 1998). The role of COX-1 in thromboxane synthesis in platelets is explained by their inability to produce an inducible enzyme in response to activating conditions in seedless platelets (Dubois *et al*., 1998). One study demonstrated that prostacyclin in vascular cells is produced by COX-2 and COX-1 under both pathological and physiological conditions (Morita *et al.,* 2002). Prostacyclin, a potent vasodilator, is one of the most important prostanoids that controls the homeostasis of the cardiovascular system. In addition, it inhibits leukocyte adhesion, platelet aggregation, and the proliferation of vascular smooth muscle cells (Mendes *et al.,* 2012). The function of COX-2 in the cardiovascular system remains largely unknown. Ischemic preconditioning has been shown to increase COX-2

expression and activity in the heart. This increase of COX-2 activity mediates protective effects against myocardial palpitation and myocardial infarction (Mohale *et al.,* 2014).

2.3.2.4. Role of COX-1 in inflammation

COX-1 recently emerged as a prominent player in CNS neuroinflammation (Garcia-Bueno *et al.,* 2009). In aged rats, brain levels of COX-1-derived TXB_2 and COX-1 expression in the hippocampus are increased and may contribute to the increased brain susceptibility to inflammation and neurodegenerative diseases. Indeed, COX-1 has been shown to support inflammatory processes and facilitate pro-inflammatory PG upregulation in several models of neuroinflammation (Aid *et al.,* 2010; Choi *et al.,* 2008). Owing to its predominant localization in microglia, brain immune resident cells, COX-1-derived products seem to be particularly important in modulating the initial phase of the inflammatory response. A recent study also identified COX-1 expression in perivascular cells (a subset of brain resident macrophages), where its expression was increased in response to a systemic LPS challenge, suggesting a role for COX-1 in mediating the immune-to-brain signaling (Garcia-Bueno *et al.,* 2009). Our group showed that COX-1 deficient mice have a decreased inflammatory response, leukocyte infiltration, oxidative stress and neuronal damage after central injection of LPS or $A\beta_{1-42}$ ((Garcia-Bueno *et al*., 2009; Choi *et al.,* 2009) and these effects were associated with altered PG levels. LPS or $A\beta_{1-42}$ –induced upregulation of brain PGE_2, $PGF_{2\alpha}$ and TXB_2 was reduced by genetic deletion of COX-1 and by SC-560, a selective COX-1 inhibitor (Choi *et al*., 2008). In agreement with these results, Matousek and collaborators recently demonstrated that COX-1 expression and PGE_2 were upregulated up to 2 months following a sustained IL-1β overexpression, a model of chronic neuroinflammation, and COX-1 genetic deletion or pharmacological inhibition completely abrogated IL-1β-mediated PGE_2 increase (Matousek *et al.,*

2010). Thus, COX-1 seems to play a role not only in acute but also in chronic neuroinflammatory conditions.

2.4. Prostaglandins and Related Mediators

Prostaglandins (PGs) play a key role in the generation of the inflammatory response. Their biosynthesis is significantly increased in inflamed tissue, and they contribute to the development of the cardinal signs of acute inflammation. Although the proinflammatory properties of individual PGs during the acute inflammatory response are well established, their role in the resolution of inflammation is more controversial.

2.4.1. Biosynthesis of Prostaglandins

PGs and thromboxane A2 (TXA2), collectively termed prostanoids, are formed when arachidonic acid (AA), a 20-carbon unsaturated fatty acid, is released from the plasma membrane by phospholipases and metabolized by the sequential actions of PGG/H synthase or by cyclooxygenase (COX) and their respective synthases. There are 4 principal bioactive PGs generated in vivo: prostaglandin E2 (PGE2), prostacyclin (PGI2), prostaglandin D2 (PGD2), and prostaglandin F2α (PGF2α). They are ubiquitously produced—usually each cell type generates 1 or 2 dominant products—and act as autocrine and paracrine lipid mediators to maintain local homeostasis in the body. During an inflammatory response, both the level and the profile of PG production change dramatically. Prostaglandin production is generally very low in uninflamed tissues but increases immediately in acute inflammation before the recruitment of leukocytes and the infiltration of immune cells. PG production depends on the activity of PGG/H synthases,

colloquially known as COXs, bifunctional enzymes that contain both COX and peroxidase activity and that exist as distinct isoforms referred to as COX-1 and COX-2 (Smith et al., 2000).

COX-1, expressed constitutively in most cells, is the dominant source of prostanoids that subserve housekeeping functions, such as gastric epithelial cytoprotection and homeostasis (Dubois et al., 1998). COX-2, induced by inflammatory stimuli, hormones, and growth factors, is the more important source of prostanoid formation in inflammation and in proliferative diseases, such as cancer.3 However, both enzymes contribute to the generation of autoregulatory and homeostatic prostanoids, and both can contribute to prostanoid release during inflammation. PGH2 is produced by both COX isoforms, and it is the common substrate for a series of specific isomerase and synthase enzymes that produce PGE2, PGI2, PGD2, PGF2α, and TXA2. COX-1 couples preferentially, but not exclusively, with thromboxane synthase, PGF synthase, and the cytosol © PGE synthase (PGES) isozymes. COX-2 prefers prostaglandin I synthase (PGIS) and the microsomal (m) PGES isozymes, both of which are often coinduced along with COX-2 by cytokines and tumor promoters (Smyth et al., 2009).

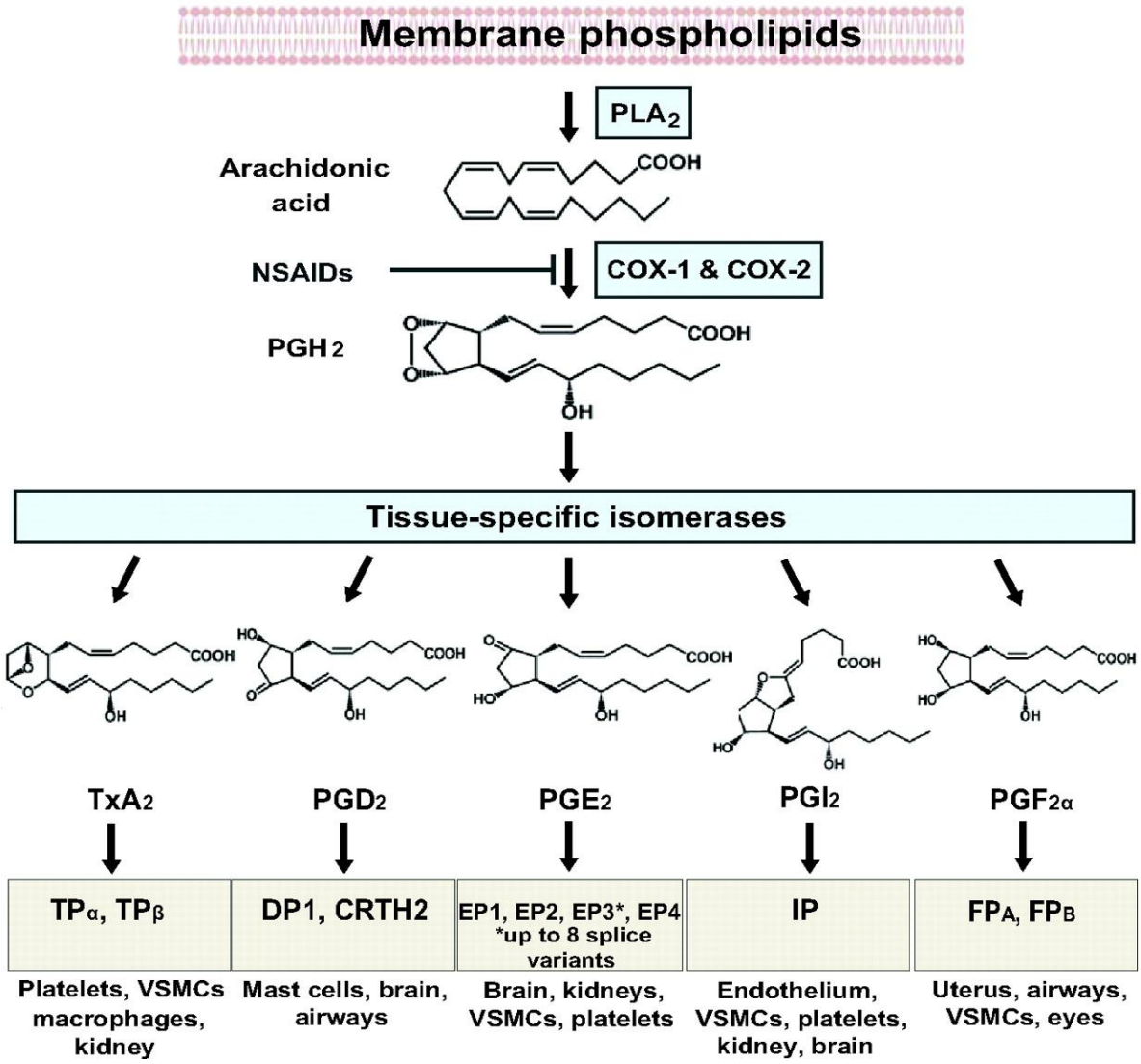

Figure 2.4. Biosynthetic pathway of prostanoids (Ricciotti and FitzGerald, 2011).

2.4.2. Roles of Prostaglandins in inflammation

PGE2 is one of the most abundant PGs produced in the body, is most widely characterized in animal species, and exhibits versatile biological activities. Under physiological conditions, PGE2 is an important mediator of many biological functions, such as regulation of immune responses, blood pressure, gastrointestinal integrity, and fertility. Dysregulated PGE2 synthesis or degradation has been associated with a wide range of pathological conditions (Legler, 2010). In inflammation, PGE2 is of particular interest because it is involved in all processes leading to the classic signs of inflammation: redness, swelling, and pain (Funk, 2001). Redness and edema result from increased blood flow into the inflamed tissue through PGE2-mediated augmentation of arterial dilatation and increased microvascular permeability. Pain results from the action of PGE2 on peripheral sensory neurons and on central sites within the spinal cord and the brain (Funk, 2001).

PGD2 is the predominant prostanoid produced by activated mast cells, which initiate IgE-mediated type I acute allergic responses (Lewis et al., 1981). It is well established that the presence of an allergen triggers the production of PGD2 in sensitized individuals. In asthmatics, PGD2, which can be detected in the bronchoalveolar lavage fluid within minutes, reaches biologically active levels at least 150-fold higher than preallergen levels (Murray et al., 1986). PGD2 is produced also by other immune cells, such as antigen-presenting DCs and Th2 cells, suggesting a modulatory role for PGD2 in the development of antigen-specific immune system responses (Urade et al., 1989). PGD2 challenge elicits several hallmarks of allergic asthma, such as bronchoconstriction and airway eosinophil infiltration (Emery et al., 1989) and mice that overexpress L-PGDS have elevated PGD2 levels and an increased allergic response in the OVA-induced model of airway hyperreactivity (Fujitani et al., 2002).

2.5. Haematological Parameters

Haematological Parameters are quantifiable constituents of blood like erythrocytes and its indices, leukocytes and platelets. These blood components originate from the haemopoeitic stem cell, they occupy the entire capacity of the bones at birth but it is been replaced with fatty marrow with increase in age, thereby affecting these blood parameters. The assessment of haematological parameters is very necessary because, they are important proxy indicators useful in the assessment of immune status, therapeutic purposes and monitoring of disease progression and treatment outcome for proper patient management. The developmental stages of life vary directly with basic biological variables of age and sex independently. In pursuant of effective health care through accurate diagnosis, haematological parameters are routinely assessed constituents of blood like erythrocytes and its indices, leukocytes and platelets (Uchechukwu *et al.,* 2021).

2.3.1 Platelets

The platelet is a small, anucleated cell that originally derives from the hematopoietic lineage via the megakaryocyte. The production of platelets from megakaryocytes is a systematic and regulated process that is thought to occur either in the bone marrow or, as has been shown more recently, the lung (Lefrancais *et al.,*2017). Due in large part to the extreme shear forces, the platelet is exposed to in the vessel as well as the limitations imposed on the platelet due to the absence of a nucleus; the lifespan of the platelet is limited to between 5 to 7 days following formation and separation from the megakaryocyte (Thon *et al.,* 2012).

Normal platelets count in adult is 140-400 x 109/ L

Children: 150-450 x 109/ L (Fischbach, 2003).

2.3.2 White Blood Cells

White blood cells, also known as leukocytes, play a central role in the immune system by providing both innate and adaptive immunity to the body. They are broadly categorized into five main types: neutrophils, monocytes, lymphocytes, eosinophils, and basophils. These cells are responsible for transporting and circulating antibodies during immune responses, as well as fighting infections and protecting the body against harmful organisms through the process of phagocytosis. A reduced white blood cell count makes an individual more vulnerable to infections, while an elevated count enhances their ability to resist disease. The typical white blood cell count ranges from 5,000 to 10,000 cells/ml for both adult males and females, and from 4,500 to 11,000 cells/ml in children (Isaac et al., 2013).

2.3.2.1. Neutrophils

Neutrophils, the most predominant type of granulocyte, comprise 40–70% of all human leukocytes (Mestas and Hughes, 2004). Approximately 100–200 billion neutrophils are generated daily in humans through hematopoiesis in the bone marrow (Ng *et al.,* 2019). According to conventional estimates, the half-life of circulating neutrophils spans 4 to 18 hours during homeostasis (Tak *et al.,* 2013). A recent study utilizing advanced technologies determined a more precise half-life of approximately 19 hours, which is still less than 1 day (Lahoz-Beneytez, 2016). Neutrophils are traditionally considered short-acting effector cells in the innate immune system and the primary line of defense against extracellular pathogens and acute inflammation (Ley *et al*., 2007). Their short lifespan contributes to the efficiency of the immune system (Ballesteros, 2020), and a substantial decrease in neutrophils in the bloodstream results in severe

immunodeficiency in humans (Dotta *et al.,* 2011). The range of a normal neutrophil count is 2.0 to 7.5 x 109/L. (Drew et al., 2004).

Differential: 0%-3% of total WBC (Ficshbach, 2003).

2.3.2.2. Lymphocytes

Lymphocytes are a type of leukocyte (white blood cell) produced in the bone marrow. They make up anywhere from 18% to 42% of circulating white blood cells in your bloodstream. Lymphocytes are roughly the size of erythrocytes (red blood cells) and continuously move through the circulatory and lymphatic systems, searching out diseases that cause the body harm. There are three types of lymphocytes that have specific functions in the human body, called T cells, B cells, and natural killer (NK) cells (Mims, 2018). The two main types of lymphocytes are B lymphocytes (B cells) and T lymphocytes (T cells), each with distinct roles in immune response. B cells are responsible for producing antibodies, which are proteins that bind to specific antigens (foreign substances) and mark them for destruction by other immune cells. This process, known as humoral immunity, is significant for neutralizing pathogens such as bacteria and viruses before they can cause harm. On the other hand, T cells lead a central role in cell-mediated immunity, where they directly attack infected or abnormal cells (Juan, 2024). Reference range; Adults and Children: 20% to 40% of total white blood cells (Ficshbach, 2003).

2.3.2.3. Monocytes

Monocytes are circulating leukocytes that are key players in tissue homeostasis and immunity. They are formed in the bone marrow and continuously enter the blood circulation, where they constitute 4% of the total leukocyte population in mice and 10% in humans (Ginhoux and Jung, 2014). One of the best known functions of monocytes is as a systemic reservoir of precursor cells

for the renewal of several populations of tissue macrophages, dendritic cells (DCs), and osteoclasts (Qu *et al.,* 2014). Reference range; Adults and Children: 2% to 8% of total white blood cells (Ficshbach, 2003).

2.3.2.4. Eosinophils

Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes including parasitic helminth, bacterial and viral infections, tissue injury, tumor immunity, and allergic diseases. In response to the diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci where they modulate immune responses through an array of mechanisms (Hogan *et al.,* 2004). Normal range of eosinophils is 0-0.7 X 109/L (Ficshbach, 2003).

2.3.3.5. Basophils

Basophils are a type of white blood cell. Basophils are the least common type of granulocyte, representing about 0.5% to 1% of circulating white blood cells. They are the largest type of granulocyte. They are responsible for inflammatory reactions during immune response, as well as in the formation of acute and chronic allergic diseases, including anaphylaxis, asthma, atopic dermatitis and hay fever (Mukai and Galli, 2013). They also produce compounds that coordinate immune responses, including histamine and serotonin that induce inflammation, and heparin that prevents blood clotting (Khurana, 2009).

Normal range for basophils is 0.02-0.05 x 109/L (Fischbach, 2003).

Differential: 0%-1.0% of total white blood cell count (Fischbach, 2003).

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 (Obazelu and Anyafulu, 2025).

3.3. Identification of *Icacina trichantha* Leaves

Icacina trichantha leaves were collected around Ekosodin community in Ovia north east area 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 with voucher number (Obazelu and Ogiza, 2024).

3.3.1. Processing of *Icacina trichantha*

The process started with removing unhealthy leaves from the sample. The leaves were then thoroughly washed, drained, and air-dried under shade for two weeks to achieve optimal dryness for grinding. Further drying was then carried out using a hot air oven at 50°c for 24hours. This

ensured the leaves were properly dried and prepared for grinding. An industrial 1000A high-speed grinder was for the grinding process (Obazelu and Evwaire, 2024).

3.4. Preparation of Plants Extract

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

3.5. Animal Care

The animals were kept in a well-ventilated room within the animal facility of the Department of Anatomy, University of Benin, Benin City. They were maintained under a 12-hour light/dark cycle and had unrestricted access to food and water. Prior to the start of the experiment, the rats were acclimatized for two weeks (Obazelu and Faluyi, 2023).

3.5.1. Inclusion Criteria

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

3.5.2. Exclusion Criteria

- Rats with excessive breathing
- Rats with reduced appetite
- Sick rats

• Rats weighing less than 150g (<150g)

3.6. Ethical Consideration

Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: HA/737/25/D/0521073 issued on 18th June, 2025).

3.7. Preparation of Aluminum Chloride and Ferrous Sulphate Drug Solution

3.7.1. Aluminium Chloride Solution

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

3.7.2 Ferrous Sulphate Drug Solution

Ferrous sulphate solution was prepared by dissolving 1000mg of the powdered drug in 50 ml of distilled water. Each animal in Group C, with an average weight of 150 g, received an oral dose of 0.3 ml of the solution every 48 hours over a 28-day period.

3.8. Research Design

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

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

Group B: This group received only aluminum chloride intraperitoneally.

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

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

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

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

3.8.1. Dosage of Ferrous Sulphate Administered

40mg/kg.bw

40mg – 1000g

? - 150g (Mean weight of rats)

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

1000mg of ferrous sulphate powder = 50mls

6mg = x

X= $\frac{6\times50}{1000}$ = 0.3ml of 6mg/ml 48 hourly for 4 weeks

3.8.2. Extract Dosing

The dosage given to each group is calculated by;

Weight of the animal = g/kg

Dose of extract = mg/kg

Stock of extract = mg/ml

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 aluminum chloride intraperitoneally

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

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

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

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

Calculating dose of extract for each group using;

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

Group D

Average weight of 10 rats = 150g

Dose = 100mg/kg

Stock = 100mg/ml

150g to kg = 0.150kg

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

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

Group E

Average weight of 10 rats = 150g

Dose = 200 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

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

Stock

$= 0.\frac{150 \times 200}{100} = 0.3ml$

Group F

Average weight of 10 rats = 150g

Dose = 400 mg/kg

Stock = 100 mg/ml

150g to kg = 0.150kg

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

$= 0.\frac{150 \times 400}{100} = 0.6ml$

3.8.3. Administered Doses of Herbal Formulation of *Icacina trichantha* Leaves Extract

Group A (control) received only standardized feed and clean water ad libitum. Group B (Aluminum chloride treated group) were administered 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days. Group C (ferrous sulphate drug solution treated group) were administered 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 6mg/ml of ferrous sulphate 48 hourly for 28 days. Group D were administered with 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of herbal formula of *Icacina trichantha* leaves extract orally using a gavage tube every 24 hours for 28 days. Group E were

administered with 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of herbal formulation of *Icacina trichantha* leaves extract orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.1ml of aluminum chloride solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of herbal formulation of *Icacina trichantha* leaves extract orally using a gavage tube every 24 hours for 28 days.

3.9. Physical Examination of Animals

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

3.9.1. Measurement of Body Weight

The body weights of the animals were measured twice during the experiment, on day 0 and day 28, representing the initial and final weights, respectively. Each animal was individually weighed using a weighing scale. This involved removing the animals from their cages, placing them on the scale, and recording the weight while the animals were resting on the scale.

3.10. Sacrifice of Animals and Collection of Samples

At the end of the experimental period, the animals were examined for general physical characteristics. Following anesthesia with chloroform and cervical dislocation, a midline incision was made along the ventral surface of each rat. Five milliliters (5ml) of blood were collected from each rat using a sterile syringe and placed in an Ethylene Diamine Tetra-acetic Acid (EDTA) container for full blood count analysis. Bone marrow samples were also obtained from the rats by opening the femur longitudinally and exposing the marrow cavity. A sterile forceps was used

to obtain the bone marrow from the cavity and placed in an Eppendorf container containing Trizol for molecular analysis.

3.11. Laboratory Analysis

3.11.1. Haematological Profile

The full blood count parameters were analyzed immediately after sample collection using the automated three parts SFRI Haematology Auto analyzers PCE-525 (Diamond Diagnostic; Holliston, USA). Calibration and standardization of the equipment, processing and analysis of the samples were done strictly according to the manufacturer's instructions.

3.11.1.1. Detection Principle of Haematology Autoanalyzer

The instrument counts and sizes the cells. It detects and measures changes in electrical resistance when a particle (such as a cell) passes through a gem aperture sensor.

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

3.11.1.2. Procedure

The whole blood sample was thoroughly mixed and introduced into the analyzer probe. A volume of 20 µL was aspirated into the instrument for analysis. The results were generated within 1–2 minutes and automatically displayed on the screen, followed by printing by the printer.

3.12.2. 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 e same staining jar.
- 98 ml of ethanol (95%) was added to the staining jar.
- The contents of the staining jar were mixed t roughly 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.
- A spreader was placed at a 45-degree angle against the blood drop while al g 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. Cyclooxygenase-1 (COX-1) Assay

3.12.1. Isolation of Total RNA

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

3.12.2. cDNA conversion

One (1µg) 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 1h, and 80°C for 5 min (Olumegbon et al., 2020).

3.12.3. PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa). PCR amplification was performed in a total of 25µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95°C for 5min, followed by 30 cycles of amplification (denaturation at 95°C for 30s, annealing for 30s and extension at 72°C for 60s) and ending with final extension at 72°C for 10 min. The amplicons were resolved on 1.0% agarose gel. The

GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al*., 2020).

3.12.4. Primer Sequences

COX-1

Forward: GCTCCAGTTTCCCCCTGCT

Reverse: TTCTGGCATGGATAGTAACAACA

GAPDH

Forward: CTCCCTGGAGAAGAGCTATGA

Reverse: AGGAAGGAAGGCTGGAAGA

3.13. Statistical Analysis

Data obtained from this research was presented and analyzed using GraphPad prism 8.02 (California, USA). Analysis of variance (ANOVA) was used to compare treatment groups of continuous variables. Tukey HSD post hoc was applied where a significant difference was observed in the ANOVA. Bar charts was used to represent the mRNA gene expression patterns. A p value of ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Table 4.1 shows the comparison of Mean±SD of white blood cell parameters of six groups namely; groups A, B, C, D, E and F, representing control, aluminum chloride, ferrous sulphate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* respectively. There was no significant difference across the white blood cells parameters.

Table 4.1: Mean Comparison of White Blood Cell Parameters among the Studied Groups

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	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

Total WBC : Total White Blood Cell Count

MID: Mid-sized Cells

Gran: Granulocytes

Table 4.2 shows the comparison of Mean±SEM of platelet parameters namely; groups A, B, C, D, E and F, representing control, aluminum chloride group, ferrous sulphate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* respectively. PDW (%) was significantly lower in group E (8.96±0.27) when compared to group D (10.6±0.31). There was no significant difference across all other platelets parameters (Platelet count, MPV, PCT and P-LCR).

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

Table 4.2: Mean Comparison of Platelets Parameters among the Studied Groups

Key: Table presented in mean±SEM. $p<0.05$ was considered significant

MPV: Mean Platelet Value

PDW: Platelet Distribution Width

PCT: Plateletcrit

P-LCR: Platelet Large Cell Ratio

Table 4.2. shows the blood morphology among the studied groups namely; 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.

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 aluminium chloride induced group (Group B), moderately present in groups A, C and D and mildly present in groups E and F. Eosinophils was mildly present in group B only, 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 aluminium chloride induced group (Group B) compared to the other groups which showed moderate and high presence of these red blood cells. Polychromatic cells were absent in all groups. Crenated red blood cells were mildly present in groups B and C. Platelets were highly present in all groups.

Table 4.3. 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 4.3 shows the body weight of groups A, B, C, D, E and F, representing control, aluminum chloride group, ferrous sulphate group, aluminum chloride + 100mg/kg *Icacina trichantha*, aluminum chloride + 200mg/kg *Icacina trichantha*, aluminum chloride + 400mg/kg *Icacina trichantha*. The weight gain of groups A, B, C, D, E and F increased slightly at day 28 when compared to day 0. The final weight of group A had significant increase when compared to the initial weight.

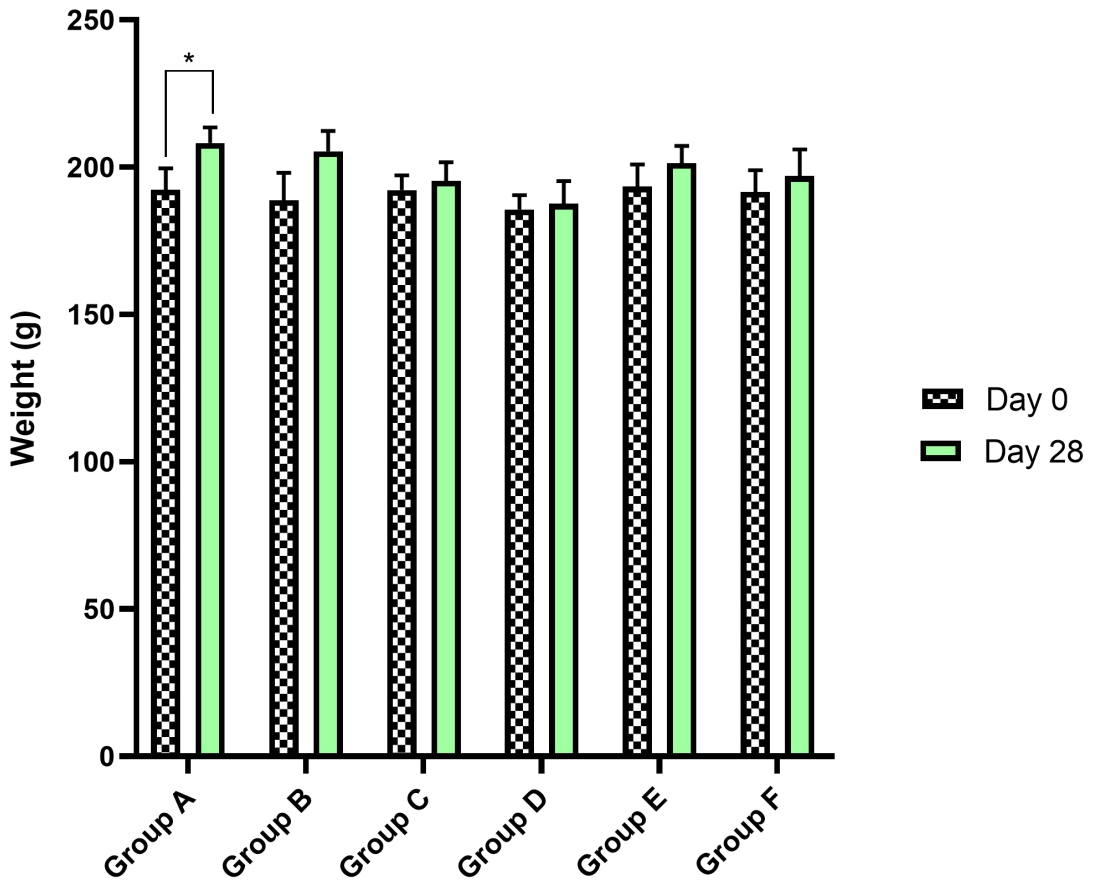

Figure 4.1: Initial and Final Body weights of Groups A, B, C, D, E and F measured at Day 0 and Day 28.

Figure 4.4 shows the expression of genes as represented by gel electrophoresis picture and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GADPH}) of mRNA expression of cyclooxygenase 1(COX-1) of groups A, B, C, D, E and F, representing control, aluminum chloride group, ferrous sulphate group, aluminum chloride + 100mg/kg *Icacina trichantha,* aluminum chloride + 200mg/kg *Icacina trichantha* and aluminum chloride + 400mg/kg *Icacina trichantha* groups respectively, represented on different bars on the bar chart. There was a significant increase in the mRNA expression of COX-1 of group B when compared to group A ($p<0.05$). Group C and D showed statistically significant lower expressions of COX-1 when compared to group B ($p<0.05$). Group C, D and E showed statistically significant lower expression of COX-1 when compared to group F ($p < 0.05$). Groups E showed a significant higher expressions of COX-1 when compared to group A, and a significant lower expressions when compared to group B ($p<0.05$). Groups F showed a statistically significant higher expressions of COX-1 when compared to group A ($p<0.05$).

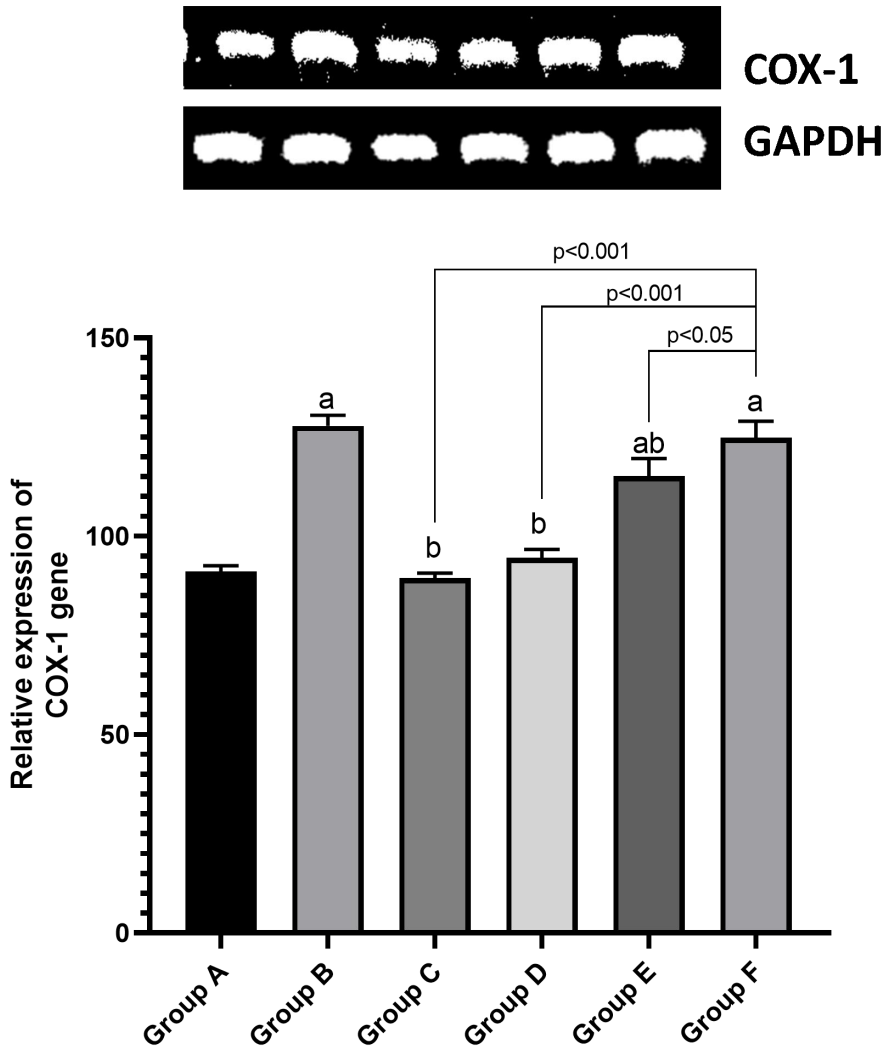

Figure 4.2: mRNA expression of COX-1 of the study group

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

Group A represents Control group

Group B represents Alcl3 group

Group C represents Alcl3 + Ferrous sulfate

Group D represents Alcl3 + 100mg/kg

Group E represents Alcl3 + 200mg/kg

CHAPTER FIVE

5.1. DISCUSSION

In recent years, there has been increasing interest in the use of natural remedies derived from medicinal plants, offering promising avenues for therapeutic interventions against various health conditions (Pandey et al., 2011). For centuries, various cultures have relied on herbal medicines for their therapeutic effects in treating numerous ailments, including blood disorders (Obazelu and Agbikimi, 2025). Among these, *Icacina trichantha*, a plant native to West Africa has garnered attention for its pharmacological potential. Traditionally known as "urumbia" or "false yam, *Icacina trichantha* has been utilized in ethnomedicine for the treatment of ailments such as gastrointestinal disturbances, fever, and infections (Burkill, 1985; Ugochukwu *et al*., 2013). Phytochemical investigations have revealed the presence of bioactive compounds including alkaloids, flavonoids, saponins, and tannins, which are believed to contribute to its antioxidant, antimicrobial, and anti-inflammatory effects (Ezugwu *et al*., 2019; Okoli *et al*., 2021). These properties support the growing scientific interest in evaluating its efficacy in managing disorders associated with oxidative stress and inflammation.

Anaemia, characterized by a deficiency in the oxygen-carrying capacity of the blood, remains a widespread global health issue, particularly in low-resource settings where nutritional deficiencies and environmental toxins are prevalent (Shubham *et al*., 2020). While conventional therapies such as iron supplementation and erythropoiesis-stimulating agents are widely used, increasing attention is being directed toward plant-based alternatives due to their accessibility and potential for fewer side effects. *Icacina trichantha*, a lesser-known but traditionally valued medicinal plant native to West Africa, has shown promise in this regard (Burkill, 1985;

Ugochukwu *et al*., 2013). A key molecular target implicated in inflammation and oxidative stress during anaemia is the cyclooxygenase (COX) pathway, particularly the constitutive isoform, COX-1. COX-1 is an enzyme responsible for the conversion of arachidonic acid into prostaglandins, which regulate physiological processes including gastric protection, platelet aggregation, and renal function (Ricciotti & FitzGerald, 2011). However, aberrant or elevated expression of COX-1 has been associated with pathological inflammation and oxidative stress, especially in toxin-induced anaemia models (Ali *et al*., 2014). The modulation of COX-1 expression by natural plant extracts, such as those from *Icacina trichantha*, may contribute to a reduction in inflammation and restoration of normal erythropoiesis. Evaluating the effect of *Icacina trichantha* on COX-1 gene expression can thus provide valuable insight into its mechanism of action and therapeutic potential in managing anaemia.

This study revealed that there was no statistically significant differences in total white blood cell (WBC) count and differential counts; lymphocytes, mid-sized cells, and granulocytes across the experimental groups. This indicates that Aluminum Chloride-induced anaemia and subsequent treatment with *Icacina trichantha* extract did not significantly affect the distribution or count of leukocytes. This finding is in agreement with the report of Ibrahim *et al*. (2020), who observed no significant alteration in WBC count in albino rats exposed to aluminum chloride, attributing the result to subacute exposure and limited hematopoietic disruption. Similarly, Okediran and Adeyemo (2021) reported that aluminum chloride caused only mild leukocytic changes in rats, with no marked deviation from normal reference values. On the other hand, the relatively stable WBC levels observed in extract-treated groups further support the immunomodulatory nature of *Icacina trichantha*, consistent with the work of Nwankwo and Oladipo (2022), who demonstrated anti-inflammatory activity in rats treated with the plant extract.

The findings from this study revealed only Platelet Distribution Width (PDW) exhibited a statistically significant difference, with 200mg/kg *Icacina trichantha* treated group, showing the lowest PDW value. A reduced PDW may indicate less variation in platelet size and reduced activation, possibly reflecting normalization or protective effects of the extract on platelet morphology. This observation suggests a possible protective effect of the extract against aluminum chloride-induced platelet stress. Similar findings were reported by Obazelu and Osazee (2024), where plant extract administration modulated platelet activation and normalized platelet indices in chemically induced anaemia models. Other platelet parameters, including platelet count, mean platelet volume (MPV), plateletcrit (PCT), and platelet large cell ratio (P-LCR) did not show statistically significant differences. Nonetheless, 200mg/kg *Icacina trichantha* treated group had lower platelet count and P-LCR compared to the control, indicating a subtle normalization effect of the extract. These findings are consistent with the study by Akinlolu *et al*. (2021), who observed that plant-based treatments stabilized platelet levels disrupted by environmental toxicants. The relative stability of these platelet indices despite exposure to aluminum chloride suggests that *Icacina trichantha* may offer a stabilizing or protective effect on platelet function, potentially reducing the risk of platelet abnormalities typically induced by oxidative or chemical stress.

The peripheral blood film analysis showed that exposure to aluminum chloride did not alter the morphology of blood cells. Comparisons between the aluminum chloride group and the other treatment groups revealed no observable differences. However, small lymphocytes were more prominent in the groups administered 200 mg/kg and 400 mg/kg of *Icacina trichantha* leaf extract. This increase may be attributed to the effect of the extract, since aluminum chloride itself did not induce any morphological changes. All other groups displayed normal blood cell

morphology. Similar observations were reported by Okediran *et al*. (2019), who noted that low to moderate doses of aluminum chloride did not significantly alter peripheral blood cell morphology in rats, despite evidence of oxidative stress.

Body weight is an important factor in studies, offering insights into different aspects of health, and experimental outcomes. In this study, all experimental groups showed a gradual increase in body weight from baseline (Day 0) to the end of the experiment (Day 28). This pattern indicates that none of the administered substances produced acute toxicity severe enough to interfere with normal growth. The control group recorded the highest weight gain when compared with the groups exposed to aluminium chloride, suggesting that aluminium-free conditions allowed for more efficient nutrient utilization and healthy physiological development. Furthermore,, the groups that received *Icacina trichantha* leaf extract alongside aluminium chloride also demonstrated steady weight increase throughout the study period. This finding suggests that the bioactive phytochemicals in the extract, known for their antioxidant and immunomodulatory effects (Zanganeh *et al*., 2018), may have played a role in reducing the negative metabolic impact of aluminium exposure. However, this protective influence was not strong enough to restore weight gain to the level observed in the control group. These results differ from those of Obazelu and Omoregie (2024), who reported more distinct weight variations between groups within a similar timeframe, a difference that could be explained by variations in dosage, extract composition, or the experimental design used.

This study revealed an elevated expression of COX-1 mRNA observed in the group administered aluminium chloride only when compared to the control group. This marked upregulation of COX-1 expression in response to aluminum chloride exposure suggests that aluminium toxicity may activate inflammatory or oxidative stress pathways, stimulating COX-1 transcription as a

compensatory mechanism. This finding supports previous work by Exley (2003) and, Yokel and McNamara (2001), who linked aluminium exposure to elevated inflammatory enzyme expression. The group administered ferrous sulphate, exhibited a COX-1 expression level significantly lower than the aluminum chloride only and 400mg/kg *Icacina trichantha* treated groups. This suggests that ferrous sulphate did not contribute to further inflammatory response and may have mildly modulated COX-1 activity. While iron is essential in treating anaemia, its oxidative potential depends on dose and context, in this case, the result indicates a protective rather than pro-inflammatory role at the given dose. This aligns with the report by Walter *et al*. (2002), which noted that iron supplementation, when well-regulated, can help reduce inflammatory stress. In the extract-treated groups, the expression of COX-1 showed a complex but defined trend. 100mg/kg *Icacina trichantha* treated group had lower COX-1 expression than aluminum chloride only and 400mg/kg *Icacina trichantha* treated groups. 200mg/kg and 400mg/kg *Icacina trichantha* groups showed expression levels higher than the control and ferrous sulphate groups. This pattern suggests that the extract exerted some suppressive effect on the aluminium-induced overexpression of COX-1, particularly at the lowest dose (100mg/kg *Icacina trichantha* group), but did not produce a clear dose-dependent inhibition. In fact, COX-1 expression in 200mg/kg and 400mg/kg *Icacina trichantha* groups appeared partially restored or stabilized, though still elevated compared to control and ferrous sulphate groups.

The result implies that *Icacina trichantha* extract may exert a modulatory rather than purely suppressive effect on COX-1 gene expression, possibly supporting physiological COX-1 activity at higher doses while still attenuating aluminium-induced spikes. This contrasts with studies like Ezeamuzie *et al*. (2004) and Sforcin *et al*. (2009), where plant extracts showed linear dose-dependent suppression of inflammatory markers. However, it agrees with findings by Okafor *et*

al. (2020), who noted dual-phase effects of *Icacina trichantha*, where lower doses downregulated pro-inflammatory markers, but higher doses tended to normalize or mildly stimulate them.

CONCLUSION

Data from this study revealed that in response to Aluminium Chloride-induced anaemia, there was no significant changes in white blood cell (WBC) parameters. Platelet parameters remained largely unchanged, with the exception of platelet distribution width (PDW), which showed a statistically significant difference between two groups. Treatment with the aqueous leaf extract of *Icacina trichantha* showed an inverse dose-dependent effect, as COX-1 mRNA expression increased progressively with higher doses, with the highest dose producing the greatest expression.

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APPENDICES

APPENDIX I

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

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Icacina trichantha* Oliv.

Family: Icacinaceae

Common Name: False Yam, Raynal

Voucher Number: UBH-I185

Student Name: Audu Winnifred Omoye

Plant Identification and Voucher Number Issued by:

03/04/2025

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

APPENDIX II

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

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

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

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

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

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

.....

ATTESTATION BY INVESTIGATOR(S)

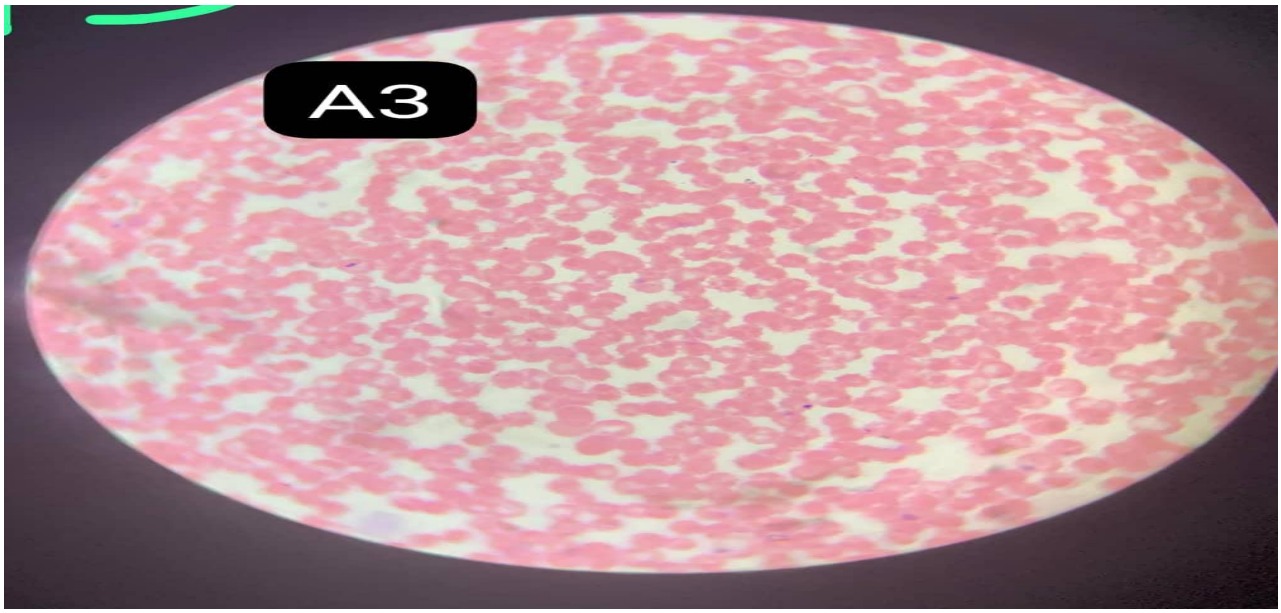
No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the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

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

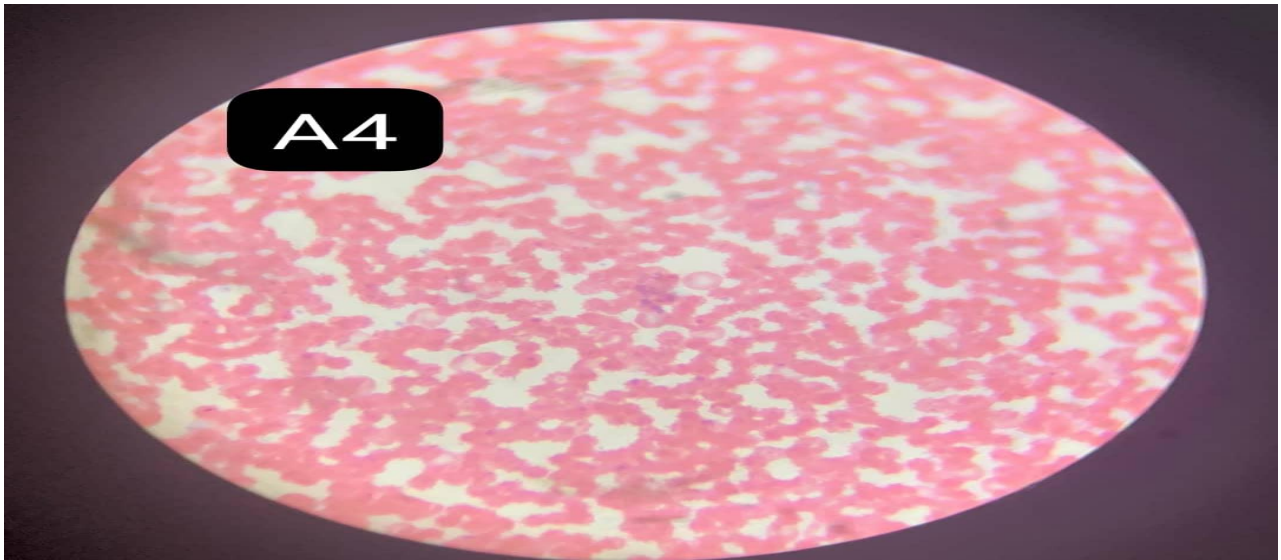
edohrec@edostate.gov.ng

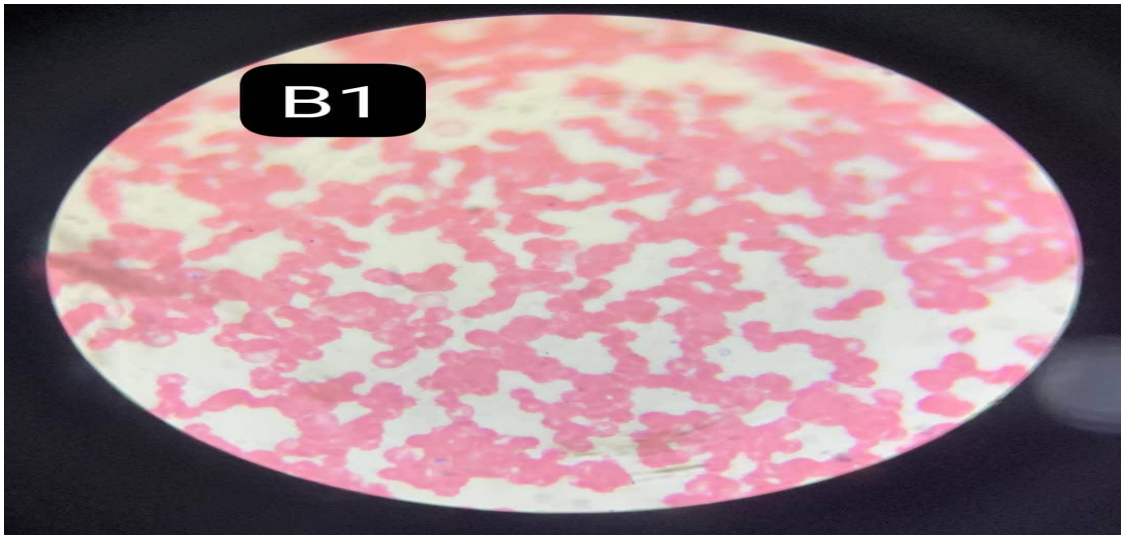
Room 16, Block D, 2nd floor, State secetariat building.

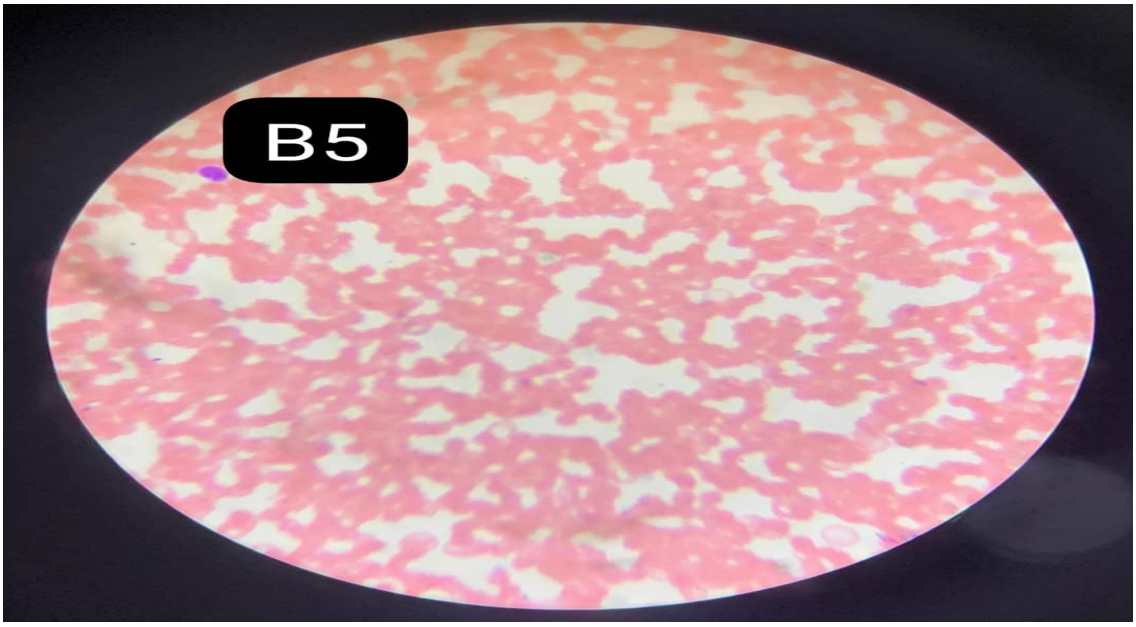
APPENDIX III



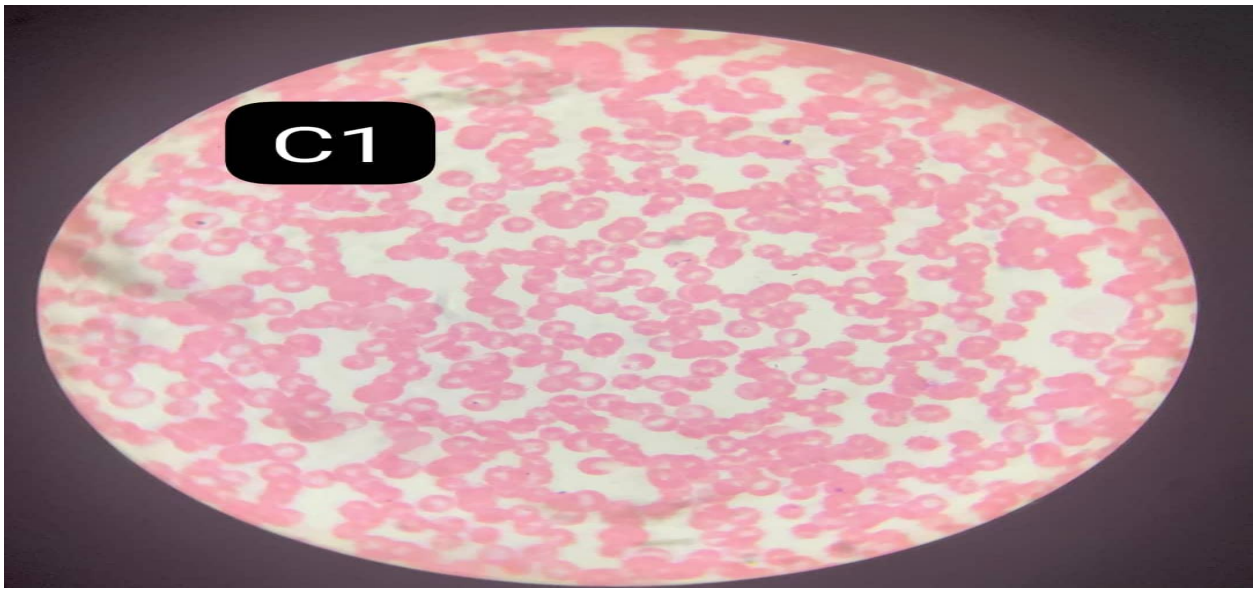
Slides showing the Blood Cell Morphology of Group A Experimental Animals

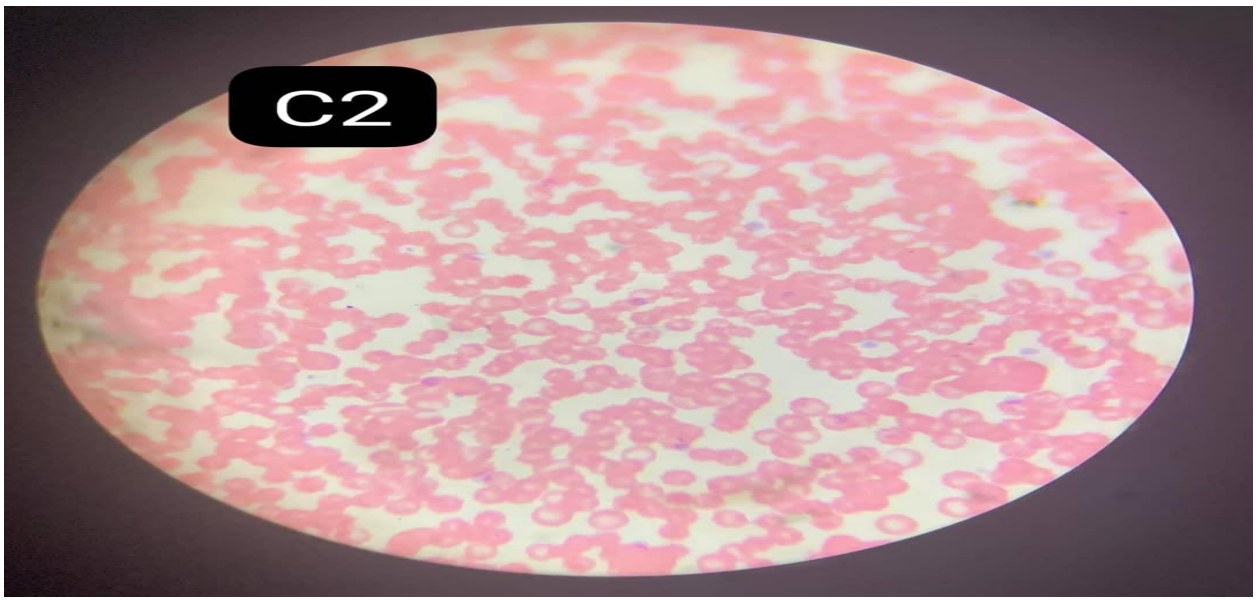




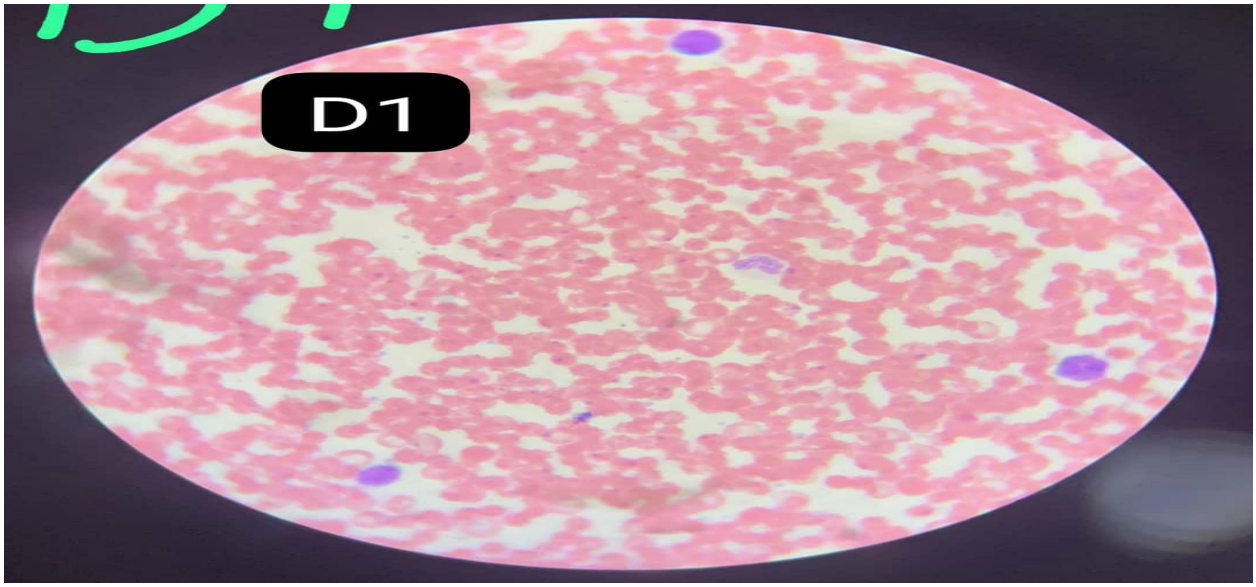


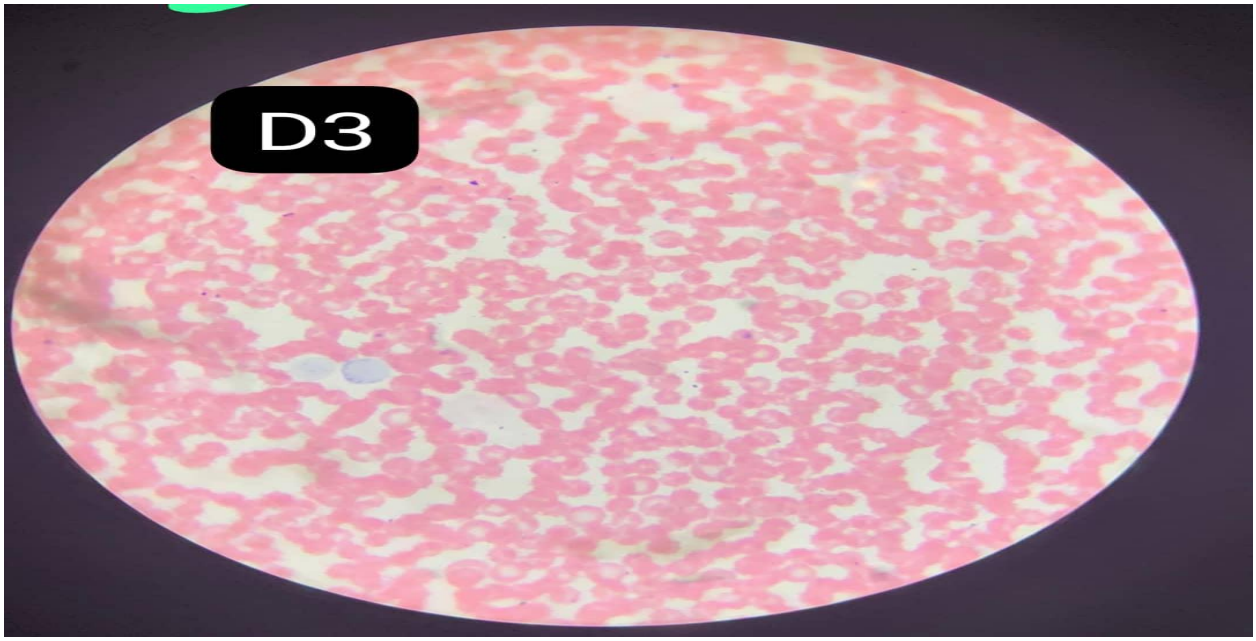
Slides showing the Blood Cell Morphology of Group B Experimental Animals



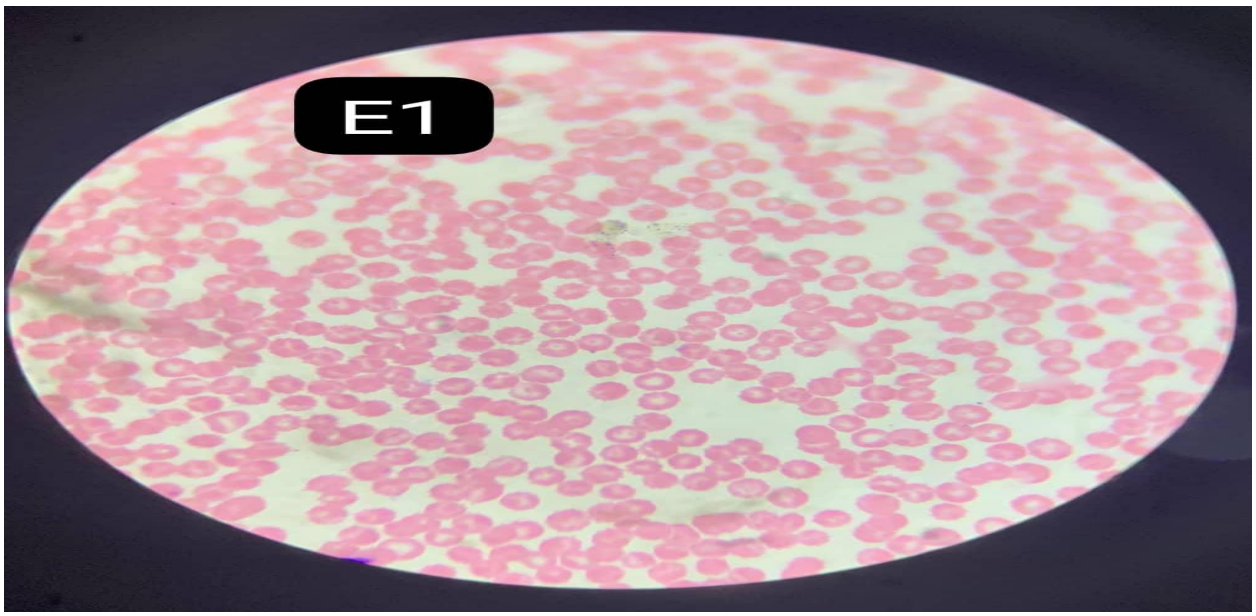


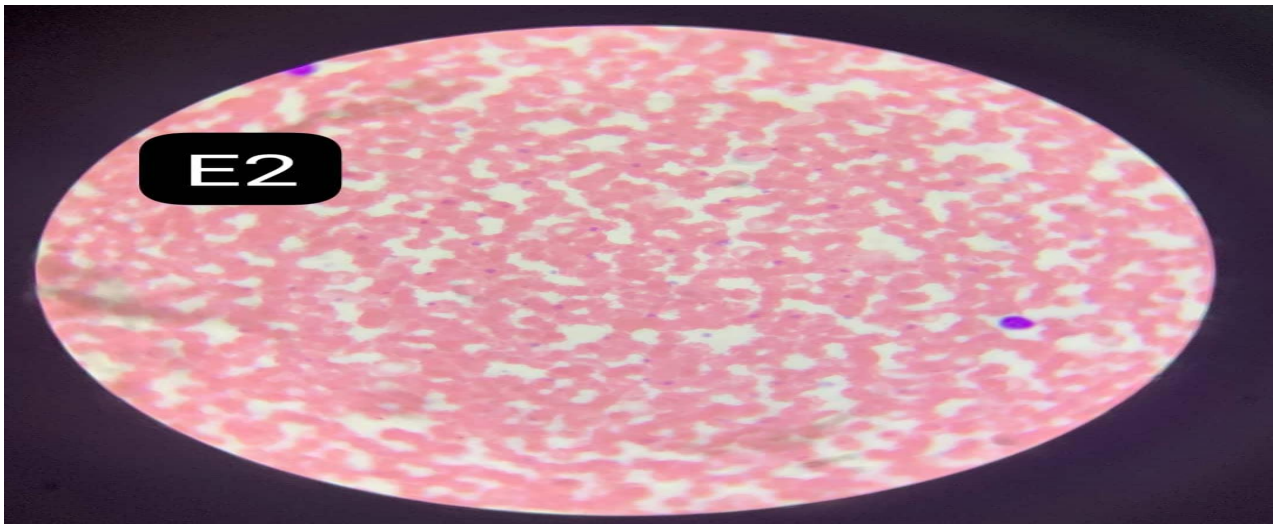
Slides showing the Blood Cell Morphology of Group C Experimental Animals



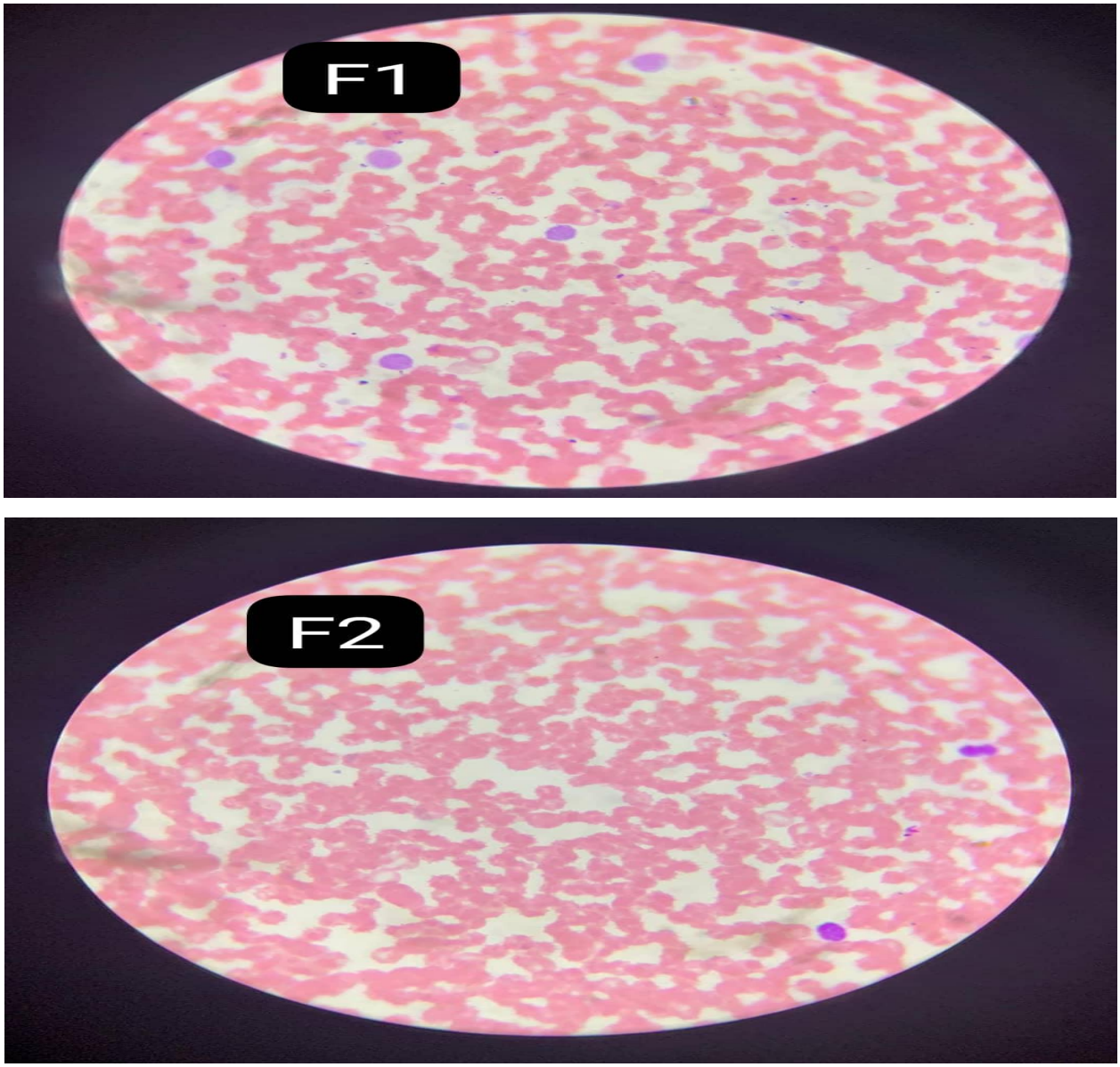


Slides showing the Blood Cell Morphology of Group D Experimental Animals





Slides showing the Blood Cell Morphology of Group E Experimental Animals



Slides showing the Blood Cell Morphology of Group F Experimental Animals

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