

**EFFECTS OF *Alluim Sativum* EXTRACT ON KIT AND FLT3 GENE
EXPRESSION IN BENZENE INDUCED HEMATOXICITY IN MALE
ALBINO WISTAR RATS**

BY

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**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES,
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BENIN CITY.**

MARCH, 2024.

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL
LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCE,
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REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL
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MEDICAL LABORATORY SCIENCE**

SUPERVISED BY: DR. A. I. ARUOMAREN

MARCH, 2024.

CERTIFICATION

This is to certify that this project work was carried out by **IGENEGBAI ANITA OLERE** with the matriculation number **BMS1802460** under the supervision of **Dr. A. I. Aruomaren** in partial fulfilment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

I dedicate this project work to God Almighty for making this seminar work a huge success and also to my lovely family for their love and support.

ACKNOWLEDGEMENT

I give thanks to God for His grace upon my life and for seeing me through this Project work.

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ABSTRACT

Allium sativum, commonly known as garlic, has been traditionally used for its medicinal properties, including anti-cancer effects. The aim of this study was to determine the effect of *Allium sativum* aqueous extract on KIT and FLT3 in male Albino Wistar rats. Twenty (20) male adult Albino Wistar rats were selected into four groups of 5 rats per group. The groups were control (group 1), group 2 (administered 0.2ml intravenous injection of benzene:2-propanol: water mixture (2:5:5 v/v) per body weight), group 3 (induced+400mg/kg extract of *Allium sativum* extract) and group 4 (400mg/kg extract of *Allium sativum* extract only). The mRNA expressions of KIT and FLT3, Haematological parameter and Cell morphology was carried out using Polymerase chain reaction, ERMA Haematology Auto analyser PCE-210N and peripheral blood film using Leishman staining technique. Data generated was analyzed using graph pad prism (version 8.02, California, USA). The findings revealed that there was significant increase ($p<0.01$) in FLT 3 expression in group 2, 3 and 4 when compared to control. Also, group 3 had a significantly higher ($p<0.001$) FLT 3 mRNA expression when compared to group 2 and 4. There was significant increase ($p<0.01$) in cKIT mRNA expression in groups 2, 3 and 4 when compared to control. In addition, group 2 had a significantly higher ($p<0.01$) cKIT when compared to groups 3 and 4. For the haematological parameter, neutrophil count was significantly higher in the control group (10 ± 0.5774) compared to group 2 (3.5 ± 0.2887), group 3 (2.667 ± 0.3333), and group 4 (3.333 ± 0.333). Neutrophil lymphocyte ratio was significantly higher in the control group (0.122 ± 0.00706) compared to group 2 (0.03885 ± 0.0029), group 3 (0.02754 ± 0.0058), and group 4 (0.03663 ± 0.004)., Lymphocyte count was significantly lower in the control group (82 ± 1.528) compared to group 2 (90 ± 1.414), group 3 (91 ± 1), and group 4 (91 ± 0.5774) ($p<0.00$). Cell morphology showed relative lymphocytosis and RBC abnormalities including macrocytic cells and target cells in the benzene + isopropanol and *A. sativum* extract-treated group and Normal leukocyte count with absolute lymphocytosis and normocytic normochromic RBCs, with some polychromatic cells in the *A. sativum* extract-only treated group at 400mg/kg dose. In conclusion, *Allium sativum* can influence leukemogenesis by modulating genes like FLT3 and KIT implicated in the development and progression of leukaemia

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

Medicinal plants have been utilized for millennia across cultures worldwide for their therapeutic properties. These plants contain a vast array of bioactive compounds, which possess medicinal properties (Mustafa *et al.*, 2017). Traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and Indigenous healing practices have long relied on the healing properties of medicinal plants to treat various ailments (Basu *et al.*, 2023). In recent years, scientific research has increasingly focused on exploring the pharmacological effects and mechanisms of action of these plant-derived compounds. This research has led to the development of pharmaceutical drugs based on natural products (Atanasov *et al.*, 2015). Additionally, many people continue to use herbal remedies and botanical supplements as complementary or alternative therapies for conditions ranging from pain and inflammation to gastrointestinal disorders and mental health issues (Kennedy, 2005).

Allium sativum (garlic) is a species in the *Allium* genus native to Central Asia and northeastern Iran. However, it has been cultivated and widely distributed throughout the world for thousands of years due to its culinary and medicinal properties. Garlic has been an integral part of various cuisines globally, adding flavor and aroma to dishes. Beyond its culinary uses, garlic has a long history of medicinal use, dating back to ancient civilizations such as the Egyptians, Greeks, and Romans (Dorrigiv *et al.*, 2020). It has been traditionally used to treat a wide range of health conditions, including infections, cardiovascular diseases, and digestive issues (Alam *et al.*, 2016). Garlic contains several bioactive compounds responsible for its medicinal properties, including allicin, diallyl disulfide, and S-allyl cysteine. These compounds exhibit antioxidant, anti-

inflammatory, antimicrobial, and cardiovascular benefits. As a result, garlic supplements are commonly used to support heart health, boost immune function, and reduce the risk of certain diseases (Ozma *et al.*, 2023). Research has shown that garlic possesses antimutagenic and antiproliferative properties that can be used in anticancer interventions for example in cases of leukaemia (Miroddi *et al.*, 2011).

Leukaemia is a type of cancer that affects the blood and bone marrow, resulting in the abnormal proliferation of white blood cells. This disease can be classified into several subtypes, with the two main categories being acute leukaemia and chronic leukaemia, depending on the speed of disease progression (Davies *et al.*, 2014). Acute leukaemias progress rapidly and require immediate treatment, while chronic leukaemias progress more slowly (Seth and Singh, 2015). Several genetic abnormalities have been identified in leukaemia, contributing to its development and progression. One example is the Philadelphia chromosome, a chromosomal abnormality resulting from a translocation between chromosomes 9 and 22, leading to the formation of the BCR-ABL1 fusion gene. This fusion gene produces a constitutively active tyrosine kinase protein, driving the uncontrolled growth of leukemic cells in chronic myeloid leukaemia (CML) and a subset of acute lymphoblastic leukaemia (ALL) (Kang *et al.*, 2016). Another example is mutations in genes such as FLT3 and KIT, which are commonly found in acute myeloid leukaemia (AML) and affect cell proliferation, differentiation, and survival pathways (Quan and Deng, 2020).

KIT, also known as the stem cell factor receptor or CD117, is a gene that encodes a receptor tyrosine kinase protein crucial for normal hematopoiesis, the process by which blood cells are formed (Escribano *et al.*, 1998). However, dysregulation of KIT signaling can contribute to the development and progression of leukaemia. Mutations in the KIT gene, particularly in the

juxtamembrane domain and activation loop of the receptor, have been identified in various types of leukaemia, including acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), and mast cell leukaemia. These mutations result in constitutive activation of the KIT protein, leading to uncontrolled proliferation, survival, and differentiation of leukemic cells (Scholl *et al.*, 2008). In particular, KIT mutations are commonly found in certain subtypes of AML, such as core-binding factor AML (CBF-AML), where they are associated with poorer outcomes (Boissel *et al.*, 2006). Targeted therapies that inhibit KIT signaling, such as tyrosine kinase inhibitors, have shown promising results in preclinical studies and clinical trials, highlighting the potential importance of targeting KIT in the treatment of leukaemia (Traxler *et al.*, 2001).

FLT3 (Fms-like tyrosine kinase 3) plays a critical role in the pathogenesis of leukaemia, particularly acute myeloid leukaemia (AML) (Pemmaraju *et al.*, 2014). FLT3 is a receptor tyrosine kinase that regulates haematopoietic stem and progenitor cell proliferation, survival, and differentiation. In normal hematopoiesis, FLT3 signaling is tightly regulated to maintain homeostasis. However, mutations in the FLT3 gene, such as internal tandem duplications (ITDs) or point mutations in the tyrosine kinase domain (TKD), can lead to constitutive activation of the FLT3 protein, driving the uncontrolled proliferation of leukemic cells (Grafone *et al.*, 2012). These mutations are found in approximately one-third of AML cases and are associated with a poor prognosis, including increased risk of relapse and reduced overall survival. As a result, FLT3 inhibitors have been developed as targeted therapies to block aberrant FLT3 signaling and improve treatment outcomes in FLT3-mutated AML (Engen *et al.*, 2014).

Understanding the effect *Allium sativum* on KIT and FLT3 in the context of leukaemia is essential for developing targeted therapies that specifically inhibit aberrant signaling pathways and improve treatment outcomes for patients with this devastating disease.

1.2. Justification of Study

KIT and FLT3 are receptor tyrosine kinases that have been implicated in the pathogenesis of leukaemia, making them important targets for research. Secondly, *Allium sativum*, commonly known as garlic, has been traditionally used for its medicinal properties, including anti-cancer effects. However, its potential impact on the expression of KIT and FLT3 in the context of leukaemia remains poorly understood. Therefore, investigating the expression of KIT and FLT3 in pre-leukemic rats administered *Allium sativum* extract could provide valuable insights into the molecular mechanisms underlying the possible anti-leukemic effects of garlic and its potential as a therapeutic agent.

1.3. Aim of Study

The aim of this study is to determine the effect of *Allium sativum* aqueous extract on KIT and FLT3 gene expressions in male preleukaemic Albino Wistar rats.

1.4. Specific Objectives

- a. To determine the effect of the effect of *Allium sativum* aqueous extract on KIT gene expression in male preleukaemic Albino Wistar rats.
- b. To determine the effect of the effect of *Allium sativum* aqueous extract on FLT3 gene expression in male preleukaemic Albino Wistar rats.
- c. To determine the effect of *Allium sativum* aqueous extract on some haematological parameters in male preleukaemic Albino Wistar rats.

1.5. Research Questions

- a. Does *Allium sativum* aqueous extract have any effect on KIT gene expression in male preleukaemic Albino Wistar rats?

- b. Does *Allium sativum* aqueous extract have any effect on FLT3 gene expression in male preleukaemic Albino Wistar rats?
- c. Does *Allium sativum* aqueous extract have any effect on haematological parameters in male preleukaemic Albino Wistar rats?

1.6. Research Hypothesis

1.6.1. Null Hypothesis (H₀)

- *Allium sativum* aqueous extract does not have any effect on KIT and FLT3 gene expressions and haematological parameters in male preleukaemic Albino Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of *Garlic (Allium sativum)*

Garlic, *Allium sativum* L. (Figure 2.1) is a member of the *Alliaceae* family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders (Londhe *et al.*, 2011). Other members of the onion genus, *Allium*, include *A. cepa* (onion), *A. schoenoprasum* (chives), *A. ascalonicum* (shallots), and *A. porrum* (leeks). *Allium sativum* is further divided into two subspecies, *Allium sativum* var. *sativum*, also known as softneck garlic, and *Allium sativum* var. *ophioscorodon*, also known as hardneck garlic (Bal *et al.*, 2023). Both varieties are composed of an underground bulb made up of cloves, which are prophylls enclosed by dry membranous skins and held together by a basal plate. The variations differ in that hard neck garlic's bulb is composed of six to eleven cloves, circled around a centralized woody stalk (Alam *et al.*, 2016). *Allium sativum* is sterile and hence is grown asexually from cloves, not requiring a pollinator. It grows best in mild climates, though hardneck varieties are better adapted to colder environments (Block, 1992). The name garlic may have originated from the Celtic word 'all' meaning pungent. Cultivated practically throughout the world, garlic appears to have originated in central Asia and then spread to China, the Near East, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa (Egypt) and Mexico (Singh and Singh, 2008). Garlic has been used for thousands of years for medicinal purposes. Sanskrit records show its medicinal use about 5,000 years ago, and it has been used for at least 3,000 years in Chinese medicine (Papu *et al.*, 2014). The Egyptians, Babylonians, Greeks, and Romans used garlic for healing purposes (Petrovska and Cekovska, 2010). In 1858, Pasteur noted garlic's antibacterial activity, and it was used as an antiseptic to

prevent gangrene during World War I and World War II (Papu *et al.*, 2014). Garlic's current principal medicinal uses are to prevent and treat cardiovascular disease by lowering blood pressure and cholesterol, as an antimicrobial, and as a preventive agent for cancer. The active constituents are several complex sulfur-containing compounds that are rapidly absorbed, transformed and metabolized (Londhe *et al.*, 2011). Pooled data from numerous randomized trials suggest that garlic lowers total cholesterol concentrations by approximately 10% and favorably alters HDL/LDL ratios. Randomized trials also support garlic's effectiveness as a mild antihypertensive which lowers blood pressure by 5-7%. Garlic also inhibits platelet aggregation and enhances fibrinolytic activity, reducing clots on damaged endothelium. In vitro data suggest antibacterial effects, but these have not been evaluated in controlled trials in humans (Tattelman, 2005).



Figure 2.1. Bulb *Allium sativum* (Garlic) (Salgado *et al.*, 2011).

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

S/N	Species
1	<i>Allium cepa</i> - Common Onion
2	<i>Allium sativum</i> - Garlic
3	<i>Allium schoenoprasum</i> - Chives
4	<i>Allium ampeloprasum</i> - Leek
5	<i>Allium fistulosum</i> - Welsh Onion
6	<i>Allium canadense</i> - Wild Onion
7	<i>Allium moly</i> - Golden Garlic
8	<i>Allium ascalonicum</i> (shallots)

Table 2.2 Botanical classification of Garlic (*Allium sativum*) (Simon and Jenderek, 2010).

Taxa	Name
Domain	<i>Eukaryote</i>
Kingdom	<i>Plantae</i>
Division	<i>Spermatophyte</i>
Sub-division	<i>Spermatophytin</i>
Class	<i>Magnoliopsida</i>
Order	<i>Aspargales</i>
Family	<i>Amaryllidaceae</i>
Genus	<i>Allium</i>
Species	<i>Sativum</i>

2.1.1. Uses of *Allium sativum*

Garlic is a popular bulb vegetable that is used to season and flavour dishes (Velisek *et al.*, 1997). Its strong flavour makes it a popular spice or condiment all throughout the world. Furthermore, Tesfaye and Mengesha (2015) discovered that garlic is employed in the preparation of dishes, notably stews, as well as the production of dried foods for preservation purposes. Garlic's pungent, lachrymatory properties, and spicy scent are caused by the presence of organosulfur compounds such as allicin and diallyldisulfide. Garlic improves the flavour of dishes while also making them easier to digest. It is a key element in the world's most popular cuisines. Garlic is used as a spice in both fresh and dehydrated forms in the culinary business. It is dehydrated into several products, including flakes, slices, and powders. Garlic, in addition to giving flavour to dishes, includes a variety of minerals, vitamins, and other elements that are beneficial to human health. It contains sugar, protein, fat, calcium, potassium, phosphorus, sulphur, iodine fibre, and silicon, in addition to vitamins. It has a high nutritional value. Its strong flavour makes it mostly employed as a spice, seasoning, and flavouring agent in foods including both green tops and bulbs (Ahmad, 1996).

2.1.2. Chemistry and Pharmacology of *Allium sativum*

Sulfur compounds are the main chemical constituents responsible for *Allium sativum*'s taste, smell, and likely for its biological effects. When a garlic clove is intact, glutamyl cysteines are the primary sulfur components (Powolynny and Singh, 2008). These are hydrolyzed to form alliin. When garlic is crushed by chewing, chopping, etc., the alliin promptly reacts with the enzyme alliinase to form allicin; after 30 seconds the reaction is 97% complete. Allicin is a diallyl thiosulfinate that accounts for 70-80% of the thiosulfinates present in *Allium sativum* (Amagase *et al.*, 2001). Allicin is also highly unstable and quickly decomposes to yield sulfur compounds

when oxidized such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, and hydrogen sulfide (Banerjee and Maulik, 2002). DAS, DADS, DATS and ajoene are all oil soluble (Miroddi *et al.*, 2011). When garlic is manipulated to produce alternate forms, the unstable sulfur compounds react and hence alter active chemical constituents. It is important to note that these changes in chemistry can alter the bioavailability of the compounds. Unfortunately, many studies do not specify the actions taken, which could possibly account for inconsistencies in reported data. Traditionally used in its raw form, garlic is now often heated, dehydrated, and aged. Only freshly crushed garlic has hydrogen sulfide, which is suspected to have significant cardioprotective effects as a vasodilator (Mukherjee *et al.*, 2009).

2.1.3. Anticancer Activity of *Allium sativum*

Several studies have established the chemopreventive efficacy of garlic utilising various garlic preparations such as fresh garlic extract, aged garlic, garlic oil, and a range of organosulfur compounds produced from garlic. The presence of organosulfur compounds in garlic is thought to contribute to its chemopreventive action (Ejaz *et al.*, 2007). The particular mechanism of action was not entirely known, although various options were presented. These include its impact on drug metabolising enzymes, antioxidant capabilities, and tumour growth inhibition. The majority of these investigations were done out using animal models. In addition, current study has focused on garlic's antimutagenic effect (Islam *et al.* 2011). Recently, it was discovered that aged garlic extract, but not fresh garlic extract, had radical scavenging action. The two primary components in old garlic, S-allylcysteine and S-allylmercapto-L-cysteine, had the strongest radical scavenging action. Furthermore, various animal models have shown that several organosulfur compounds produced from garlic, such as S-allylcysteine, inhibit the formation of

chemically created and transplantable tumours. Thus, garlic ingestion may give some protection against cancer growth (Lau *et al.*, 1990).

2.1.4. Wound Healing Activity of *Allium sativum*

Successful wound healing depends upon angiogenesis, and impaired angiogenesis is a hallmark of the chronic wounds encountered with diabetes and venous or arterial insufficiency. To intervene and improve wound closure, it is essential to investigate the effects of different natural remedies in wound healing (Alam *et al.*, 2016). Study was done on the chicken dorsum skin excision wound assay to investigate the influence of different concentrations of aged garlic solution (AGS) on wound healing. Gross, histopathology, scanning electron microscopy (SEM) and computer-based three-dimensional (3D) image probing techniques were utilized to determine the effects of AGS on wound closure, re-epithelialization, dermal matrix regeneration and angiogenesis (Jalali *et al.*, 2009).

2.1.5. Antidiabetic Activity of *Allium sativum*

In diabetic patients, it was reported that garlic oil can correct hyperglycemia. In addition, a precursor of various allyl sulfide constituents of garlic oil, S-allylcysteine sulfoxide (allin), was shown to have a hypoglycemic effect similar to that of glibenclamide. Garlic has been found to be effective in lowering serum glucose levels in streptozotocin (STZ)-induced as well as alloxan-induced diabetic rats and mice (Liu *et al.*, 2006). Most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits. It is not clear how garlic actually works in alleviating hyperglycaemia. The hypoglycaemic action of garlic could possibly be due to an increase in pancreatic secretion of insulin from β -cells, release of bound insulin or enhancement of insulin sensitivity (Khayatnouri *et al.*, 2011). It has been previously suggested that garlic (allicin) can enhance serum insulin by effectively combining with compounds like

cysteine, which would spare insulin from SH group reactions which are a common cause of insulin inactivation. Another mechanism proposed by researcher states that the antioxidant effect of S-allyl cysteine sulfoxide, an isolated product from garlic, may contribute to its beneficial effect in diabetes (Thomson *et al.*, 2007). Research postulate garlic may act as an antidiabetic agent by increasing either the pancreatic secretion of insulin from the β -cells or release of bound insulin (Mahesar *et al.*, 2010).

2.1.6. Antibacterial Properties of *Allium sativum*

The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium* (Yin and Cheng, 2003). Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic. Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl Sulfides isolated and identified the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur compound which they termed allicin from the Latin name of the garlic plant, *Allium sativum* (Ankri and Mirelman, 1999).

2.1.7. Antifungal Activity of *Allium sativum*

Ajoene is an active compound found in garlic which plays a great role as topical antifungal agent (Ledezma and Apitz-Castro, 2006). Garlic has been shown to inhibit growth of fungal diseases as equally as the drug ketoconazole, when tested on the fungi *Malassezia furfur*, *Candida albicans*, *Aspergillus*, *Cryptococcus* and other *Candida* species (Shams-Ghahfarokhi *et al.*, 2006). A report from a Chinese medical journal delineates the use of intravenous garlic to treat a potentially fatal

and rare fungal infection of the brain called *Cryptococcus* meningitis. In the report, the Chinese compared the effectiveness of the garlic with standard medical treatment which involved a very toxic antibiotic called Amphotericin-B. The study revealed that, intravenous garlic was more effective than the drug and was not toxic regardless of its dosage (Lemar *et al.*, 2007). A study found that *Candida* colonies were substantially reduced in mice that had been treated using liquid garlic extract. The study also revealed that garlic stimulated phagocytic activity. This implies that infections such as *Candida* may be controlled because garlic stimulates the body's own defenses. Garlic oil can be used to treat ringworm, skin parasites and warts if it is applied externally. Lesions that were caused by skin fungi in rabbits and guinea pigs were treated with external applications of garlic extract and began to heal after seven days (Sabitha *et al.*, 2005).

2.1.8. Antioxidant Activity of *Allium sativum*

In vivo antioxidant effects of several organosulfur compounds derived from garlic have been studied. In one study, two lipophilic organosulfur compounds, diallyl sulfide (DAS) and diallyl disulfide (DADS) and two hydrophilic organosulfur compounds, s-ethyl cysteine (SEC) and n-acetyl cysteine (NAC), protected against lipid related oxidations by activating associated antioxidant enzymes (Yoshida *et al.*, 1987). The in vivo antioxidant effects of four test organosulfur compounds against lipid-associated oxidations have been studied by researcher reported that these antioxidant effects were due to the activation and modification of several enzymes such as 3-hydroxy-3-methylglutaryl-CoA reductase, glutathione-s-transferase and catalase (Tsai *et al.*, 2005).

2.1.9. Dosage of *Allium sativum*

A commercial garlic product should provide a daily dose equal to at least 4000mg (one to two cloves) of fresh garlic. The cloves may be diced and mixed with wildflower honey for

palatability. This dosage translates to at least 10mg alliin or a total allicin potential of 4000ug (Bongiorno *et al.*, 2008). In dried form this would be 300-mg of garlic powder tablet (standardized to 1.3 percent alliin or 0.6 percent allicin yield) two to three times per day, or 7.2 g of aged garlic extract per day. In tincture form from fresh bulb as a 1:2 in 95 % alcohol, the dosage can be 40 drops up to six times per day (Tattelman, 2005).

2.1.10. Adverse Effects of *Allium sativum*

The most prevalent side effect of garlic consumption is bad breath, particularly when the plant is raw. Other prominent side effects include nausea and vomiting, so use caution when taking large doses. Although a full bulb generates little juice, it is effective and can serve as a severe emetic, even in tiny doses. Although garlic offers few safety concerns, there have been sporadic reports of topical garlic burns (Friedman *et al.*, 2006) and allergy (Yin and Li, 2007). Rare garlic allergies have been linked to the protein allinase, which causes immunoglobulin E (IgE)-mediated hypersensitivity reactions in skin prick tests (Kao *et al.*, 2004). As a result, the literature has generally cautioned against using garlic while using anticoagulant therapy (Saw *et al.*, 2006).

2.2. Leukaemia

Leukaemia is a malignancy that develops in the body's blood-forming tissues, specifically the bone marrow and lymphatic system. It is classified either with the WHO or FAB system (figure 2.2). It is distinguished by the fast creation of aberrant white blood cells, which are critical to the immune system's function. These aberrant cells push out good blood cells, causing a weakened immune system, anaemia, and bleeding issues. Leukaemia can be acute or chronic, and it is further classified according to the kind of white blood cells involved. Symptoms may include weariness, recurrent infections, easy bruising or bleeding, swelling lymph nodes, and weight loss

(Manisha, 2012). Leukaemia treatment options often include chemotherapy, radiation therapy, targeted therapy, or stem cell transplantation, depending on the kind and stage of the illness.

While medical improvements have increased survival rates, leukaemia remains a severe and sometimes fatal condition that need continued study and novel therapies (Pollyea *et al.*, 2014).

Leukaemia is one of the most frequent malignant conditions impacting the global population.

Globally, leukaemia was the fifteenth most prevalent cancer diagnosed in 2018, with 437,033 cases and 309,006 deaths, making it the eleventh leading cause of death from malignant illnesses.

Leukaemia has a global geographic distribution, with greater frequency and fatality rates in industrialised nations. However, poorer nations have a higher death rate (Bray *et al.*, 2018).

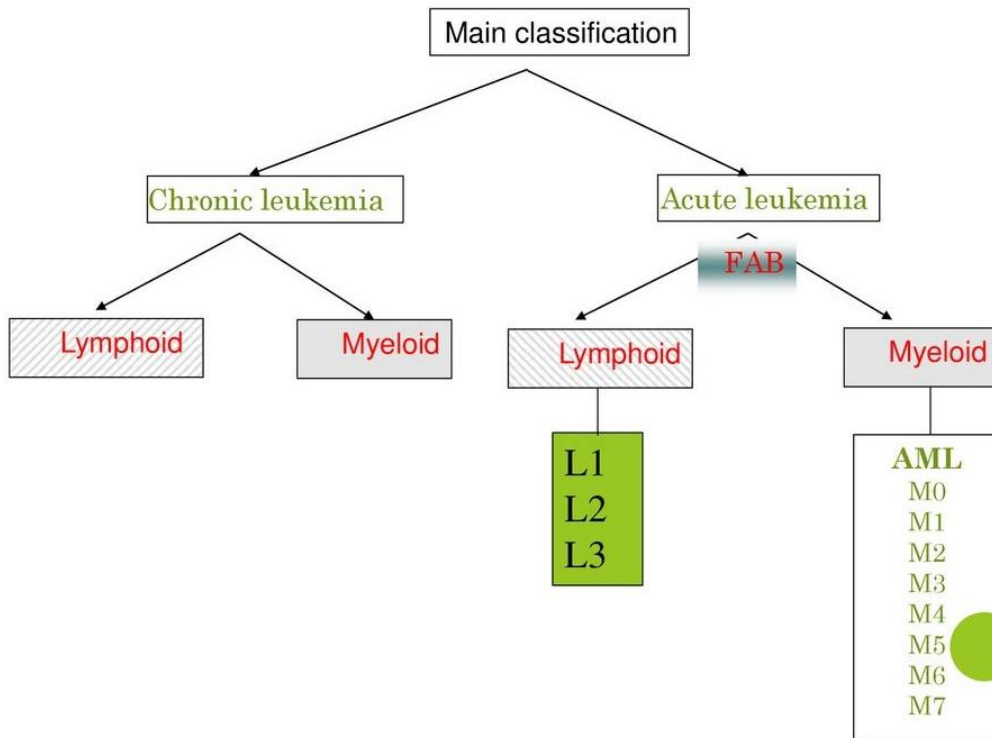


Figure 2.2. French-American-British (FAB) Classification of Leukaemia (Estey and Dohner, 2006).

2.2.2. Acute Lymphoblastic Leukaemia

Acute lymphoblastic leukaemia (ALL) is a type of cancer that affects the blood and bone marrow, characterized by the abnormal proliferation of immature lymphoid cells, called lymphoblasts. ALL is the most common type of leukaemia in children, although it can also occur in adults. The disease typically arises from genetic mutations that disrupt the normal development and function of lymphoid progenitor cells, leading to their uncontrolled growth and accumulation (Cortes and Kantarjian, 1995). Common symptoms of ALL include fatigue, fever, easy bruising or bleeding, bone pain, and frequent infections. Diagnosis is made through blood tests, bone marrow biopsy, and imaging studies. Treatment for ALL usually involves chemotherapy, often in combination with other therapies such as radiation therapy, targeted therapy, and stem cell transplantation (Desmond *et al.*, 2019).

2.2.3. Acute Myeloid Leukaemia (AML)

Acute myeloblastic leukaemia (AML) has two peaks in incidence: in early infancy and later in adulthood. The median age of newly diagnosed individuals with AML is 66 years. While the condition can affect anybody, it is uncommon to be diagnosed before the age of 40. The aetiology of AML is uncertain. A wealth of information and understanding on leukemogenic medicines, particularly chemotherapy regimens used to treat a wide range of malignant illnesses, has collected (Tebbi *et al.*, 2007). Certain molecular abnormalities, such as the t (8;21) translocation and chromosomal 16 inversion, have been linked to AML. In addition to genetic abnormalities, epigenetic modifications such as promoter silencing by hypermethylation of the p15/INK4b and other genes in the aetiology of AML have been identified. Certain genetic variables, including as genetic abnormalities, are thought to be associated with AML, particularly in youngsters. Patients with various genetic diseases, such as Down's syndrome,

have a much greater risk of developing malignant conditions, including AML. Children with Trisomy 21 have a 10- to 20-fold greater risk of having acute leukaemia, particularly AML (Khan *et al.*, 2011).

2.2.4. Chronic Lymphoblastic Leukaemia (CLL)

Chronic lymphocytic leukaemia (CLL) is a type of cancer that affects the blood and bone marrow, characterized by the abnormal proliferation of mature lymphocytes, typically B cells, which accumulate in the blood, bone marrow, and lymphoid tissues. CLL is the most common type of leukaemia in adults, typically occurring in older individuals. It often progresses slowly, and many patients may not experience symptoms in the early stages of the disease (Yee and O'Brien, 2006). Common symptoms of CLL may include fatigue, swollen lymph nodes, unintentional weight loss, and frequent infections. Diagnosis is made through blood tests, bone marrow biopsy, and imaging studies (Méhes, 2005). While CLL is not usually curable, treatment options are available to help manage the disease and control symptoms, including chemotherapy, immunotherapy, targeted therapy, and stem cell transplantation. The prognosis for CLL varies depending on various factors, including the stage of the disease, the presence of specific genetic abnormalities, and the overall health of the patient (Shadman, 2023).

2.2.5. Chronic Myeloid Leukaemia

Chronic myeloid leukaemia (CML) is a type of cancer that affects the blood and bone marrow, characterized by the abnormal proliferation of myeloid cells, particularly granulocytes, in the bone marrow. CML is characterized by the presence of the Philadelphia chromosome, resulting from a reciprocal translocation between chromosomes 9 and 22, leading to the formation of the BCR-ABL1 fusion gene. This fusion gene produces a constitutively active tyrosine kinase protein, driving the uncontrolled growth of myeloid cells (Cardama and Cortes, 2006). CML

typically progresses slowly through three phases: chronic phase, accelerated phase, and blast crisis. Common symptoms of CML may include fatigue, abdominal discomfort due to an enlarged spleen, unintentional weight loss, and fever. Diagnosis is made through blood tests, bone marrow biopsy, and genetic testing (Cardama and Cortes, 2006). Treatment for CML has been revolutionized by the development of tyrosine kinase inhibitors (TKIs), which specifically target the BCR-ABL1 protein and have dramatically improved outcomes for patients with this disease (Braun *et al.*, 2020).

2.3. KIT Gene

KIT, short for Kirsten rat sarcoma viral oncogene homolog, is a crucial gene that encodes a type III receptor tyrosine kinase protein, known as c-KIT or CD117. This protein plays a pivotal role in various cellular processes, including cell survival, proliferation, and differentiation. The KIT gene is particularly significant in the context of cancer, as mutations in this gene have been implicated in the development of several malignancies (Quesada and Amato, 2012). One of the most well-known cancers associated with KIT mutations is gastrointestinal stromal tumors (GISTs), where aberrant activation of the KIT protein contributes to tumor growth and progression (Xu *et al.*, 2014). Additionally, mutations in the KIT gene have been identified in other cancers such as acute myeloid leukaemia (AML) and mastocytosis (Patnaik *et al.*, 2007). Targeted therapies that specifically inhibit the activity of the KIT protein have been developed and utilized in the treatment of these KIT-driven cancers, offering new hope for patients with these diseases. An essential role for c-Kit in haematopoietic cells is indicated by the phenotypes caused by mutations in the loci of either the receptor or the ligand (White spotted and Steel mutants, respectively) (Woodman and Davies, 2010). Mutations in these genes have been

observed to cause lethal anaemia, haematopoietic stem cell defects and mast cell deficiency in mice (Russell, 1979).

2.3.1. Structure of c-KIT

The proto-oncogene c-kit, mapped to chromosome 4q11-12 in humans and chromosome 5 (W locus) in mice, was discovered in 1986 as the cellular homolog of the transforming viral oncogene v-kit in the Hardy-Zuckerman 4 feline sarcoma virus. Wild-type c-kit encodes for a 145 kDa, 976 amino acid type IIIa RTK protein known as c-KIT, which is often referred to as CD117 or stem cell factor (SCF) receptor due to its association with its ligand SCF (Mol *et al.*, 2004). The c-KIT protein resides in the cell membrane and is comprised of extracellular domain (EC), transmembrane (TM), and intracellular (IC) regions. Like all class III RTKs, the EC portion of c-KIT comprises five Ig-like domains (D1-D5). The first three domains are essential for c-KIT binding to SCF, whereas D4 and D5 are involved in dimerizing adjacent c-KIT monomers (Lennartsson and Rönstrand, 2012). The EC region is followed by a single spanning TM helix that connects with the IC domain, including a JM domain coupled to a TK domain and a C-terminal tail region. The JM domain is essential for c-KIT receptor control and modulation, particularly in the relay of IC downstream signaling (Chan *et al.*, 2003). The TK domain is split into the proximal amino-terminal lobe (N-lobe) TK1 with an ATP binding region, and a distal carboxy-terminal lobe (C-lobe) TK2 with a phosphotransferase domain (Gilreath *et al.*, 2019) (Sheikh *et al.*, 2022).

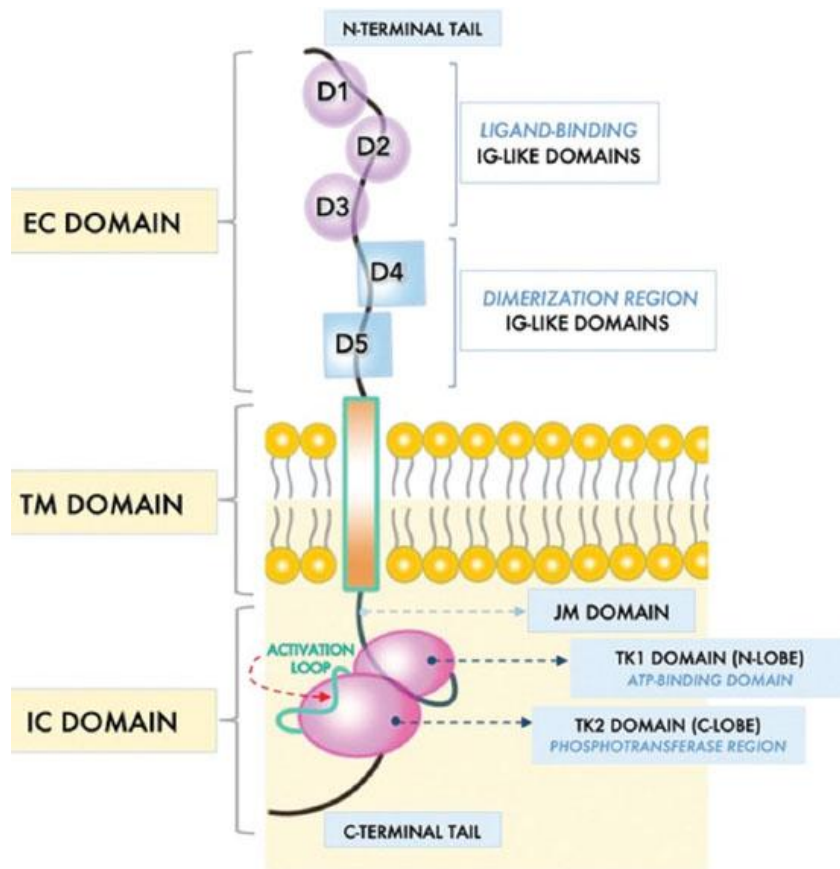


Figure 2.3. Structural Organization of the Human c-KIT Receptor (Sheikh *et al.*, 2022).

2.3.2. Biological Functions Of c-KIT

c-KIT is expressed by various cells in the body, and signaling pathways stimulated by its activation by stem cell factor (SCF) under physiologic conditions are implicated in regulating cellular processes such as cell proliferation, survival and migration (Lennartsson and Rönstrand, 2012). In the normal bone marrow, c-KIT is expressed by haematopoietic stem cells, playing an important role in self-renewal and differentiation into various blood cells. c-KIT expression is gradually lost during haematopoietic differentiation and only retained or increased in mast cells, natural killer (NK) cells and dendritic cells (DCs), suggesting an essential function in inflammation and immunity (Reber *et al.*, 2006). Moreover, different studies have shown that CD117/c-KIT is not only expressed by bone marrow-derived stem cells, but also by those found in other organs in adults, such as prostate, liver and heart, suggesting that SCF/cKIT signaling pathways may contribute to stemness in some organs (Sheikh *et al.*, 2022). Furthermore, c-KIT has been linked to many different biological processes in other cell types. For instance, c-KIT signaling has been shown to regulate oogenesis, folliculogenesis and spermatogenesis, exerting critical functions in female and male fertility (Hutt *et al.*, 2006).

c-KIT is also critical to the proliferation, survival and migration of melanocytes from the neural crest to the dermis (Wehrle, 2003). Loss-of-function mutations in c-kit can cause piebaldism, an autosomal dominant disorder characterized by congenital absence of melanocytes in patches of skin and hair, similar to the “dominant white spotting” observed in mice with mutations in the same gene (Spritz, 1994).

2.3.3. c-KIT and Leukaemia

c-KIT expression is seen in myeloblasts in 65-90% of acute myeloid leukaemia (AML) patients and, in some cases, co-expressed with SCF, suggesting a potential autocrine activation (Ikeda *et*

al., 1991). Furthermore, c-kit mutations are found in AML patients, predominantly associated with core-binding factor leukaemias, an AML variant that involves chromosomal abnormalities t (8;21) (q22; q22) or inv (16) (p13q22)/t (16;16) (p13; q22) (Allen *et al.*, 2013). Most of these constitutive activating mutations mainly reside in exon 8 (as in-frame insertions or deletions that affect an EC domain involved in c-KIT dimerization) or exon 17 (as missense mutations that affect the activation loop in the c-KIT TK domain) (Allen *et al.*, 2013).

2.4. Fms-like Tyrosine Kinase 3 (FLT3)

FLT3 is a gene that encodes a receptor tyrosine kinase protein involved in regulating hematopoiesis, the process of blood cell formation. The FLT3 protein is primarily expressed on the surface of haematopoietic stem and progenitor cells and plays a crucial role in their proliferation, survival, and differentiation (Grafone *et al.*, 2012). Mutations in the FLT3 gene are commonly found in acute myeloid leukaemia (AML), one of the most aggressive forms of leukaemia. These mutations lead to constitutive activation of the FLT3 protein, promoting the uncontrolled growth and survival of leukemic cells (Hasserjian *et al.*, 2013). As a result, FLT3 has emerged as a promising therapeutic target in AML, and targeted therapies that inhibit FLT3 activity have been developed and are being used in clinical practice (Engen *et al.*, 2014).

2.4.1. Structure of FLT3 Receptor

The FLT3 receptor (figure 2.4) (Fms-like tyrosine kinase 3), also known as FLK2 (fetal liver tyrosine kinase 2), STK-1 (stem cell tyrosine kinase 1) or CD135, is encoded by the FLT3 gene located on chromosome 13q12 (Rosnet *et al.*, 1991). This gene consists of 24 exons and covers approximately 96 kb; the exact size is unknown because of the presence of a large intron (>50 kb)

located between exons 2 and 3. The length of the transcript is 3.7 kb and it contains a pseudogene in the open reading frame of 2979 bp. The protein encoded is a transmembrane receptor of 933 amino acids with a molecular weight of 155-160 kDa that belongs to the class III family of receptor tyrosine kinase (RTK) (Rosnet, 1999). The structure consists of four regions:

- A N-terminal extracellular region (541aa) consisting of five immunoglobulin-like domains, of which the three most distal from the plasma membrane are involved in ligand binding, while the proximal domains are involved in dimerization of the receptor
- A transmembrane portion (21aa)
- A juxta membrane (JM) domain
- An intracellular C-terminal region (431aa) with a split kinase domain (Grafone *et al.*, 2012).

The two substructures of this domain are called N-lobe and C-lobe and are connected by an interkinase domain. These lobes consist of a TKD and are also indicated as first tyrosine kinase (TK1) and second tyrosine kinase (TK2) domain, respectively (Griffith *et al.*, 2004). The extracellular region is highly glycosylated and contains a binding domain with high affinity for its ligand (FLT3 ligand or FL). The non-glycosylated isoform has a molecular weight of 130-143 kDa and is not associated with the plasma membrane (Markovic *et al.*, 2005).

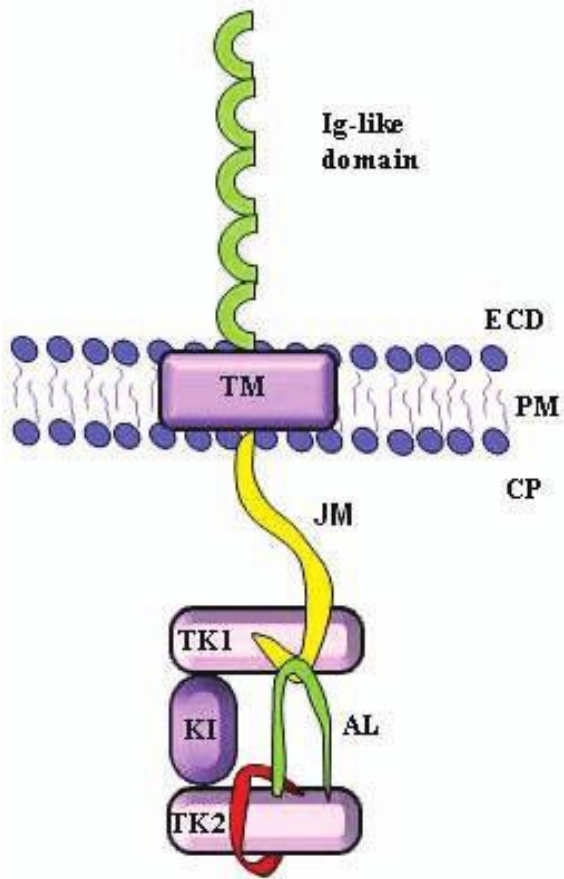


Figure 2.4. Schematic presentation of FLT3 receptor monomer. ECD, extracellular domain; PM, plasma membrane; CP, cytoplasm; TM, transmembrane domain; JM, juxtamembrane domain; TK1, first tyrosine kinase domain, N-lobe; KI, kinase insert; TK2, second tyrosine kinase domain, C-lobe; AL, activation loop (Grafone *et al.*, 2012).

2.4.2. FLT3 Expression and Function in Normal Haematopoietic Cells

FLT3, or Fms-like tyrosine kinase 3, is a critical regulator of hematopoiesis, the process by which blood cells are formed. In normal haematopoietic cells, FLT3 expression is predominantly observed on haematopoietic stem and progenitor cells, where it plays a key role in maintaining their self-renewal and differentiation potential (Boyer *et al.*, 2011). Upon binding of its ligand, FLT3 activates intracellular signaling pathways involved in cell proliferation, survival, and differentiation. Through these mechanisms, FLT3 helps orchestrate the orderly production of various blood cell lineages, including red blood cells, white blood cells, and platelets (Scheijen *et al.*, 2004). The precise regulation of FLT3 expression and activity is crucial for maintaining homeostasis within the haematopoietic system (Tsapogas *et al.*, 2017).

2.4.3. FLT3 Expression in Human Leukaemias

FLT3 is expressed at high levels in 70% to 100% of cases of AML and in a high percentage of ALL cases. For example, Rosnet and colleagues reported expression of FLT3 on leukaemia blasts in 18 of 22 AML and 3 of 5 ALL cases (Rosnet *et al.*, 1996). Carow and coworkers found FLT3 RNA expressed at higher levels than in normal bone marrow in 33 of 33 B-lineage ALL, 11 of 12 AML, and 3 of 11 T-cell ALL cases. Western blotting did not detect FLT3 expression in normal bone marrow, but identified FLT3 protein in 14 of 14 B-cell ALL, 36 of 41 AML, and 1 of 4 T-cell ALL cases (Carow *et al.*, 1996). These data indicate that FLT3 expression may play a role in the survival or proliferation of leukemic blasts. FLT3 is also expressed at high levels in leukaemia and lymphoma cell lines, including pre-B, myeloid, and monocytic cell lines, and FLT3 expression can be detected in a majority of cell lines (Meier *et al.*, 1995).

2.5. Haematological Parameters

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013). Haematological parameters are quantifiable constituents of blood like erythrocytes and its indices, leukocytes and platelets. These blood components originate from the haemopoietic 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 indicators useful in the assessment of immune status, therapeutic purposes and monitoring of disease progression and treatment outcome for proper patient management. In pursuant of effective health care through accurate diagnosis, haematological parameters are routinely assessed (Azuonwu *et al.*, 2017).

2.5.1 Red blood cells

Red blood cells are the main cellular component of the blood. Red cells are flexible and biconcave in shape (Fischbach, 2003). This shape provides a large surface area for the exchange of oxygen to the tissues and organs in the body. The human body contains about 5 litres of blood out of which about 2 litres are red cells (Fischbach, 2003). Each litre of blood contains about 5x red cells, the number varying with state of health, age and gender of an individual. Red cells are produced in the bone marrow (Cheesbrough, 2006), with a span of 120 days in blood after which they are destroyed from circulation and new ones are synthesized.

2.5.2 Packed cell volume (PCV)

Packed cell volume (PCV) also known as haematocrit (HCT) or erythrocyte volume fraction (EVF) is the percentage of red blood cells in blood. Packed cell volume is involved in the transport of oxygen and absorbed nutrients.

Normal range of packed cell volume for males is 40-52 (%), females is between 36-48 (%) (Hoffbrand *et al.*, 2016).

2.5.3 Haemoglobin

Haemoglobin is the iron-containing oxygen-transport metalloproteinase in red blood cells (NseAbasi *et al.*, 2013). It gives the red cell its colour (Fischbach, 2003), with its main function being to transport oxygen to tissues and return carbon dioxide from tissues to the lungs (Hoffbrand *et al.*, 2016). Normal range of Haemoglobin in males is 135-175g/L and females 115-155g/L (Hoffbrand *et al.*, 2016).

2.5.4 Platelets

Platelets also known as thrombocytes are the smallest of the formed elements in the blood. The cells are non-nucleated, round, flattened, disk-shaped structures. The activity of platelets is necessary for blood clotting, vascular integrity and vasoconstriction. They have a life span of about 7.5 days. In coagulation profile, platelets count is consequential (Fischbach, 2003).

Normal platelets count in adult is $140-400 \times 10^9$ /L

Children: $150-450 \times 10^9$ /L (Fischbach, 2003).

2.5.4.1. Mean Platelet Volume (MPV)

Mean platelet volume (MPV) is the haematological parameter that indicates the uniformity in size of platelet population. The parameter can be used for the differential diagnosis of thrombocytopenia.

Mean platelet volume: Adults: 7.4- 10.4fL

Children: 7.4-10.4fL (Fischbach, 2003).

2.5.5. Red Cell Indices

2.5.5.1. Mean Cell Haemoglobin Concentration (MCHC)

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

$$\text{MCHC (g/l)} =$$

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

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

2.5.5.2. Mean Cell Volume (MCV)

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

$$\text{MCV} = (\text{fl})$$

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

2.5.5.3. Mean Cell Haemoglobin (MCH)

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

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

$$\text{MCH} =$$

2.5.5.4. Red Cell Distribution Width (RDW)

The red cell distribution width is used to assess the degree of anisocytosis (abnormal variation in size of red blood cells). The measurement can be used in the investigation of some haematological disorders and in monitoring response to therapy. Normal range is between 11.5-14.5 coefficients of variation (CV) of red cell size (Fischbach, 2003).

2.5.6. White Blood Cells (WBCs) Count

White blood cells (WBC), or leukocytes, are the main immune system components that offer an organism both innate and adaptive immunity. White blood cells count is the number of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and immature or atypical cells present in 1 mL of blood. Leukocytosis, or elevation of the WBC, can be seen in a broad range of conditions, including both benign and malignant conditions. Elevation of the WBC requires accurate differential count and morphologic evaluation of the peripheral blood smear along with clinical information to determine the cause. (Chabot-Richards and George, 2014). Leukopenia, or decrease of the WBC, can also be caused by several conditions and requires accurate differential and morphologic examination to determine which cell line is decreased and to assess whether rare atypical or abnormal cells are present (Fischbach, 2003). The normal number of WBCs in the blood is 4,500 to 11,000 WBCs per microliter (4.5 to $11.0 \times 10^9/L$) (Chabot-Richards and George, 2015). The differential count measures the percentage of each of the five types of white blood cells (Cheesbough, 2006).

2.5.6.1. Neutrophils

Neutrophils are a type of white blood cells (leukocyte) that is characterized histologically by its ability to be stained by neutral dyes and functionally by its role in mediating immune responses against infectious microorganisms. About 50 to 80 percent of all the white blood cells occurring in the human body are neutrophils. The neutrophils are fairly uniform in size with a diameter between 9 and 15 μ m. Within the body the neutrophils migrate to areas of infection or tissue injury. An abnormally high number of neutrophils circulating in the blood is called neutrophilia. An abnormally low number of neutrophils is called neutropenia (Hoffbrand *et al.*, 2016). Reference range; Adults: 40% to 60% of total white blood cells, Children: 25% to 40% of total white blood cells (Ficshbach, 2003).

2.5.6.2. Lymphocytes

Lymphocyte is a type of white blood cell (leukocyte) that is of fundamental importance in the immune system because lymphocytes are the cells that determine the specificity of the immune response to infectious microorganisms and other foreign substances (Hoffbrand *et al.*, 2016). In human adult lymphocytes make up roughly 20 to 40 percent of the total number of white blood cells. They are found in the circulation and also are concentrated in central lymphoid organs and tissues, such as the spleen, tonsils and lymph nodes, where the initial immune response is likely to occur. The lymphocytes are the immunological competent cells that assist the phagocytes in defence of the body against infection and other foreign invasion. The immune response depends upon two types of lymphocytes; B and T cells which are derived from the haematopoietic stem cell. The B cells mature in the bone marrow and circulate in the peripheral blood while the T cells develop from cells that have migrated to the thymus where they differentiate into mature T cells (Hoffbrand *et al.*, 2006). Natural killer (NK) cells are another

type of lymphocytes. They are large cells with cytoplasmic granules and typically express surface molecules CD16 (Fc receptors), CD56 and CD57. They are designed to kill cells that have low level of expression of HLA class molecules such as may occur during viral infection or on a malignant cell (Hoffbrand *et al.*, 2006). Reference range; Adults and Children: 20% to 40% of total white blood cells (Ficshbach, 2003).

2.5.6.3. Monocytes

Monocytes are the largest cells of the blood (averaging 15–18µm in diameter), and they make up about 7 percent of the leukocytes. The nucleus is relatively big and tends to be indented or folded rather than multilobed. Monocytes are actively motile and phagocytic. The cytoplasm contains many fine vacuoles giving a ground-glass appearance (Ficshbach, 2003). They are capable of ingesting infectious agents as well as red cells and other large particles, but they cannot replace the function of the neutrophils in the removal and destruction of bacteria. Monocytes usually enter areas of inflamed tissue later than the granulocytes. Often, they are found at sites of chronic infections (Thiele *et al.*, 2001). Reference range; Adults and Children: 2% to 8% of total white blood cells (Ficshbach, 2003).

2.5.6.4. Eosinophils

Eosinophils are a type of white blood cell, specifically a granulocyte, that play a role in the immune system's response to certain types of infections, allergic reactions, and other inflammatory conditions. They are characterized by the presence of large, uniform granules within their cytoplasm, which can be stained with eosin, a red dye (Hoffbrand *et al.*, 2016). Eosinophils are produced in the bone marrow and then released into the bloodstream, where they circulate for a short period before migrating to tissues throughout the body. They are particularly prevalent in tissues associated with the respiratory system, gastrointestinal tract, and skin, as

these areas often come into contact with potential allergens and parasites. They contain histamine (one third of all histamine in the body) and respond to allergic and parasitic diseases (Fischbach 2003). Normal range of eosinophils is $0-0.7 \times 10^9/L$.

2.5.6.5. Basophils

Basophils are a type of white blood cell, specifically a granulocyte, that play a role in the immune system's response to various stimuli, particularly in allergic reactions and defense against parasites (Yamanishi *et al.*, 2017). They have many dark cytoplasmic granules which overlie the nucleus and contain heparin and histamine and is characterized histologically by its ability to be stained by basic dyes. It plays an active role in mediating hypersensitivity reactions of the immune system. Basophils are the least numerous of the granulocytes and account for less than 1 percent of all white blood cells occurring in the human body (Hoffbrand *et al.*, 2016). Basophils also incite immediate hypersensitivity reactions in association with platelet, macrophages, and neutrophils. They are only seen occasionally in normal peripheral blood.

Normal range for basophils is $0.02-0.05 \times 10^9/L$ (Fischbach, 2003).

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

2.5.7. Platelets

Platelets (thrombocytes) are colourless, nonnucleated blood component that is important in the formation of blood clots (coagulation) (Hoffbrand *et al.*, 2016). Platelets help to stop bleeding and repair damage to the blood vessels by forming blood clots. It results from a chemical “cascade” which begins with the prothrombin activators released by platelets (Cheesbough, 2006). Sometimes referred to as platelet thromboplastin, these chemicals cause the macromolecule prothrombin to break down into smaller units including thrombin. Thrombin acts on fibrinogen, a soluble polymer present in the plasma, and breaks it into monomers which re-

polymerize into insoluble fibrin. The fibrin forms threads which knit the platelets and other cells into a clot (Stock and Hoffman, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

This study was carried out in the University of Benin City, Edo state, Nigeria. The University of Benin (UNIBEN) is a public research university located in Benin City, Edo State, Nigeria. It was founded in 1970 and is one of Nigeria's foremost institutions of higher education.

3.2. Study Population

In this study, animal (rats) model was used. A total of twenty-four (24) male rats of the Albino Wistar strain were purchased from the animal holdings from the department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed at TRIGAS Research Laboratory, University of Benin. Animals were exposed to 12 hours dark and light cycles with access to feed and water *ad libitum*. The rats were allowed to acclimatize for two weeks for the commencement of the study.

3.3. Identification of the *Allium sativum*

Allium sativum bulb were purchased from Aduwawa market in Benin city, Edo State, Nigeria. It was further transported to the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, where it was identified and authenticated.

3.3.1. Preparation of Plant Extracts

The gloves (parchment skin) were removed from the bulb, after which the bulbs were weighed. Then a measured weight of thirty grams (300g) of the peeled garlic bulbs was grounded using the standard laboratory mortar and pestle. 1000ml of distilled water was added to the grounded garlic, and allowed for 30minutes. After which the solution of garlic was sieved, using 1mm

sieve to obtain a homogenous extract. The filtrate when measured weighed 100g. Therefore, the garlic extract solution contained 200g of garlic.

3.3.2. Preparation of Test Drug from the Extract

300g of grounded garlic was reconstituted into 1000ml of distilled water, the filtrate weighed 100g. The concentration of garlic in the solution will be equal to 200g (300g-100g) of garlic in 1000ml of distilled water. Therefore,

1ml of extract = 200mg/ml = 0.2g of garlic.

2ml of extract = 400mg/ml = 0.4g of garlic.

3.4. 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.

3.4.1. Inclusion Criteria

- Apparently healthy Wistar rats weighing between 150-250g
- Male rats

3.4.2. Exclusion Criteria

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

3.5. Ethical Consideration

Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: ha/737/23/b/200600149 issued on 14/12/2023).

3.6. Research Design

Twenty-four (24) male adult albino wistar rats were selected into a negative control group 1 and positive control group 2 and also two experimental groups 3 and 4. Each group consisted of six animals each. Afterwards, they were fed for 1 month following the established feeding routine. Group 1 was the control group which was fed with normal standardized feed and water while group 2 were given grower mash and water, and administered 0.2ml intravenous injection of benzene:2-propanol: water mixture (1:5:5 v/v) per body weight of the rat to induce leukaemia without treatment. Group 3 were given grower mash and water, administered with 0.2ml intravenous injection of benzene:2-propanol: water mixture (1:5:5 v/v) and treated with 400mg/kg extract of *Allium sativum* extract after 7 days of administering benzene and propanol. Group 4 were given grower mash and water, administered with 400mg/kg extract of *Allium sativum* for 7 days. The experimental period lasted for five (5) weeks and body weight of animals were taken weekly and recorded.

3.7. 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 =

100g of the extract was weighed

100g is equivalent to 100,000mg

100g of the extract is dissolved in 1000ml of distilled water

Concentration/Stock of extract = = 100mg/ml

Volume to administer =

Group 3

Average weight of 6 rats = 222g

Dose = 400mg/kg

Stock = 100mg/ml

222g to kg = 0.222kg

Volume to administer =

= = 0.89ml

Group 4

Average weight of 6 rats = 252g

Dose = 400 mg/kg

Stock = 100 mg/ml

252g to kg = 0.252kg

Volume to administer =

= = 1.01ml

3.8. Sacrifice of the Animals

At the end of the experimental period, the animals were grossly observed for general physical characteristics, and were weighed using a weighing balance. A midline incision was made through the ventral wall of the rats under mild anesthesia using chloroform.

3.9. Collection of Samples

A midline incision was made through the ventral wall of the rats after anaesthetizing (using chloroform) and cervical dislocation. 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 obtained from the experimental animals by opening of the femur cavity after which a sterile forceps was used to obtain the marrow. The bone marrow sample was placed in an Eppendorf container containing trizol.

3.10. Laboratory Analysis

3.10.1. RNA Extraction And Semi-Quantitative Polymerase Chain Reaction (PCR)

RNA was isolated from the bone marrow with TRIzol Reagent (ThermoFisher Scientific) and converted to cDNA using ProtoScript First Strand cDNA Synthesis Kit (NEB). PCR amplification of KIT and FLT3 was done using OneTaq® 2X Master Mix (NEB).

3.10.1.1. Procedure

After sacrificing the animals, the tissues were imbedded in 0.3mL of TRIzol (Invitrogen Life Technologies, Inc., Carlsbad, CA) reagent inside an empendoff tube for proper tissue preservation. The tissues were homogenized using a plastic pestle. RNA lyase buffer was then added to the homogenate to further break down the tissue cell membranes, after which it was spun at 10000 rpm for 10 minutes. The supernatant which contains the RNA was carefully removed and placed in a separate empendoff tube. The RNA precipitating buffer was then centrifuged at 10000 rpm for 30 minutes. The supernatant was carefully removed remaining the

RNA precipitate at the bottom of the tube. RNA wash buffer was added and centrifuged again at 10000 rpm for 5 minutes. This step was repeated 3 times to remove excess solutions and buffers previously added. Nuclease free water was added to break down the RNA in a pellet form and the phosphodiester bond of the RNA. The solution also has a nuclease inhibitor, which removes other DNA or RNA contaminants from the medium. The RNA was then quantified using a UV spectrophotometer at 260nm.

3.10.1.2. Complimentary DNA (cDNA) Synthesis

All component of the cDNA kit was added to the RNA following manufacturer's instruction, these components included random primer, oligonucleotide, primer or deoxynucleotides, reverse transcriptase buffer. After adding all components to the RNA, the mixture was then incubated at 42⁰C in a thermocycler for 1 hour. Then the temperature was increased to 75⁰C to denature the reverse transcriptase. Thereafter all the RNA was converted to cDNA. For RQ-PCR on rats sample the expression levels of KIT and FLT3 were normalized to the levels of GADPH housekeeping gene.

3.10.1.3. Gene Amplification

An equal volume of both forward and reverse primer was added, PCR mix (master mix), taq polymerase, and magnesium was also added. The mixture will be placed in a thermo-cycler for amplification. The thermo-cycler programed for 30 cycles.

3.10.1.4. Gel Electrophoresis

After the PCR process, the DNA gel loading dye was added to the mixture. The agarose gel was prepared by dissolving 1% of agarose gel in TBE buffer. The gel was then allowed to solidify and then the sample was loaded and the gel connected for electrophoresis. Thereafter, a snap shot

will be taken. The image will then be transferred to ImageJ. The intensities of the bands from agarose gel electrophoresis will be quantified densitometrically using ImageJ software.

3.10.2. Full Blood Count Analysis

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

3.10.2.1. 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.

3.10.2.2. Procedure

The whole blood was properly mixed and inserted into the probe. Then 20 μ L 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.

3.10.3. Peripheral Blood Film

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."

3.10.3.1. 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 $\times 100$ objective lens.
- Different cellular components such as red blood cells, white blood cells, and platelets were observed for morphology and abnormalities.

3.11. Statistical Analysis

Data obtained from this research was presented and analysed using graph pad prism (version 8.02, California, USA). Analysis of variance (ANOVA) was used to compare means and results was expressed in Mean \pm Standard error of mean. $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

There have been previous findings by researchers on the toxic effects of benzene of haemopoietic tissues, although its effects on FLT 3 and cKIT genes hasn't been fully confirmed. In this study, FLT3 and cKIT genes were analysed in the bone marrow of male albino rats.

Figure 4.1 shows the PCR and agarose gel analysis expression pattern of FLT 3. There was significant increase ($p < 0.01$) in FLT 3 expression in the group administered the induction regiment (benzene + isopropanol), induction regiment + 400mg/kg of *A. sativum* aqueous extract and 400mg/kg of *A. sativum* aqueous leaf extract administered only group when compared to control. Also, the group administered the induction regiment treated with 400mg/kg of *A. sativum* aqueous extract had a significantly higher ($p < 0.001$) FLT 3 mRNA expression when compared to the group administered the induction regiment (benzene + isopropanol) and 400mg/kg of *A. sativum* aqueous extract administered only group.

In Figure 4.2, there was significant increase ($p < 0.01$) in cKIT mRNA expression in the group administered the induction regiment (benzene + isopropanol), induction regiment treated with 400mg/kg of *A. sativum* aqueous extract and 400mg/kg of *A. sativum* aqueous extract administered only group when compared to control. In addition, the experimental group that received the induction regiment only had a significantly higher ($p < 0.01$) cKIT when compared to

the group that received induction regiment + 400mg/kg of *A. sativum* aqueous leaf extract and 400mg/kg of *A. sativum* aqueous extract administered only group.

Table 4.1 presents the results of the blood parameters analysis in male rats in different experimental groups compared to the control group. The total white blood cell count (TWBC), monocyte count, eosinophil count, RBC count, haemoglobin concentration, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, and platelet lymphocyte ratio showed no significant differences between the experimental and control groups ($p > 0.05$). However, the neutrophil count was significantly higher in the control group (10 ± 0.5774) compared to group 2 (3.5 ± 0.2887), group 3 (2.667 ± 0.3333), and group 4 (3.333 ± 0.333) with a p-value of < 0.0001 . Similarly, the neutrophil lymphocyte ratio was significantly higher in the control group (0.122 ± 0.00706) compared to group 2 (0.03885 ± 0.0029), group 3 (0.02754 ± 0.0058), and group 4 (0.03663 ± 0.004) with a p-value of < 0.0001 . Additionally, the lymphocyte count was significantly lower in the control group (82 ± 1.528) compared to group 2 (90 ± 1.414), group 3 (91 ± 1), and group 4 (91 ± 0.5774).

Blood smear analysis revealed various findings in different groups. In plate 4.1, leukocytes appeared adequate with relative lymphocytosis, large lymphocytes, and hyper segmented neutrophils. RBCs showed normocytic normochromic cells, polychromatic cells, and a few crenated cells. Plate 4.2 showed adequate leukocyte numbers with absolute lymphocytosis and large lymphocytes. RBCs appeared as normocytic normochromic cells, with some abnormalities like tear drop cells, macrocytic cells, and polychromatic cells. Plate 4.3 displayed relative lymphocytosis in leukocytes and abnormalities in RBCs such as macrocytic cells, polychromatic cells, stomatocytes, target cells, and crenated cells in the group treated with benzene + isopropanol and *A. sativum* extract. Plate 4.4 showed normal leukocyte count with absolute lymphocytosis and normocytic normochromic RBCs with some polychromatic cells in the group treated with only *A. sativum* extract at a dose of 400mg/kg.

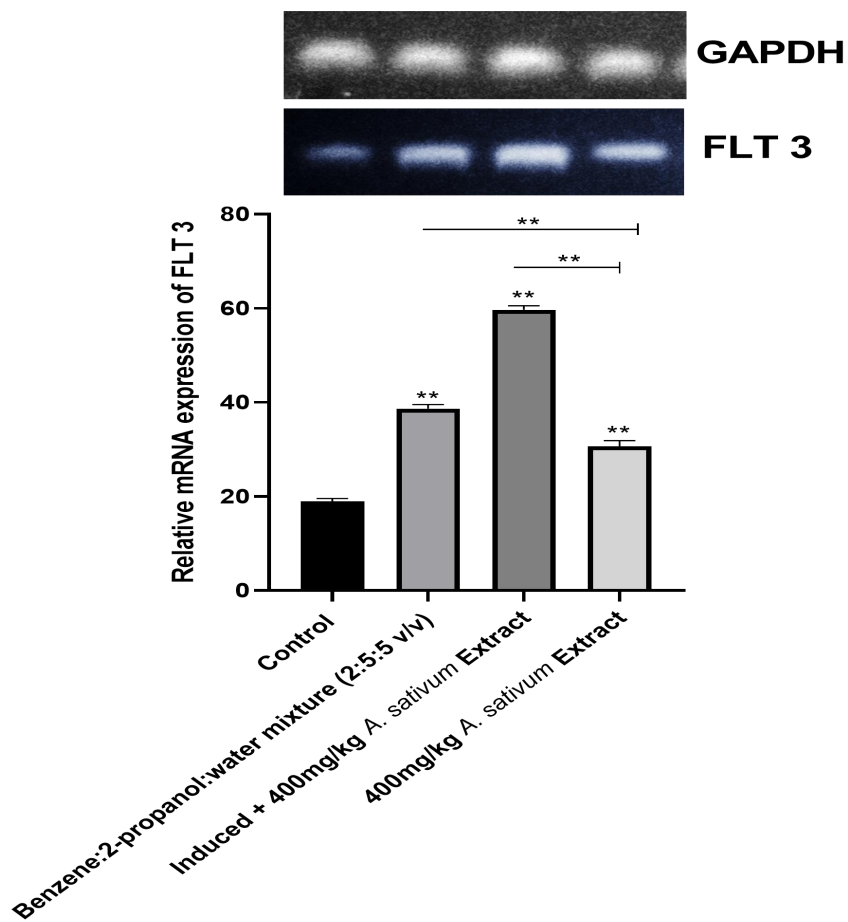


Figure 4.1: PCR and agarose gel analysis of FLT 3 mRNA from bone marrow of male rats induced with benzene and administered 400mg/kg of *A. sativum* aqueous extract. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001).

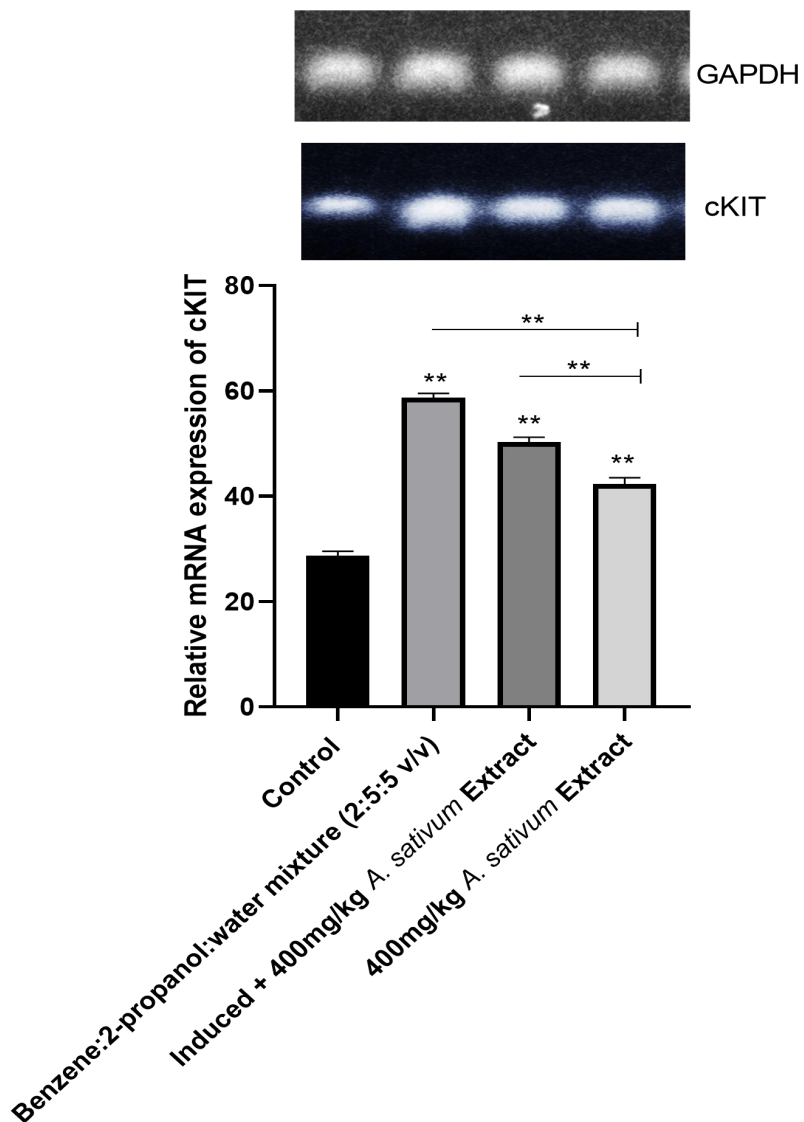


Figure 4.2: PCR and agarose gel analysis of cKIT mRNA from bone marrow of male rats induced with benzene and administered 400mg/kg of *A. sativum* aqueous extract. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001).

Table 4.1: Effect of *A. sativum* Aqueous Extract on Complete Blood Count of Male Albino Wistar Rats Exposed to Benzene

Parameters	Group 1	Group 2	Group 3	Group 4	F value	P value
TWBC (X10 ⁹ /L)	8.267±1.906	10.05±1.19	6.7±0.9074	6.467±0.9821	1.765	0.2235
Neutrophil Count (%)	10±0.5774	3.5±0.2887 ^a	2.667±0.3333 ^a	3.333±0.333 ^a	74.75	<0.0001
Lymphocyte Count (%)	82±1.528	90±1.414 ^a	91±1 ^a	91±0.5774 ^a	10.6	0.0037
Monocyte Count (%)	6.667±1.764	5.75±1.436	5.333±0.33	4.333±0.33	0.5685	0.6495
Eosinophil Count (%)	1.33±0.3333	0.75±0.25	1.5±0.5	1.333±0.3333	1.118	0.3974
Red Cell Count (X10 ⁹ /L)	6.017±0.7828	6.103±0.1364	6.315±0.485	6.633±0.2577	0.3912	0.7627
Haematocrit (L/L)	37.5±4.106	38.2±1.393	36.7±3.2	39.93±2.546	0.2173	0.8817
Haemoglobin (g/dL)	12.5±1.401	13.5±0.2483	12.95±0.25	13.47±0.7623	0.3535	0.7881
MCV (fL)	62.8±1.646	60.43±1.246	58.15±0.65	60.17±1.844	1.292	0.3418
MCHC (g/L)	33.3±0.7234	35.28±0.6663	35.45±2.45	33.7±0.2517	1.325	0.3322
MCH (pg)	20.87±0.5548	21.28±0.6343	20.55±1.15	20.23±0.4485	0.5075	0.688
Platelet Count (X10 ⁹ /L)	690±147.8	1255±212	980.3±148.9	668.3±76.35	2.887	0.0949
NLR	0.122±0.00706	0.03885±0.0029 ^a	0.02754±0.0058 ^a	0.03663±0.004 ^a	81.52	<0.0001
PLR	8.477±1.907	14.04±2.604	10.79±1.557	7.336±0.7942	2.291	0.147

Results presented in Mean±SEM. a represents statistical significance with Control.

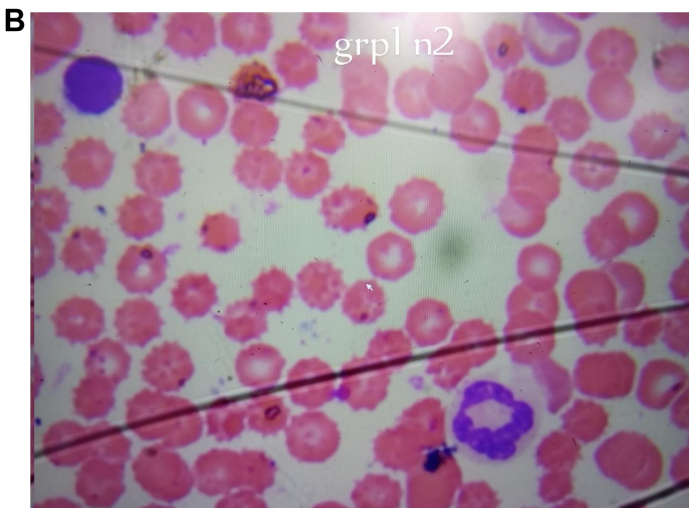
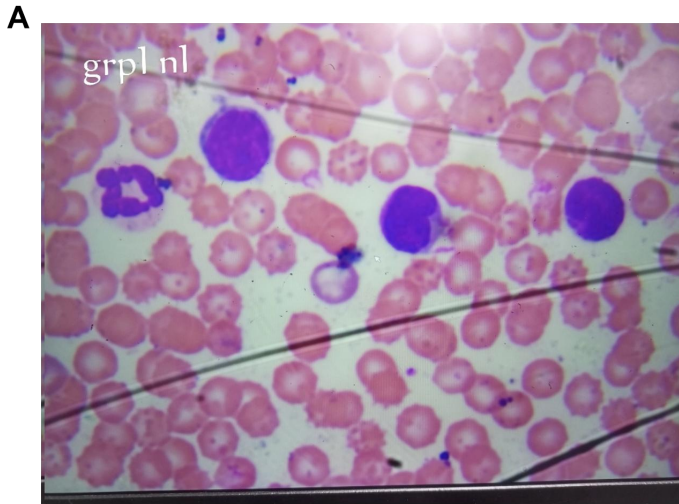


Plate 4.1: Panel A and B represents the blood picture stained with Leishman stain of male albino wistar rats.

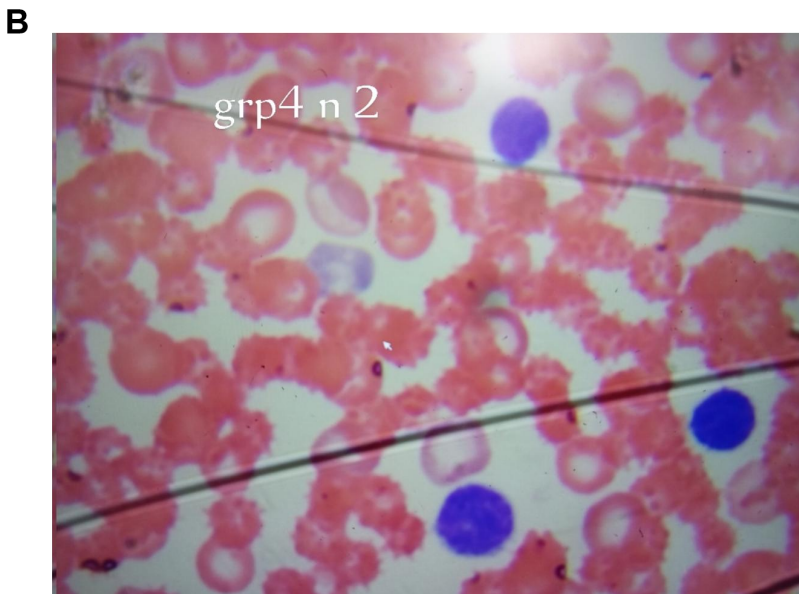
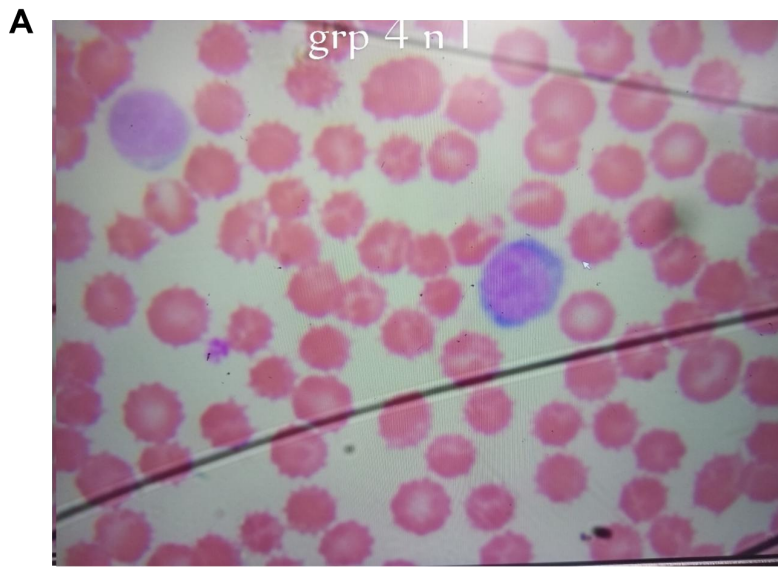


Plate 4.2: Panel A and B represents the blood picture of male albino wistar rats administered with benzene and isopropanol.

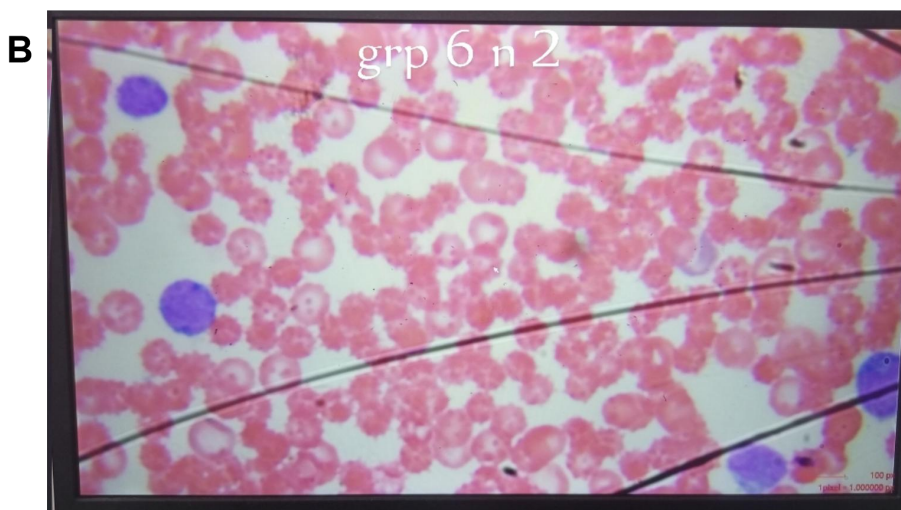


Plate 4.3: Panel A and B represent the blood picture of male albino wistar rats administered with the induction regiment (benzene + isopropanol) and 400mg/kg of *A. sativum* aqueous extract. The blood picture showed relative lymphocytosis, large and small lymphocyte. RBC showed normocytic normochromic cells, macrocytic cells, poly chromatic cells, stomatocytes, target cells and crenated cells.

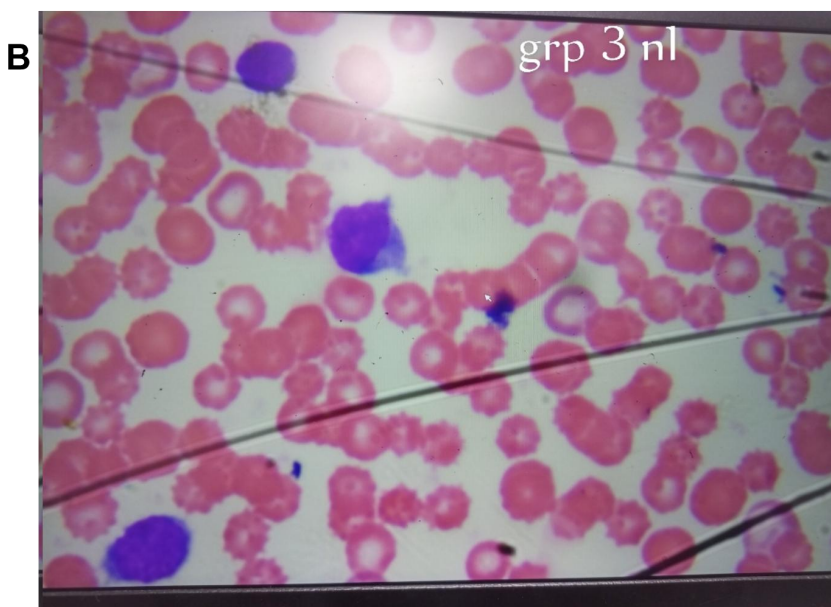


Plate 4.4: Panel A and B represent the blood picture of male albino wistar rats administered with the 400mg/kg of *A. sativum* aqueous extract. Leucocyte appear normal with absolute lymphocytosis. RBC showed normocytic normochromic cells and poly chromatic cells.

CHAPTER FIVE

DISCUSSION

Leukemia is a cancer that develops from hematopoietic cells and is still a major global health concern. The aetiology and pathophysiology of leukemia are complicated, requiring ongoing investigation of innovative therapeutic strategies despite breakthroughs in treatment (Warner *et al.*, 2004). Pre-leukemic models are essential for comprehending the initial molecular processes that cause leukemia and for assessing possible treatment or preventive measures (McHale *et al.*, 2012). It is well recognized that the expression of several genes, like KIT and FLT3, is essential for leukemogenesis and hematopoiesis. The receptor tyrosine kinase KIT controls a number of biological functions, such as the survival, differentiation, and proliferation of hematopoietic progenitor cells. Likewise, the proper growth of hematopoietic stem and progenitor cells depends on FLT3, another receptor tyrosine kinase (Birg *et al.*, 1994). Aberrant expression or mutations in these genes have been implicated in the pathogenesis of leukaemia (Masson and Rönstrand, 1994).

In this study, we investigated the effects of *Allium sativum* extract on KIT and FLT3 gene expression in benzene induced male albino wistar rat. *Allium sativum*, known for its diverse pharmacological properties, has been reported to exhibit anti-leukemic effects through various mechanisms, including modulation of gene expression (Khawaji *et al.*, 2021). The significant increase in FLT3 expression observed in the groups administered the induction regimen (benzene + isopropanol) and induction regimen + 400mg/kg of *A. sativum* aqueous extract, as well as in the group treated with 400mg/kg of *A. sativum* aqueous leaf extract alone, compared to the control group, suggests a potential modulation of FLT3 expression by both the induction regimen and *A. sativum* extract. This modulation could be due to the cellular stress induced by the

benzene + isopropanol induction regimen. Benzene is a known hematotoxic and leukemogenic agent, while isopropanol is often used as a solvent and may potentiate the toxic effects of benzene (Kong *et al.*, 2024). The induction regimen likely triggers molecular pathways involved in cellular proliferation and survival, which could lead to upregulation of FLT3 expression as a compensatory mechanism. The significant enhancement of FLT3 expression in the group administered the induction regimen treated with 400mg/kg of *A. sativum* aqueous extract compared to both the induction regimen-only group and the group treated with *A. sativum* extract alone suggests a potential synergistic effect between the induction regimen and *A. sativum* extract. *A. sativum* is known for its antioxidant, anti-inflammatory, and anti-cancer properties, which may counteract the toxic effects of benzene and isopropanol while simultaneously modulating FLT3 expression (Gurumallu and Javaraiah, 2021). Previous research has demonstrated the anti-leukemic effects of *A. sativum* through various mechanisms, including induction of apoptosis, inhibition of proliferation, and modulation of gene expression (Hassan, 2004). The result obtained in this study however is in contrast to the study of Thomas *et al.* (2014) who reported a downregulation of FLT3 due to benzene exposure.

The significant increase in cKIT mRNA expression observed in the groups administered the induction regimen (benzene + isopropanol), induction regimen treated with 400mg/kg of *A. sativum* aqueous extract, and the group treated with 400mg/kg of *A. sativum* aqueous extract alone compared to the control group suggests a potential induction of cKIT expression by both the induction regimen and *A. sativum* extract. Benzene can lead to cellular stress and activation of survival pathways, including those involving cKIT expression (Spatari *et al.*, 2021). Increased cKIT mRNA expression in response to the induction regimen may reflect an attempt by the cells to mitigate the toxic effects and promote survival (Faivre *et al.*, 2007). The significant difference

observed between the group receiving the induction regimen only and the group receiving the induction regimen along with *A. sativum* aqueous leaf extract suggests a potential modulatory effect of the extract on cKIT expression. In agreement to this study, research carried out by Chiang *et al.* (2013) reported that garlic extract including diallyl disulfide (DADS) and diallyl trisulfide (DATS) led to upregulation of c-KIT.

The study indicated that most haematological parameters, including TWBC, monocyte count, eosinophil count, RBC count, haemoglobin concentration, haematocrit, MCV, MCH, MCHC, platelet count, and platelet lymphocyte ratio, did not show significant differences between the experimental and control groups ($p>0.05$). This is in contrast to other studies that has shown alterations in haematological parameters after benzene and garlic extract administration. Qian *et al.*, 2019, found that benzene causes haematotoxicity by deacetylation. Benzene has also been shown to suppress the haemopoietic myeloid progenitor cells which can lead to a stoppage in the production of new white blood cells (Yoon *et al.*, 2001). Oluwole (2001) reported alterations in some red cell parameter after 100mg of *Allium sativum* extract administration. There were notable differences in neutrophil count, neutrophil lymphocyte ratio, and lymphocyte count. Specifically, the control group exhibited significantly higher neutrophil count and neutrophil lymphocyte ratio compared to groups administered the induction regimen with or without *A. sativum* extract. Conversely, the lymphocyte count was significantly lower in the control group compared to the experimental groups. These findings suggest a potential modulatory effect of *A. sativum* extract on immune cell populations, particularly neutrophils and lymphocytes as reported by Arreola *et al.* (2015).

The blood picture of male albino Wistar rats administered with the induction regimen (benzene + isopropanol) and 400mg/kg of *A. sativum* aqueous extract reveals relative lymphocytosis with

both large and small lymphocytes. Red blood cell (RBC) abnormalities include normocytic normochromic cells, macrocytic cells, polychromatic cells indicative of active erythropoiesis, stomatocytes suggesting potential liver disease or alcoholism, target cells indicative of altered RBC membrane structure, and crenated cells (Thachil and Bates, 2017). These findings suggest complex haematological responses to the induction regimen and *A. sativum* extract.

The blood picture of male albino Wistar rats administered with 400mg/kg of *A. sativum* aqueous extract revealed several that the leukocyte population appears normal, indicating no significant abnormalities in overall leukocyte morphology. However, there is a notable observation of absolute lymphocytosis, suggesting an increase in the absolute number of lymphocytes in the blood. Lymphocytosis can occur in response to various stimuli, including infections, inflammation, and stress (Padgett and Glaser, 2003). Regarding red blood cells (RBCs), the blood picture shows the presence of normocytic normochromic cells, indicating RBCs with a normal size and coloration, suggesting adequate haemoglobin content and oxygen-carrying capacity. This finding is reassuring as it suggests no significant abnormalities in RBC morphology or function. Additionally, the presence of polychromatic cells among the RBCs is observed, which are indicative of active erythropoiesis. These cells represent immature RBCs containing residual ribosomal RNA and are typically seen in response to increased RBC turnover or demand (Harvey, 2008).

5.2. CONCLUSION

The result of this showed that administration of aqueous extract of *Allium sativum* in pre leukaemic rats influences expression of key genes involved in haematopoiesis and leukemogenesis namely FLT3 and cKIT. While most haematological parameters remained unaffected, by the treatment, alterations were observed in neutrophil count, lymphocyte count and neutrophil to lymphocyte ratio.

5.3. RECOMMENDATIONS

1. Since it is a new study it is recommended to conduct a detailed research on the therapeutic effects of garlic for a longer period of time
2. Evaluate the safety profile of garlic and its potential interactions with other medications
3. The experiment can be conducted with other dosage and concentrations to optimize the full effectiveness of garlic.

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