EFFECT OF UZIZA LEAF EXTRACT *(Piper guineense)* ON TRANSFERRIN RECEPTOR ONE GENE IN DROSOPHILA MELANOGASTER

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OCTOBER, 2025

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELORS DEGREEE IN THE MEDICAL LABORATORY SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

OCTOBER, 2025.

CERTIFICATION

This is to certify that this work was carried out by **ESEBANMHEN, IMHANDE STEPHANIE** with matriculation number **BMS1906872** under the supervision of DR. J.O OSAKUE and is being submitted to the department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree

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DEDICATION

I dedicate this work to God Almighty for His guidance, grace and strength. I also dedicate it to my lovely family for their love and support throughout my journey in this study

ACKNOWLEDGMENT

I am sincerely grateful to God Almighty for His grace upon my life and for seeing me through this study amidst all the challenges and obstacles I faced.

My profound gratitude goes to my supervisor, Dr J.O. OSAKUE for his constructive and supportive ideas, his invaluable guidance, encouragement, and support throughout the course of this work.

Special thanks to the Head of Department, Medical Laboratory Science, DR. MRS. ZAINAB OMORUYI and also to the entire staff of the department for their support, guidance and providing me with the foundational knowledge and resources necessary tocarry out this study.

My deepest appreciation goes to my father, Mr. Stephen Esebanmhen, my mother, Mrs.Veronica Esebanmhen and my siblings Sinon, Sebastian and Sabella Esebanmhen, for their constant encouragement, prayers, and moral support, which kept me motivated during this journey.

Finally, I appreciate the efforts of my friends as well and all those who contributed in one way or another to the successful completion of this project. Your support and collaboration were invaluable, and I am deeply thankful for your contributions.

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ABSTRACT

Iron homeostasis is vital for numerous physiological processes, including oxygen transport, cellular respiration, and erythropoiesis, and its imbalance can result in anemia or iron overload. The transferrin receptor 1 (TfR1) plays a central role in this regulation by mediating iron uptake at the cellular level. Given the limitations of synthetic modulators of iron metabolism, the search for natural alternatives has gained scientific attention. Piper guineense, commonly known as uziza, is a West African spice rich in phytochemicals with reported hematopoietic and antioxidant properties. This study therefore aimed to investigate the effect of varying concentrations of aqueous *Piper guineense* leaf extract on TfR1 gene expression in Drosophila melanogaster, a model organism widely used due to its conserved genetic similarity with humans. Flies were divided into five groups: a control group and four treatment groups receiving 100, 200, 300, and 400 mg/ml of the extract, respectively. Survival rate was monitored for 21 days, while molecular analysis was conducted through RNA isolation, cDNA synthesis, and semi-quantitative PCR. The 100 mg/ml group demonstrated the highest survival rate and a TfR1 expression level comparable to the control (2.28 ± 0.07 vs 2.30 ± 0.10), suggesting maintenance of normal iron uptake. At 200 mg/ml, a slight decline in TfR1 expression (2.10 ± 0.08) was observed relative to the control, while 300 mg/ml produced a more pronounced reduction (1.97 ± 0.06). The 400 mg/ml group showed the lowest expression (1.89 ± 0.05), indicating significant dose-dependent downregulation. These findings implied that low concentrations may enhance or preserve normal iron metabolism, whereas higher doses may suppress transferrin receptor activity, potentially disrupting iron uptake. It is therefore recommended that *Piper guineense* extract be used in low doses for beneficial hematologic modulation, and further studies be conducted to isolate its active compounds and assess safety thresholds in mammalian systems.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The term "medicinal plants" refers to those species that are well known for their capacity to both prevent and treat a wide range of diseases and health issues. When utilized properly, these plants are generally safe for human consumption despite their natural origins. These plants fall into two categories: domesticated species, which have been cultivated and maintained by human actions like selective breeding or agricultural management, and wild species, which naturally occur in ecosystems without direct human interference (Oladeji,2016).

Additionally, the WHO (2001) defines a medicinal plant as herbal preparations made by extracting, fractionating, purifying, concentrating, or undergoing other physical or biological processes. These preparations might be made for immediate consumption or as a basis for herbal products.

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care (Silva and Júnior, 2010).

In medicine, plants are used to treat certain disorders and illnesses as well as to preserve and improve mental, spiritual, and physical health. It has been discovered that traditional medicine helps some of the main healthcare needs of nations in Latin America, Asia, and Africa. For instance, up to 80% of people in Africa receive their primary medical care

through traditional medicine. Traditional medicine adaptations in developed nations are referred to as "alternative" or "complimentary" medicine. The use of traditional medicine is expanding quickly in developed nations, while it remains prevalent in all parts of the developing world .

Plants are used in medicine to maintain and augment health-physically, mentally and spiritually as well as to treat specific conditions and ailments. It has been found that countries in Africa, Asia and Latin America use traditional medicine to help meet some of their primary health care needs. In Africa, for example, up to 80 percent of the population uses traditional medicine for primary health care. In industrialized countries, adaptation of traditional medicines is termed “complimentary??” or “alternative” medicine. Traditional medicine has maintained its popularity in all regions or the developing world and its use is rapidly spreading in industrialized countries (So *et al.,* 2018).

Many antioxidants found in plants aid in providing defense against illnesses linked to free radicals. The majority of the antioxidant chemicals are produced by plants as secondary metabolites. The term "plant-chemicals" actually refers to phytochemicals. These are the non-nutritive chemical components of plants that have several health advantages and the ability to prevent disease. Their nutrients are non-essential, meaning the body does not need them to maintain life. These substances are created by plants to support life, and when consumed by humans, they improve their health. Based on their function in plant metabolism, more than a thousand phytochemicals have been identified and categorized as major or secondary ingredients. Common sugars, amino acids, chlorophylls, purines, and pyrimidines of proteins and nucleic acids are among the phytochemicals categorized as primary components.Others classified as the secondary constituents are the chemicals consisting of

alkaloids, flavonoids, terpenes, phenolics, lignans, plant steroids, curcumines, saponins, glucosides.(Nwozo *et al.,* 2023).

The inherent medicinal potentials of these plants lie in their bioactive constituents which includes nutrients such as minerals, vitamins, and non-nutrients such as phytochemicals. One ofsuch plants with medicinal potential is *Piper guineense* (Amadi *et al.,* 2019).

The perennial climbing vine plant *Piper guineense* belongs to the Order Piperales, Division Magnoliophyta, Class Magnoliopsida, and Kingdom Plantae. It is a member of the genus Piper and the family Piperaceae. widely used in West Africa as a spice. In English, it is referred to as fake cubeb, Guinea pepper, Ashanti pepper, Benin pepper, and West African black pepper. Locally, the Hausa name it "masoro," the Yoruba call it "iyere," the Igbo call it "uziza," and the Ghanaians call it "Soro wisa." The plant climbs up to 12 meters using adventitious rootlets. It has a corky lower stem and simple, opposite leaves that are ovate, pointed at the tip (acuminate), heart-shaped at the base (cordate), and have five main veins. Its flowers are arranged in 4–6 cm long spikes, are greenish-yellow, and spiral along the spine. The small, oval fruits grow in clusters, turn red-brown when ripe, and become black when dry.(Alagbe *et al.,* 2021)

The seeds of P. guineense are used in traditional herbal medicine for a number of purposes. For example, in some regions of Nigeria, women consume the seeds after giving birth in order to increase uterine contractions, which aid in the expulsion of the placenta and other remnants from the womb. They are also used as an adjuvant to treat rheumatic pains, an antiasthmatic, and to help control weight. In concentration-related ways, the extracts from seeds and leaves can show depolarizing neuromuscular activity. There have also been reports of P. guineense leaves and seeds having antiparasitic, antibacterial, and antifungal properties.(Ojinnaka *et al.,* 2016)

The protostome and ecdysozoan arthropod Drosophila melanogaster is a member of the Drosophilidae family, a group of dipteran insects distributed worldwide. Evolutionarily, arthropods diverged from the vertebrate lineage over 600 million years ago , indicating a distant relationship between Drosophila and humans. Nevertheless, extensive genetic, molecular, and behavioral research conducted for more than a century has revealed significant biological similarities. As a result, Drosophila has become a key model organism in the study of multicellular eukaryotes. Its use has led to major scientific breakthroughs, including support for the chromosomal theory of inheritance and the first experimental identification of the gene as a functional unit (Hirth, 2010).

The fruit fly, or Drosophila melanogaster, is still one of the most widely used model organisms in biomedical research. The fly has been an essential tool for fundamental research for over a century due to its inexpensive cost, quick generation time, and superior genetic tools. The model system has been able to stay up to date with the most recent developments thanks to the integration of multiple molecular tools. Several contributors in this issue give examples of present applications for Drosophila as well as their thoughts on the system's future directions (Tolwinski ,2017).

The primary justification for using Drosophila melanogaster as a model organism is its distinctive traits, which include its quick and short life cycle, cost-effectiveness, and dependability, as well as the fact that female adults become fertile 12 hours after eclosion and that a single pair of flies can give birth to hundreds of offspring in a matter of days. In addition, the genome of Drosophila melanogaster has been fully sequenced, including over 13,600 genes spread across 4 chromosomes. Given that over 75% of the genes implicated in human disorders share sequences with D. melanogaster, it is noteworthy that Drosophila has also been shown to have genetic similarities to humans (Jennifer *et al.*, 2021).

In the life cycle of Drosophila melanogaster at 25 °C, fertilized eggs are laid after adult female and male flies mate, and the embryo takes about 24 hours to mature into a first instar larva. The larvae then develop and go through two more larval stages (second and third instars), each lasting around 24 hours. In order to undergo the metamorphosis, D. melanogaster exhibits significant rates of glycolytic flux, lactate generation, glycogen synthesis, and triglyceride (TAG) buildup during the larval stages. Larvae pupate at the conclusion of the third instar, which lasts for two to three days. Adult fly tissues develop and metamorphosis takes place during the pupal stage, which lasts three to five days. Eclosion from the puparium occurs at the end of the metamorphosis, and adult flies become fertile after about 24 hours.

Flies live for 60–90 days depending on the rearing conditions which is temperature and diet composition (Brischigliaro *et al.,* 2023).

The hematopoiesis of Drosophila and vertebrates is comparable. The two waves of hematopoiesis that they both display are known as definitive hematopoiesis and primitive hematopoiesis. As early as two hours after gastrulation, fruit flies go through the primitive wave in the embryo's procephalic mesoderm. Around stage 5 of embryonic development, definitive hematopoiesis, or the second wave, occurs in the lymph gland (LG), the organ where the second wave of blood cell development takes place. However, it becomes more noticeable at stage 17, when it is seen as a kidney-like structure flanking either side of the dorsal vessel, an organ that functions similarly to the heart muscles in pumping hemocytes to maintain their circulation (Koranteng *et al.,* 2022).

Multipotent hematopoietic progenitors, also known as prohemocytes, are the source of Drosophila blood cells. These progenitors can develop into three distinct mature cell types, collectively referred to as hemocytes. Two of these cell types, known as plasmatocytes and

crystal cells, are constitutively produced, while the third, known as lymphocytes, differentiates in response to immune challenges. Prohemocyte production in Drosophila occurs in two stages, which is similar to vertebrate hematopoiesis. When prohemocytes separate from the head mesoderm and develop into crystal cells and plasmatocytes, the first wave of development takes place in the early embryo. To put it briefly, the panhematopoietic GATA transcription factor Serpent (Srp) is necessary for prohemocyte emergence, and the expression of the lineage-specific transcription factors Glide/Glial Cell Missing and the RUNX transcription factor Lozenge (Lz) determines whether a cell differentiates into a plasmatocyte or a crystal cell.Actually Serpent and Lozenge cooperate to control crystal cell differentiation and are reiteratively employed at all phases of Drosophila hematopoiesis , which is reminiscent of the situation in vertebrates where GATA and RUNX also functionally interact and control several steps of blood cell development (Letourneau *et al.,* 2016).

Once defined, plasmatocytes, which make up approximately 90% of blood cells, travel along traditional pathways in response to chemoattracting cues, whereas crystal cells, which make up approximately 5% of all embryonic blood cells, stay concentrated in the anterior region of the gut. These embryo-derived hemocytes subsequently populate the larvae where they remain, either as circulating cells in the hemolymph (the circulatory fluid that fills the body cavity of arthropods) or as patches of cells attached to the integument (sessile hemocytes).

In a specialized organ called the lymph gland, which develops during embryogenesis, a second wave of hematopoiesis occurs during the larval stages. The following describes lymph gland ontogeny and the molecular mechanisms governing lymph gland hematopoiesis both in the absence of wasp parasitism and after it occurs. The majority of lymph gland cells, if not all of them, differentiate before being discharged into the bloodstream when the lymph gland

malfunctions. Normally, lymph gland prohemocytes produce crystal cells and plasmatocytes that remain inside the gland until the larva to pupa transition.

In adult flies, hemocytes produced from the lymph glands and embryos coexist. The imago is dotted with plasmatocytes and a few crystal cells, which are primarily sessile cells. A tiny percentage of differentiated hemocytes appear to duplicate their DNA in response to bacterial infection, even if plasmatocyte phagocytic capacity and total hemocyte counts decrease with age. It's interesting to note that Ghosh et al. also found that adult plasmatocyte and crystal cell development occurs in so-called hematopoietic hubs, which are found in the abdomen near the heart and have a high hemocyte count. Functional transferrin, also known as ovotransferrin, is present in all vertebrates and, in birds and reptiles, it exhibits antibacterial and iron-binding qualities. One of the important proteins involved in cellular iron uptake is the transferrin receptor (TfR).Human Transferrin is a 76-kDa glycoprotein that is mainly produced in the liver and has a half-life of approximately 8 days in the serum (Kawabata, 2018).

In the human body, iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds (flavin-iron enzymes, transferring, and ferritin). The body requires iron for the synthesis of its oxygen transport proteins, in particular hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions.Almost two-thirds of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store, and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions (Abbaspour *et al.,*2014).

Iron is a critical metal for several vital biological processes. Most of the body's iron is bound to hemoglobin in erythrocytes. Iron from senescent red blood cells is recycled by macrophages in the spleen, liver and bone marrow. Dietary iron is taken up by the divalent metal transporter 1 (DMT1) in enterocytes and transported to portal blood via ferroportin (FPN), where it is bound to transferrin and taken up by hepatocytes, macrophages and bone marrow cells via transferrin receptor 1 (TfR1). While most of the physiologically active iron is bound hemoglobin, the major storage of most iron occurs in the liver in a ferritin-bound fashion (Vogt *et al.,* 2021).

Transferrin receptor (TFR) 1 and 2 are expressed in the liver; TFR1 levels are regulated by cellular iron levels while TFR2 levels are regulated by transferrin saturation (Herbison *et al.,* 2009).

Tfr1 is essential for erythropoiesis, which consumes the vast majority of body iron.5 Thus, mice with pathologically reduced erythroid Tfr1 expression develop microcytic anemia (Fillebeen *et al.,* 2018).

Transferrin receptor 1 (Tfr1) facilitates the uptake of iron at the cell surface by internalizing diferric transferrin. Tfr1 is ubiquitously expressed in mammalian tissues and has been called the "cellular iron gate"..(Wang *et al.,* 2019) . The transferrin receptor (TfR1), which mediates cellular iron uptake through clathrin-dependent endocytosis of iron-loaded transferrin, plays a key role in iron homeostasis. Since the number of TfR1 molecules at the cell surface is the rate-limiting step for iron entry into cells and is essential to prevent iron overload, TfR1 expression is precisely controlled at multiple levels (Gammella *et al.,* 2017).

1.2 Statement of the Problem

Iron plays a central role in human physiology, serving as a cofactor in oxygen transport, cellular respiration, and DNA synthesis. When its balance is disrupted, a wide range of

clinical conditions can arise. Iron deficiency remains the most common nutritional disorder worldwide and is a leading cause of anemia, especially in low- and middle-income countries. On the other hand, excessive iron accumulation results in iron overload syndromes such as hemochromatosis, which can damage organs including the liver, pancreas, and heart. At the cellular level, transferrin receptor 1 (TfR1) is one of the key proteins responsible for regulating iron uptake. Any alteration in its activity or expression has the potential to disturb iron metabolism.

While various synthetic supplements and pharmaceutical agents are available to address iron-related conditions, they often come with limitations such as high cost, reduced accessibility in resource-constrained regions, and side effects from prolonged use. This has encouraged the exploration of natural plant products as possible modulators of biological pathways. *Piper guineense* (commonly known as uziza), a plant widely used in West Africa for both culinary and medicinal purposes, is known to contain numerous phytochemicals. These compounds may influence important molecular processes, including those involved in iron metabolism.

However, little is known about the direct impact of uziza on gene expression, particularly on genes such as TfR1 that are critical for iron regulation. This knowledge gap makes it difficult to establish whether uziza has potential therapeutic applications at the molecular level. Investigating its effect using a reliable model organism such as Drosophila melanogaster may therefore provide useful insight.

1.3 Aim of the Study

The overall aim of this research is to examine the effect of uziza (Piper guineense) leaf extract on the expression of the transferrin receptor 1 (TfR1) gene in Drosophila melanogaster.

1.4 Specific Objectives

The study is guided by the following objectives:

1. To analyze samples for transferrin gene expression in Drosophila melanogaster
2. To compare the values obtained from survival analysis.
3. To evaluate changes in TfR1 gene expression using semi-quantitative polymerase chain reaction (PCR).

1.5 Research Questions

This study seeks to answer the following questions:

1. Does uziza leaf extract cause a measurable change in TfR1 gene expression?
2. At which concentration is the effect of uziza extract on gene expression most pronounced?
3. Is Drosophila melanogaster an appropriate model for studying the effect of plant extracts on genes related to iron metabolism?

1.6 Research Hypotheses

Null hypothesis (H0): Uziza leaf extract has no significant effect on the expression of the transferrin receptor 1 gene in Drosophila melanogaster.

Alternative hypothesis (H1): Uziza leaf extract significantly affects the expression of the transferrin receptor 1 gene in Drosophila melanogaster.

1.7 Significance of the Study

This study is relevant for several reasons. First, it provides scientific evidence on the molecular effects of uziza, a plant already valued in traditional medicine, thus bridging indigenous knowledge with modern biomedical science. Second, by examining how uziza influences TfR1 expression, the research offers possible insights into the management of

iron-related disorders such as anemia and iron overload. Third, the findings may serve as a preliminary step toward pharmacogenomic investigations into plant-derived compounds as regulators of gene expression. In the long term, this could guide the development of cost-effective, plant-based interventions that are particularly valuable in developing economies.

1.8 Scope of the Study

The investigation is limited to the use of Drosophila melanogaster as an experimental model. The focus is on one gene transferrin receptor 1 while other transferrin receptor isoforms and unrelated metabolic pathways are excluded. Molecular techniques, specifically RNA extraction, cDNA synthesis, and semi-quantitative PCR, are employed to assess gene expression changes.

1.9 Limitations of the Study

Like most scientific studies, this work faces certain constraints. The chemical composition of uziza leaves can vary depending on environmental and seasonal factors, which may affect the reproducibility of results. Access to advanced laboratory equipment and specialized reagents is also limited, which may restrict the depth of analysis. Furthermore, the time available for the research is relatively short, preventing the observation of long-term or multigenerational effects of the extract on gene expression.

CHAPTER TWO

LITERATURE REVIEW

2.1 Plant of Study *(Piper guineense)*

Due to its many ethno-medical applications, African guinea pepper (*Piper guineense* Schumach & Thonn) is a spice and medicinal plant that is highly prized in Africa (Mgbeahuruike *et al.,* 2018).

The spice plant *Piper guineense* belongs to the genus Piper and the family Piperaceae. About 1050 species of tropical shrubs, lianas, and tiny trees make up the genus Piper; many of these are used as flavorings, spices, and medicinal plants. According to Isikhuemen *et al.* (2020), P. guineense is a perennial vine that may reach a height of 12 meters and climbs up tree boles via adventitious roots. Its leaves are pale greenish while fresh and deeper green when frozen or dried, and its ripe, red or redbrown fruits turn black when dry.

The seeds are smooth and are prolate-elliptically shaped. The seeds of the plant are commonly known in English-speaking countries as “West African black pepper”, “uziza” in Igbo “iyeree” in Yoruba and “poivrie” in French (Ojinnaka *et al.,* 2016).

The fruits of *Piper guineense* are marketed as condiments in local markets, and the spice is mostly used to enhance regional cuisine in West African nations. According to El-Hack *et al.,(*2022), this medicinal plant has been traditionally used to treat a number of illnesses and infectious disorders, such as bronchitis, diarrhea, and rheumatoid arthritis.

Traditionally, the seeds are used as postpartum tonic for women to stimulate uterine contraction which is assumed to clear the womb of remains of the placenta and other remains after the birth of the child. A recent review by Morufu et al. (2016) revealed the many nutritional and non-nutritional benefits of various parts of the plant. Aqueous extracts of the

seed have also been shown to have cholesterol lowering effects and significant increase in hemoglobin level, white blood cell and red blood cell counts of albino Wistar rats (Ene-Obong *et al.*, 2016).

According to Juliani *et al*.,(2013), the fruits—the portion of the plant that has historically been used are abundant in a variety of natural compounds, including as volatile oils, lignans, amides, alkaloids, flavonoids, and polyphenols. In Nigerian marketplaces, the leaves and seeds are typically offered as edible medicinal plants or as a flavoring and scent ingredient in food. The seeds, leaves and sometimes the stems are used in preparing soup. It imparts "heat" and a spicy pungent aroma to food. The medicinal properties of *Piper guineese* exert bacteriostatic and bacteriocidal effects on some bacteria.The leaves are considered aperitive, carminative and eupeptic.(Okoye and Ebeledike, 2013; Sumathykutty *et al.*, 1999).The leaves are also used for female infertility while,the fruits are used as an aphrodisiac .It was reported that the leaf extracts of *P. guineese* have antimicrobial effect on some test organisms.(Ojinnaka, Ubbor, *et al.,* 2016).

Several physiologically active substances can be found in solvent extracts of P. guineense fruits and leaves. Myristicine, linalool, and dillapiole are some of P. guineense's main physiologically active substances. These substances have antioxidant, anti-diabetic, and anti-cancer qualities. Along with other antimicrobial phytochemicals, it also includes piperine, which gives Piper species their reputation for being hot. Promoting fertility and having anti-inflammatory properties are two of P. guineense's unusual potential qualities. It works well as an antibacterial against pathogenic organisms and food deterioration. Fruits include many of the biologically active substances that are present in leaves or their derivatives.(Ojimelukwe, 2021).

The phytochemical analysis of *Piper guineense* (Uziza leave) serves as a good complement for food and also contains some considerable amount of anti-nutrient alkaloids, tannins, spaonins which could also have some health benefit to its consumers, and also help to reduce the intake of starchy foods, enhances nourishment and protection, prevent constipation. Thus it is also noticed that *Piper guineense* (Uziza leave) is cheap vegetables that can help in our nutritive values (Stephen *et al.,* 2016).

2.1.1 Scientific Classification of *Piper guineense* (Taxonomy)

Piper guineense is scientifically classified as follows:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Piperales

Family: Piperaceae

Genus: Piper

Species: Piper guineense

2.1.2 Plant Morphology and Botanical Description

Although *Piper guineense's* physical characteristics are typical of the Piperaceae family, it differs from closely related species in a few key ways. It belongs to the Division Magnoliophyta, Class Magnoliopsida, Order Piperales, genus Piper, family Piperaceae, and Kingdom Plantae. Common names for it include Ashanti pepper, Guinea pepper, Benin

pepper, West African Black pepper, and fake cubeb. Hausa-speaking tribes in Ghana call it "masoro," Yoruba call it "Iyere," and Igbo-speaking tribes in Nigeria call it "uziza."(Attah *et al.,* 2012).

With slender stems that frequently cling to supports, *Piper guineense* is a dioecious perennial climbing vine or shrub that can reach a height of 10 meters. Stem cuttings or seeds are used to reproduce the plant .The plant grow up tall climbing up bole of trees by means of adventitious roots (Ugoma *et al.,* 2023).

This plant has simple, alternating, ovate to oblong leaves that are 3–7 cm wide and 5–15 cm long. They have a cordate base with a smooth, glabrous surface and an acute apex. (Essien *et al*., 2007).

The fresh leaves had an aromatic odour and a hot and very pungent taste.(African Journal of Pharmacy and Pharmacology," 2024).

The flowers are greenish-yellow and arranged in a spiral on the spine. The fruit is oval, occurs in clusters are small (5mm in diameter) and redbrown when ripe but black when dry (Okwute and Egharevba, 2013).

Venation is pinnate, and the margins are entire. The flowers are tiny, unisexual, and arranged on slender, pendulous spikes measuring 3–6 cm. Male spikes are generally longer than female spikes.Flowers lack petals and sepals, and are subtended by bracts. The fruits are small, globular berries, measuring 3–5 mm in diameter . Each fruit contains a single seed and is characterized by its pungent aroma due to the presence of essential oils (Ekanem *et al.,* 2004). The roots are fibrous, aiding the plant's climbing habit and absorption of nutrients from various soil types.

Fig 2.1: *Piper guineense* seeds and leaves (Alagbe *et al.*, 2021).

2.1.3 Origin, habitat, description and distribution of *Piper guineense*

The plant is widely distributed in the tropical regions of central and western Africa and can be found countries such as Nigeria, Guinea, Ghana and Uganda.It grows in closed forests, forest edges and generally wet places in forests clearings (Oyemitan,2017).

2.1.4 Ethnomedical Uses of *Piper guineense*

In various regions of Africa and Asia, the leaves and seeds of *Piper guineense* Schumach & Thonn have long been used in ethnomedicine and as spices to improve the flavor of food and drink. It is frequently used as an aphrodisiac; women eat the seeds after giving birth to increase uterine contractions and help regulate weight. They are also used to treat rheumatism, digestive disorders, bronchitis, and cough. The extracts from seeds and leaves have the ability to depolarize neuromuscular activity (Kpomah *et al*.,2019).

A ubiquitous plant in West Africa, shanti pepper is well-known for it's culinary and therapeutic qualities. It has long been used to treat ailments like fever, malaria, and discomfort. Piperine, a bioactive substance found in Ashanti peppers, has been shown to have strong anti-inflammatory and antioxidant properties. By combating oxidative stress and inflammation, two factors that have been linked to neuronal damage and behavioral changes, the use of the plant extract has the potential to mitigate stress-induced neurobehavioral changes in the context of chronic stress. The extract has the potential to preserve neural tissues by scavenging free radicals and lowering oxidative stress in the brain.Additionally, the extract may possess the ability to modulate immune response and decrease brain inflammation, potentially contributing to the amelioration of stress-induced behavioral changes (Oluwaseun *et al.,* 2024).

The fruits are used also as a tonic, to easy childbirth, for tumours, insecticide and for haemorrhoids.Traditionally, the herb is prepared in several forms including decoctions, powders or tinctures. Roots have been also used as an aphrodisiac, treatments for colds, respiratory diseases and caries. It was reported that a mixture of leaves, roots and fruits are incorporated in preparations for the treatment of infectious diseases as an antibacterial agent (Iwu, 2014). Abdominal problems, intestinal colic, bronchitis, chickenpox, cough, headache, lumbar discomfort, gingivitis, chest complaints and illnesses, and antihelmintic properties are all treated with leaves. A yellow soup believed to treat diarrhea is made in Cameroon by combining the leaves of Pentas shimperana spp. occidental with those of West African peppers (Focho *et al.,* 2009). In Southeast Nigeria, the leaves are used to stimulate the womb to contract, to promote the removal of the placenta and other leftovers from the womb, and as a pre-labor stimulant . In China, *P. guineense* fruit extract is used in the treatment of epilepsy . The seed is used as an adjuvant for the treatment of asthma and weight control while the root is used as chewing stick for healthy teeth (Sulaimon *et al.,* 2020).

Piper guineense is employed for various ethno-medicinal purposes. It is used to treat gastrointestinal upsets, sickle cell anemia, dental caries and respiratory diseases like asthma. *Piper guineense* is also used as anti-hypertensive, anti-cancer, anti-allergic, anti-inflammatory and antimicrobial agent (Jonah *et al.,* 2018).

2.1.5 Phytochemical Uses of *Piper guineense*

The different parts of the plant have been characterized and its constit-uents determined. Phytochemicals are bioactive compounds found inplants.They are not vitamins or minerals but are constituents in the plant that work with other nutrients and dietary fibers to prevent and protectagainst diseases (Okoye and Ebeledike, 2013)

The phytochemical analysis of *P. guineense* leaves showed the presence of alkaloids (wisamine, dihydrowisamine, piperine and dihydropiperine) , saponins, tannins, flavonoids, resins, essential oil (dillapiol, elemicine, myristicine and safrole) and hydrogen cyanides which have a lot of pharmacological properties in diseases such as diabetes mellitus .(Sulaimon *et al.,* 2020).

The root contains mainly alkaloids such piperine, trichostachine and wisanine. Terpenes and phenylpropanoids are the major phytochemicals found in the fruit of *Piper guineense.*(Ohemu *et al.,* 2024).

Cardiac glycosides found in *P. guineense* are helpful in the treatment of cardiovascular disease. The plant's flavonoids have anti-inflammatory, anti-tumor, anti-allergic, antiplatelet, and antioxidant qualities. Essential oils found in *P. guineense*, such as dillapiol, piperine, elemicine, myristicine, and safrole, exhibit antibacterial and bactericidal properties against specific microbes. Five to ten percent of the substance is piperine, which is known to give plants of the Piper family heat. Additionally, the plant's substantial β-caryophyllene content is being investigated for its possible anti-inflammatory properties. (Ogbunugafor *et al.,* 2017).

Together with nutrients and fibers, phytochemicals found in plants—such as alkaloids, saponins, tannins, flavonoids, and polyphenols—help prevent disease. Among these substances include HCN and essential oils such as safrols, myristicin, and elemecin. Both leaves and seeds contain alkaloids, which are known to have therapeutic advantages against cancer, malaria, hypertension, arrhythmia, and pain. Additionally, alkaloids stimulate the central nervous system. A significant concentration of saponins (1.88%) in the leaves supports the plant's ability to combat malaria by demonstrating its antimalarial properties.

2.1.6 Nutritional Constituents of *Piper guineense*

Studies on *Piper guineense* have revealed that its leaves and seeds are both excellent nutrient sources. Proteins, carbs, fats, fiber, beta-carotene, vitamin C, and several minerals, including calcium and magnesium, are all present in them. Important trace elements including copper, iron, sodium, potassium, manganese, selenium, and zinc are also said to be present in the fruits. Although often eaten smaller amounts than daily requirements, proximate studies conducted in Nigeria show that the leaves include vitamins, minerals, calcium, copper, iron, magnesium, potassium, phosphorus, sodium, and zinc, as well as amino acids. However, the seeds are particularly abundant in phytochemicals known to have potential health advantages, including as flavonoids, saponins, anthraquinones, cardiac glycosides, terpenes, alkaloids, and deoxy-sugars.Secondary metabolites such as tannins and saponins have also been detected in the leaves.

In traditional diets across West Africa, different parts of the plant are commonly consumed. The leaves and stems are used as condiments to enhance the taste of soups and sauces. In Côte d'Ivoire, for example, the leaves are boiled, ground, and added to sauces, while in other areas they are eaten as vegetables in soups. The dried fruits are often milled into powder and used as a spice or to season meat and fish dishes.(Ake *et al.,*2019)

When comparing nutrient content, the leaves are found to contain more protein than the seeds, although the seeds still provide a fair amount. This shows that both plant parts contribute to dietary protein, which is needed for growth, tissue repair, enzyme function, hormone production, and energy supply.

Mineral analysis also reveals differences between seeds and leaves. More chromium, zinc, iron, potassium, sodium, and phosphorus are found in the seeds, but higher amounts of

magnesium, calcium, manganese, and copper are found in the leaves. But the most prevalent mineral in both leaves and seeds is calcium. This implies that *Piper guineense* can help avoid calcium deficiency-related issues like osteoporosis, muscle spasms, and twitching, as well as support bone strength, muscle function, and enzyme functioning. All things considered, *Piper guineense* is a natural source of nutrients and bioactive substances that can support health and wellbeing in addition to being a spice and food enhancer.(Imo *et al.,*2018)

2.2 Biological Activities of *Piper guineense*

2.2.1 Antimicrobial properties

A dimorphic opportunistic pathogenic yeast called Candida albicans can cause significant fungal illnesses in humans, especially in those with acquired immunodeficiency syndrome. Extracts from Piper guineense were discovered to be highly effective against Candida albicans. Results showed that the leaf and fruit extracts of Piper guineense inhibited the growth of different strains of Candida (such as C. albicans, C. tropicalis, C. parapsilosis, and C. glabrata), further supporting the plant's potential as an antifungal agent and defining its use in the treatment of STDs. Additionally, P. guineense shown potent inhibitory effects against human infections brought on by flamentous fungi, such as Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Aspergillus favus, Scopulariopsis brevicaulis, and the yeast Cryptococcus neoformans.(Brown *et al.,* 2014).

Additionally, *P. guineense* may be a significant source of bactericidal chemicals on a variety of bacteria, according to (Tekwu *et al.*, 2012). Its use in the treatment of ailments by traditional healers in various African and global civilizations can be determined from the numerous reports on the inhibitory action of *P. guineense's* leaves, stem, and seeds on microorganisms.

The plant's extracts have demonstrated inhibitory effects on a variety of harmful microbes, such as fungus and bacteria. For example, studies show that *P. guineense's* methanol leaf and seed extracts include substances such terpenes, alkaloids, glycosides, tannins, and flavonoids that enhance its antibacterial activity (Alagbe *et al.,* 2021).

2.2.2 Antibacterial effect

O. gratissimum and *P. guineense* leaf aqueous and ethanol extracts were tested for antibacterial activity against Staph. aureus and E. coli in this investigation. It was discovered that both extracts showed specific inhibition against the isolates.(Mouafo *et al.,* 2012).

2.2.3 Anti-oxidant activity

According to a number of in-vitro and in-vivo investigations, *Piper guineense* has a strong reducing power and scavenging ability. This could be because the plant contains polyphenols (Okon *et al.,* 2013; Omodamiro and Ekeleme, 2013) (Moukette *et al.,*2015). Additionally, they stopped the antioxidant system from collapsing by delaying the oxidation of LDL by lengthening the lag period (Alagbe *et al.,* 2021).

2.2.4 Anti-parasitic activity

The in vivo antiplasmodial effect of the crude ethanol extract of *P. guineense* against rodent malaria parasite *P. berghei* was investigated by Kabiru *et al.,* (2016), and they reported a reduction in parasitemia in a dose-dependent pattern. They also reported the analgesic effect which was dose-dependent but was not as effective as aspirin, the positive control drug(Ogbunugafor *et al.,* 2017).

2.2.5 Aphrodisiac Potentials

P. guineese showed potential value as a natural sexual enhancement (aphrodisiac) agent as shown by significant increase in some of the libido indicator parameters assessed (Ochei *et al.,* 2017).

2.2.6 Anti inflammatory Potential

Ethanol and aqueous extract of leaves at doses 100 and 200mg/kg possesses antiasthmactic activity on histamine induced bronchoconstriction in guinea pig and histamine induced dose dependent contraction of guinea pig tracheal chain and isolated guinea pig ileum preparation.(Gbekley *et al.,* 2016).

2.2.7 Fertility Effect

According to this study, *Piper guineense* stimulates the testes, epididymis, and seminal vesicles, which improves male reproductive measures such testicular hormones, sperm count, spermatocyte count, spermatid count, and sperm morphology. This implies that it could be able to undo the effects of toxins on these metrics. The pharmacological evidence of *Piper guineense* on libido was also reported in this study, indicating that it may be able to prevent the negative effects of some toxins on libido and that it may be used as an aphrodisiac. Additionally, studies from the survey indicate that it has a good effect on females by improving female reproductive performance and elevating specific reproductive hormones.(Onwuka *et al.,* 2022).

2.2.8 Anti-hyperlipidemic Effect

The results from the present study indicate that the aqueous extract of *Piper guineense* exerts a hypolipidemic effect, reduces cholesterol-induced body weight gain, and increases the body's antioxidant defense system in experimental hypercholesterolemia in rats(Nwozo *et al*.,2017).

2.2.9 Anti-tumor Effect

Though more research is required to confirm these findings and clarify the mechanisms involved, preliminary studies have suggested that *Piper guineense* may have anti-tumor properties. The plant's bioactive compounds may potentially inhibit the growth of cancer cells, and the use of several plant parts, including its seeds, was found to benefit the management of these cancer cells (Ohemu *et al.,* 2024).

2.2.10 Contractile Effect

Uterine contractions have long been induced using *piper guineense, e*specially in postpartum treatment. Extracts from the plant are thought to promote smooth muscle contraction, which facilitates the involution of the uterus following childbirth (Alagbe *et al.,* 2021).

2.2.11 Anti atherogenic effect

Present study test the hypothesis that *Piper guineense* prevent the deleterious effect of atherogenic diet on hamsters as measured by antioxidant activities and lipid profiles of cardiac, hepatic and renal tissues.(Agbor *et al.,* 2012)

Nearly ten years ago, it was reported that the leaf extract of *P. guineense* has anti-atherosclerotic properties in hamsters fed an atherogenic diet. According to the study's findings, *P. guineense* exhibited strong anti-atherogenic and antioxidant properties in opposition to harmful atherogenic diets. In a separate investigation, Nwozo et al. demonstrated that *P. guineense* aqueous extract was a strong antioxidant preparation that shown hepato-protective qualities in male albino rats with ethanol-induced liver damage. (Franklyn *et al.,* 2021).

2.2.12 Insecticidal Activity

The concentration, exposure duration, and organic solvent utilized for extraction all affected the *P. guineense* extract fraction's insecticidal activity. The most hazardous extracts against

D. maculatus larvae were ethyl-acetate fractions, which were followed by n-Haxane and methanolic fractions. (Jatau *et al.,* 2023) . Similar to the findings of Ajayi and Peter (2016), who reported that an extract of *P. guineense* produced oil that has been reported to be very effective in the control of stored product pests, the efficacy of ethyl acetate and n-Haxane may be attributed to their oily appearance in contrast to the solid methanolic extracts.

2.2.13 Effect on Hematological Parameters

An increase in hemoglobin concentration could be a direct result of eating more plants that are high in vitamins and minerals, which could promote hemoglobin synthesis. The type and amount of protein in the *Piper guineense* seed extract may have contributed to the rise in the WBC and RBC counts. (Friday *et al.,* 2015).

The effects of both short-term and long-term *P. guineense* administration on haematological values shown that the extract of *P. guineense* raised RBC, WBC, PCV, and MCHC levels. (Hassan *et al.,*2010).

2.3 Organism of Study (Drosophila melanogaster)

2.3.1 Introduction

Drosophila melanogaster, commonly known as fruit fly or vinegar fly, is originally an African species, with all non-African lineages having a common origin . The term “drosophila” means “dew-loving”: it is a modern scientific Latin adaptation from the Greek words δρόσος, drósos, “dew”, and φιλία, philía, “lover”. The term “melanogaster” means “black-belly”, and comes from the Greek words μέλας, mélas, “black”, and γᾰστήρ, gastḗr, “belly”. (Giansanti *et al*., 2025).

Charles William Woodworth of Harvard University spearheaded the development of Drosophila melanogaster as a crucial model organism in biological research in the early 1900s. Woodworth was the first to breed the fruit fly in captivity. Frank Eugene Lutz, a fruit

fly researcher at the Carnegie Institution's Experimental Evolution Station, became interested in Woodworth's work after being influenced by William Ernest Castle's genetic research. Thomas Hunt Morgan, who sought a less expensive substitute for mice in genetic studies, was introduced to Drosophila by Lutz. In January 1910, Morgan found the first Drosophila mutant, a white-eyed male linked to an X chromosome abnormality, after isolating visible mutants by 1909 and starting gene localization research.

The common fruit fly is a species of Dipterans called Drosophila melanogaster. Although Drosophila have home ranges on all of the main continents in the wild, humans are responsible for their current worldwide dispersion since they are commensal organisms that spread by eating on human fruits and other food sources. Drosophila had a more limited range in equatorial Africa in the past, and it's unclear what they ate before human activities spread. The fact that Drosophila can be raised on a wide range of food sources may have had a role in their original selection as a model organism.(Wangler and Bellen, 2017).

Drosophila genome is 60% homologous to that of humans, less redundant, and about 75% of the genes responsible for human diseases have homologs in flies (Ugur et al., 2016). These features, together with a brief generation time, low maintenance costs, and the availability of powerful genetic tools, allow the fruit fly to be eligible to study complex pathways relevant in biomedical research(Mirzoyan *et al.,* 2019)

Furthermore, Drosophila continues to be a crucial model organism for research on genetic pathways in other insect species, including tropical disease vectors like Anopheles gambiae (malaria) and Aedes aegypti (yellow fever, dengue fever). Fruitflies are a model organism that permits genetic modifications not achievable in the majority of other systems.

2.3.2 Scientific Classification of *Drosophila melanogaster*

The genus was first described by Johann Wilhelm Meigen in 1830 and continues to be a focal point in scientific research due to its genetic accessibility and ecological importance.

Kingdom: Animalia.

Phylum: Arthropoda.

Class: Insecta (Insects).

Order: Diptera (True Flies).

Family: Drosophilidae (Vinegar Flies, Pomace Flies, or Fruit Flies).

Genus: Drosophila.

(Vivekanandhan *et al*.,2024)

2.3.3 Morphology

The adult fruit fly's body is composed of three parts: the head, thorax, and abdomen, in accordance with standard insect morphology. There are a number of sensory organs in the head, including the proboscis, which is the gustatory organ for food detection, taste, and intake and which can extend and retract to pump food into the gut, and compound eyes, which have primary pigments that are characteristic of various mutants. Three components make up the thorax: the mesothorax (middle), which has one pair of legs and one pair of wings, the metathorax (posterior), which has one pair of legs and one pair of halters (modified wings), and the prothorax (anterior), which has one pair of leg**s.**

Females and males can be easily differentiated by morphological attributes, especially, females are generally bigger and possess an abdomen that has a pointed tip whereas males show a rounded abdomen with black pigmentation in the posterior segment with an epandrium (male external genitalia) (Žurovec, 2014)

The brain, peripheral nervous system, heart, trachea system (similar to the lung), esophagus, Malpighian tubules (similar to the kidneys), fat body with oenocytes (combining the functions of adipose tissue and the liver), gut, and gonads are among the organ systems in the fly's anatomy that have comparable functions to those of mammalian organisms (Baenas *et al.,* 2019).

2.3.4 Life Cycle

The Drosophila life cycle, which lasts about ten days at 25°C, begins with embryogenesis, which takes around 24 hours and involves laying hundreds of eggs. During this time, essential genes are expressed to establish the larval body plan, including proteins from maternally derived mRNAs. These proteins are then transcribed into gene cascades, splitting the embryo into segments, regions, and structures.

After emerging from the egg, a larva in its first instar begins to feed in order to grow and convert it into sugars and fats that may be stored in the fat body. Prothroacicotropic hormone (PTTH), juvenile, and ecdysone are the hormones that control molting, the process by which larvae lose their exoskeleton as they age. Before undergoing a final molt to produce a pupa, the larva goes through three instars. Larvae can expand because the release of ecdysone is regulated by the quantity of PTTH that increases. This process is also influenced by the development of imaginal discs, which are sac-like structures made of monoepithelial cells, and larval organs. A new cuticle, or exoskeleton, is created when PTTH causes the prothoracic glands to secrete ecdysone into the hemolymph.The actual molt, where the larva loses its exoskeleton and enters a new instar stage, is initiated by another hormone called eclosion hormone (EH). Larvae, known as "wandering larvae," start searching for a pupation site near the end of the third instar stage.The pupal stage is when metamorphosis occurs, and

the animal must activate developmental autophagy, a "self-eating" signaling mechanism that transforms the fat body's stored nutrients into energy for survival. (Allocca *et al.,* 2018).

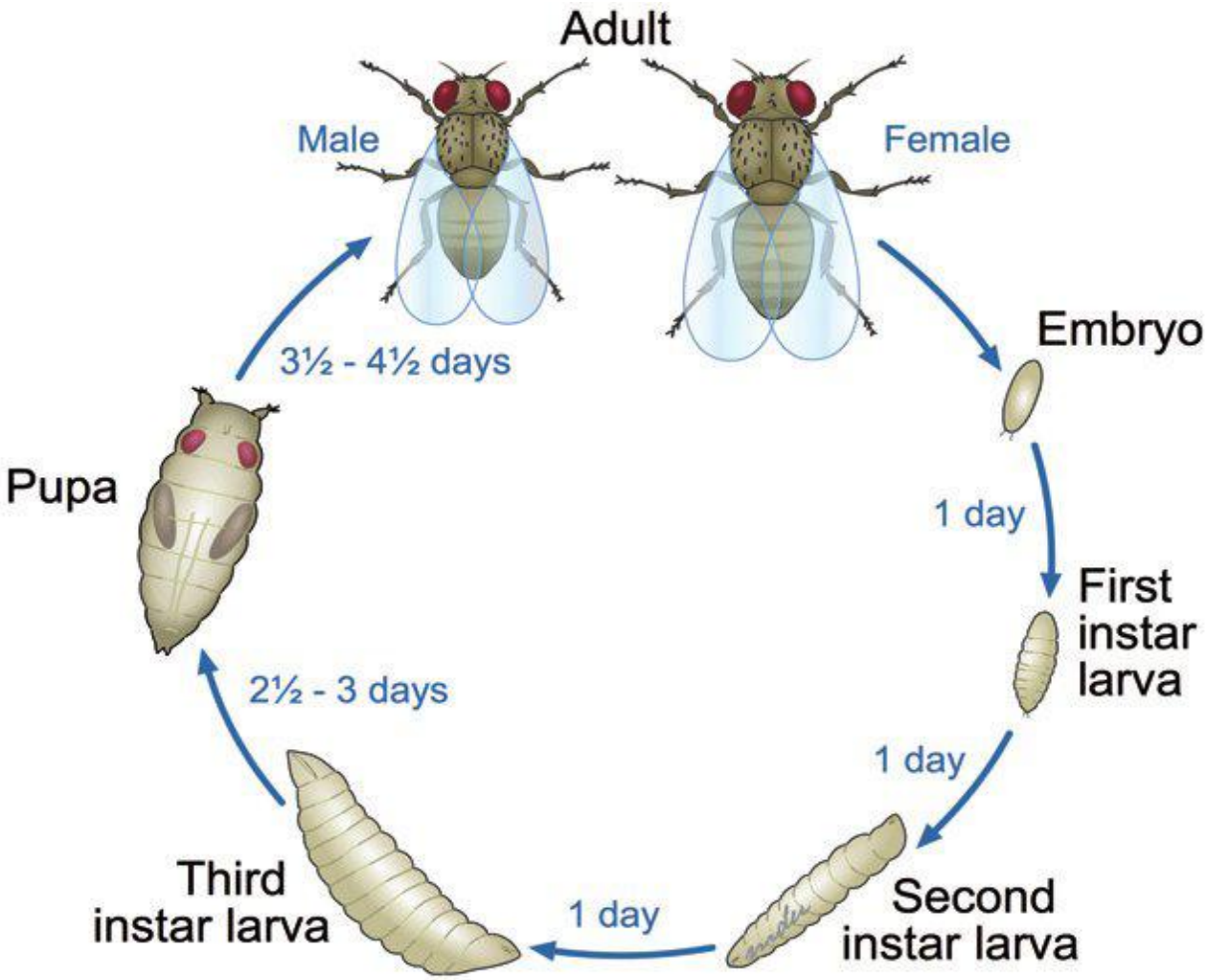

Fig 2.2: The whole life cycle of the fruit fly Drosophila is relativelyrapid and takes only approximately 10–12 days at 25 C. The Drosophila development is divided into various stages: embryo, larva (first instar,second instar and third instar), pupa and adult.(Ong *et al.*, 2014).

2.3.5 Drosophila melanogaster Culture and Media

For rearing and culturing the drosophilids, the collected samples need to be transferred from bait bottles to the narrow culture vials (with culture media) While shifting flies from the bottles to collection tubes the openings of the bottles must be kept under bright light directing the flies toward the light and thus making their transfer easy. For culturing, the flies are kept at room temperature (25°C). If room temperature is not adequate the culture tubes could be kept in the BOD (Biological Oxygen Demand) incubators. This would help the culture media to last longer until the drosophilid colony begins to establish.(Topal *et al.,* 2021).

Cultures can be maintained by transferring adult flies to fresh media. Varying the temperature will speed up or slow down the life cycle of the flies. Higher temperatures speed up the life cycle. Higher temperatures also promote bacterial and fungal growth in the culture vials. If virgins are needed for genetics testing, collect every 8 hours if culturing at higher temperatures or every 12 hours for lower temperatures.

The use of Drosophila cell culture provides the rapid testing of potential in vivo transgenic constructs for proper biochemical and cellular functions before creating transgenic flies,the ability to follow the immediate responses to a stimulus in a population of homogenous cells .A vast set of molecular reagents available for genetic manipulation, including CRISPR-Cas9 editing tools and many expression-ready tagged ORF cDNA collections also the amenability to high-throughput functional genomics, including the well-developed protocols for chemical screens and RNA interference (RNAi) (Luhur *et al.,* 2018).

Fig 2.3: Culture of inbred flies in corn meal solid culture media (Mohapatra and Pandey, 2018)

2.3.6 Drosophila as a Model Organism to Study Human Diseases

For more than a century, Drosophila has been successfully employed as a model organism to investigate a wide range of biological phenomena, such as learning, behavior, aging, embryonic development, organ regeneration, genetics and heredity, and more. Many examples show the value of adopting Drosophila as a model system since the majority of the basic biological processes and signaling pathways that govern development and survival have been preserved throughout evolution. (Cheng *et al.,* 2018). About 75% of the genes causing human diseases have fly homologs, while the genomes of Drosophila and humans are 60% similar, albeit less redundant. (Mirzoyan *et al.,* 2019).

Drosophila melanogaster models of human brain disorders have various benefits for studying the cellular and molecular processes that underlie human illness. The advantages of using Drosophila as a model organism include its short lifespan, high quantity of progeny, numerous genetic approaches, well-known anatomical condition, and diverse range of mutations (Jeibmann and Paulus, 200**9).**

Numerous human illnesses and conditions, such as those linked to neurodegeneration, tumor growth, muscle deterioration, intestinal malfunction, and inflammation, can be replicated in Drosophila. When it comes to researching the development of cancer and the relationships between tumor suppressors and oncogenes, Drosophila have proven to be especially useful. Following the discovery that Drosophila tumors exhibit many or all of the characteristics of human cancers, flies were recognized as a useful model for quickly analyzing the molecular pathways underlying carcinogenesis. (Verheyen, 2022).

2.3.7 Advantages of Drosophila in the Study of Human Diseases

Drosophila melanogaster is a unique, powerful genetic model organism for studying a broad range of biological questions. Human studies that probe the genetic causes of rare and undiagnosed diseases using massive-parallel sequencing often require complementary gene function studies to determine if and how rare variants affect gene function.The fruit fly, Drosophila melanogaster allows studies related to genetics, development, neural function and maintenance,physiology, metabolism, wiring of the brain, as well as molecular and cellular mechanisms (Bellen *et al.,* 2019).

D. melanogaster models of human diseases provide several unique features such as powerful genetics, highly conserved disease pathways, and very low comparative costs. The fly can effectively be used for low- to high-throughput drug screens as well as in target discovery.(Pandey *et al.,*2011).

An effective and trustworthy experimental model for studying a variety of genetic problems and creating treatment plans for uncommon and ultra-rare diseases is Drosophila melanogaster. It is an excellent research model for researching genetic abnormalities because of its short life cycle, quick life cycle, ease of laboratory care, and fully sequenced genome. For the study of muscular disorders, especially those affecting muscle growth, function, and degeneration, Drosophila is an appropriate model organism. The structural conservation of muscle tissues is one of the many benefits of using Drosophila models to research muscular illnesses.

2.4 Haematopoiesis in Drosophila melanogaster

2.4.1 Haematopoiesis in Adult Flies

The lymph gland is a specialized organ where Drosophila hematopoiesis takes place. We identify the developmental stages in the generation of blood cells (hemocytes) from their antecedents in this methodical examination of lymph gland anatomy and gene expression. During hematopoietic progression, specific zones of hemocyte maturation, signaling, and proliferation in the lymph gland are characterized. A medullary zone, a cortical zone, and a zone known as the posterior signaling center are the developmental niches into which several stages of hemocyte development have been categorized based on marker expression. This gives a genetic foundation for the spatial and temporal events that control hemocyte formation and creates a framework for identifying Drosophila blood cells at different stages of maturity (Jung *et al.,* 2005).

There are two stages in the creation of Drosophila blood cells. Hemocytes form from the early embryo head mesoderm during the first, "primitive," phase, contributing to the pool of circulating blood cells. The lymph gland, a tiny organ, produces adult hemocytes during the second phase. Various cell-specific markers have been used to study the differentiation of hemocytes and the larval lymph gland. The posterior signaling center (PSC), medullary zone (MZ), and cortical zone (CZ) are the divisions of the larval lymph gland's primary, biggest lobe. It has been hypothesized that the MZ contains a comparatively homogeneous population of pluripotent prohemocytes (PH), also known as stem-like cells. As these cells develop into plasmatocytes (PM), crystal cells (CC), and lamellocytes, they go into the CZ (cortical zone). The homeostasis between prohemocytes and differentiated blood cells is maintained by the Posterior signaling center.(Minakhina and Steward, 2009).

2.4.2 Similarities between Drosophila and Human Haematopoiesis

Between Drosophila and vertebrates, hematopoiesis is highly preserved. The locations of hematopoiesis change during Drosophila development, just like in vertebrates. During hematopoietic waves in the embryo and the Drosophila lymph gland, blood cells (hemocytes) are created from scratch. The larva's hematopoietic wave, on the other hand, is predicated on differentiated hemocytes that develop in the embryo colonizing resident hematopoietic sites. This is comparable to vertebrates' primitive yolk sac macrophages colonizing peripheral tissues or hematopoietic stem and progenitor cells seeding the fetal liver, spleen, and bone marrow.(Makhijani and Brückner, 2012).

The cells that make up the Drosophila blood system are all involved in wound healing, tissue integrity, innate immunity, and other types of stress response; as a result, they behave similarly to mammalian myeloid cells. The main cell types have particular roles in melanization, encapsulation, and phagocytic processes. Drosophila exhibits many sites of hematopoiesis, similar to mammalian systems, and the mechanisms underlying this process use many of the same molecular pathways that demonstrate human blood formation (Banerjee *et al.*, 2019).

2.5 Iron Metabolism in Living Organism

Because it is essential for oxygen transport, oxidative metabolism, cellular proliferation, and other catalytic activities, iron is a key trace element for humans. Iron levels in the human body must be kept within the optimal range in order to be advantageous. Iron homeostasis depends on the interplay of numerous organs and tissues, including iron metabolism, one of the most intricate processes. There is no active iron excretion mechanism. In order to balance the daily losses, the amount of iron absorbed by the intestine is strictly regulated.

The reticuloendothelial system is in charge of iron recycling through erythrocyte phagocytosis, while the bone marrow is the body's primary iron consumer and the location of erythropoiesis.The liver has important synthetic, storing, and regulatory functions in iron homeostasis.(Yiannikourides and Latunde-Dada, 2019).

Iron is one of the most important nonorganic substances that make life possible. Iron plays major roles in oxygen transport (eg, hemoglobin; ~67% of total body iron [TBI]), short-term oxygen storage (eg, myoglobin; ~3.5% of TBI), and energy generation (eg, cytochromes; ~3% of TBI).1 Iron also serves vital roles in various nonheme-containing enzymes (~2% of TBI).(Winter *et al.,* 2014)

One of the most distinguishing features of iron metabolism is the degree to which body iron is conserved. Of the typical 3 to 4 g of iron contained in the normal adult human, only about 0.03% (or ~1 mg) is lost per day, mainly the result of obligatory losses of exfoliated mucosal cells, bile, and extravasated red cells. To replace these basal losses and remain in iron balance, the body must absorb a roughly equivalent amount ofiron from the diet. This relatively small daily exchange of iron between body and environment contrasts sharply with the comparatively large exchange of this metalbetween internal organs.(Kühn, 2014).

2.5.1 Role of Iron in Haematopoiesis And Metabolism

Iron is an essential nutrient, but its concentration and distribution in the body must be tightly controlled due to its inherent toxicity and insolubility in aqueous solution. Living systems have successfully overcome these potential limitations by evolving a range of iron binding proteins and transport systems that effectively maintain iron in a nontoxic and soluble form for much, if not all, of its time within the body.

Iron is carried to the intended organs in the bloodstream via the serum iron binding protein transferrin. Using both transferrin receptor 1-dependent and independent routes, individual cells adjust how much transferrin-bound iron they absorb according on their iron needs. Once inside the cell, iron can be stored in ferritin if there is too much of it or chaperoned to areas of need. The ferroxidase activity of ceruloplasmin or hephestin is necessary for the iron export protein ferroportin1 to safely load iron onto transferrin, which releases iron from cells. Hepcidin is the primary regulator of iron homeostasis, and it primarily regulates iron export at the systemic level.

Hepcidin, in turn, responds to changes in body iron demand, making use of a range of regulatory mechanisms that center on the bone morphogenetic protein signaling pathway. (Frazer and Anderson, 2013).

2.5.2 Iron Regulation

Iron is an essential trace element in the human body, but excess iron is toxic as it contributes to oxidative damage. To keep iron concentration within the optimal physiologic range, iron metabolism at the cellular level and the whole systemic level are tightly regulated. Balance of iron homeostasis depends on the expression levels and activities of iron carriers, iron transporters, and iron regulatory and storage proteins. (Gao *et al.,* 2019).

Many redundant systems that are frequently preserved throughout the mammalian kingdom closely regulate iron metabolism to modify iron concentrations at the cellular and systemic levels. Iron homeostasis is regulated and maintained by a coordinated interaction between iron-processing cells, such as tissue macrophages, hepatocytes, erythrocytes, and duodenal epithelial cells, in response to iron and red blood cell (RBC) demand. As cellular components of iron metabolism, macrophage populations in particular are essential for

preserving the equilibrium between the availability of adequate iron levels and the avoidance of hazardous iron levels in the body. Red pulp macrophages (RPMs) in the spleen, central nurse macrophages in bone marrow (BM), and Kupffer cells (KCs) in the liver are examples of tissue macrophages that are specifically trained to recycle iron.(Sukhbaatar and Weichhart, 2018).

Delivery of iron-loaded transferrin into target cells is accomplished by receptor-mediated endocytosis . Endosomal acidification facilitates release of iron, and the apotransferrin-transferrin receptor complex is recycled to the cell surface. Ferric iron released from transferrin is reduced in the endosome by the ferrireductase STEAP3 and subsequently transported into the cytoplasm by Divalent metal transporter 1. From this point, the fate of iron depends on cellular needs. Iron can be used in the biosynthesis of heme, a tetrapyrrole molecule serving both as a prosthetic group for metalloenzymes and as the oxygen-binding moiety of hemoglobin.(Cassat and Skaar, 2013).

Iron sensing proteins, also referred to as iron regulatory proteins (IRPs) or iron responsive elements (IRE)-binding proteins, interact to regulate the expression of proteins involved in iron metabolism and homeostasis, such as ferritin or transferrin receptors, at the cellular level. (Vogt *et al.,* 2021).

2.6 Transferrin Receptor Gene

Transferrin receptors (TFRs) encoded by TFRC is a membrane glycoprotein, which can import iron by binding a plasma glycoprotein, transferrin (TF) . TF was first referred to as serum protein, with two specific sites binding Fe (III), so it is an iron source for synthesizing hemoglobin. Meanwhile, TF-bound iron undergoes cellular uptake requiring interaction between this protein and a specific TFR . The molecular weight of TFR as a homodimer is

180 kDa. Each monomer contains a TF-binding C-terminal domain, a short N-terminal domain and a single transmembrane domain.

The transcription of TfR gene is regulated by intracellular iron concentration via binding of iron regulatory proteins (IRPs) to the iron response elements (IREs) on the 50 untranslated region of TfR transcript.(Kazan et al., 2017).

A blood plasma glycoprotein called transferrin is essential for iron metabolism because it transports ferric ions to the liver, spleen, and bone marrow, among other tissues. Since transferrin binds nearly all plasma iron, there is little free iron in the body due to its strong affinity for ferric iron. As the body's most important ferric iron pool, transferrin is a crucial biochemical indicator for assessing the iron status of the body.

Transferrin receptors have two subtypes, transferrin receptor 1 (TFR1) and transferrin receptor 2 (TFR2). TFR1 is a homodimeric type II transmembrane glycoprotein that is expressed ubiquitously on the surfaces of most cells while another member of TFRs, TFR2 is mainly expressed in the liver.(Shen *et al*.,2018).

2.6.1 The Transferrin Receptor 1 (TfR1)

TfR1, also known as cluster of differentiation 71 (CD71), which is widely expressed and binds Tf with higher affinity and the less common TfR2, which is predominantly expressed in hepatocytes.(Kawabata, 2018).

The ability of TfR1 to mediate cell entrance of its molecular ligands is based on a clathrin-mediated endocytosis process. As one of the principal gatekeepers of iron metabolism and homeostasis, its vital function necessitates strict molecular regulation to preserve the proper iron balance in the body. By combining the control of TfR1 gene expression with the ability to fine-tune its activity through binding to various ligands, this degree of control is attained.

The internalization of the Tf-iron complex (Fe-Tf) through a receptor-mediated endocytosis mechanism via a clathrin-dependent pathway is the most well-understood method of iron uptake mediated by TfR1. (Testi *et al.*, 2019).

TfR1 consists of a dimer on the surface of the cell. Each receptor monomer binds one Tf molecule that consists of lobes (the N- and C-lobes). Each lobe binds one iron atom. Diferric Tf, also known as holo-Tf, contains two atoms of iron and binds to the receptor with high affinity.(Candelaria *et al.*, 2021).

Transferrin receptor 1 (Tfr1) facilitates the uptake of iron at the cell surface by internalizing diferric transferrin.Tfr1 is ubiquitously expressed in mammalian tissues and has been called the “cellular iron gate”.Tfr1 is essential for erythropoiesis, a process that consumes the majority of circulating iron. (Wang *et al.,* 2019).

TfR1 is expressed on malignant cells at levels several fold higher than those on normal cells and its expression can be correlated with tumor stage or cancer progression .This high expression of the receptor on malignant cells, its ability to internalize, and the necessity of iron for cancer cell proliferation make this receptor a widely accessible portal for the delivery of drugs into malignant cells and thus, an attractive target for cancer therapy (Daniels *et al.,* 2011).

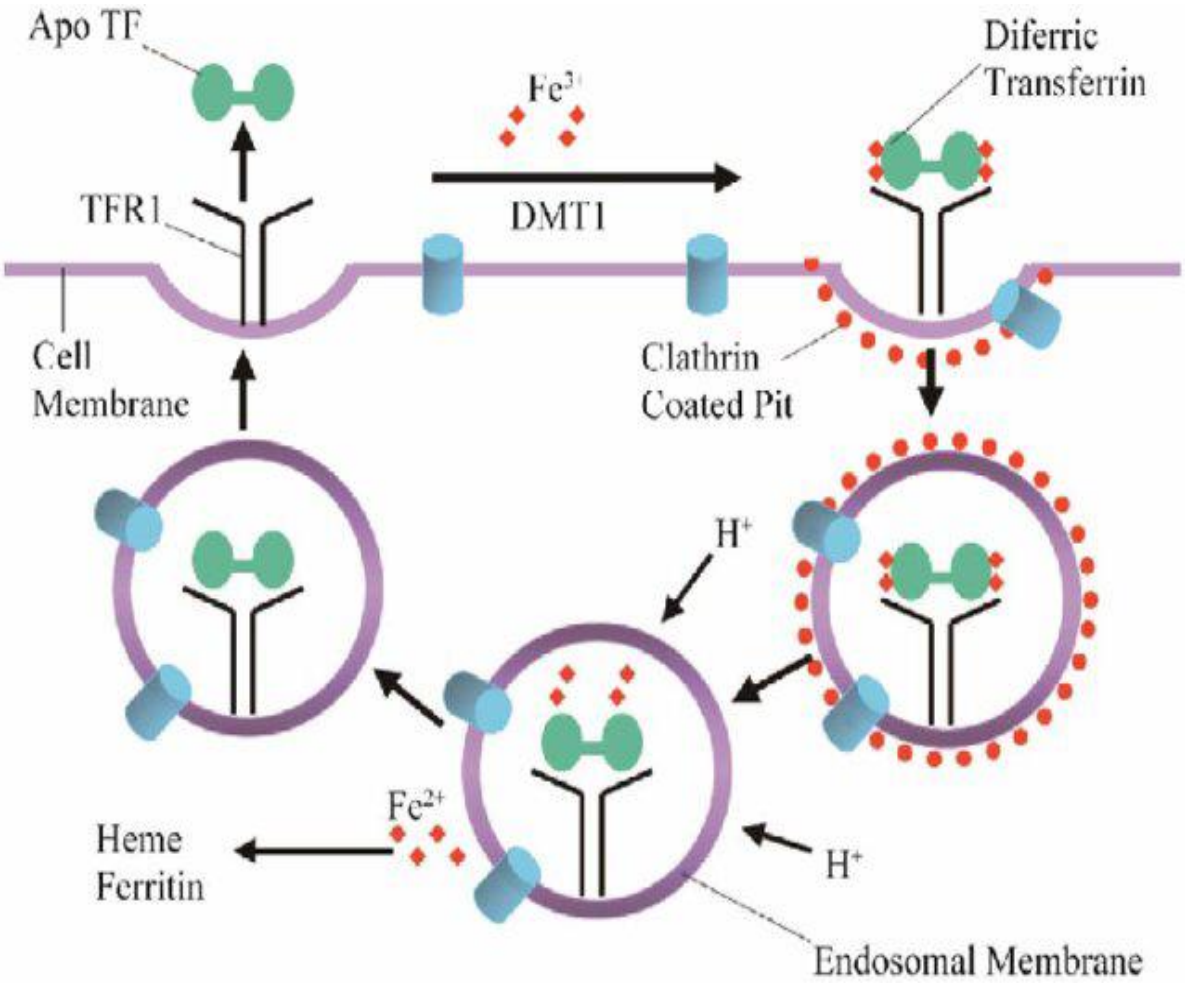

Fig 2.4: The transferrin cycle and the transferrin receptor 1-mediated cellular iron uptake (Elliott and Head, 2012).

2.6.2 Regulation of Transferrin Receptor Gene 1 in Erythropoiesis

Transferrin receptor 1 (TFR1), also known as CD71 and encoded by the TFRC gene, is expressed throughout the body but is most abundant in red blood cell precursors, where millions of copies may be present per cell.

To support the synthesis of red blood cells and preserve iron balance, its expression is strictly controlled. At the transcriptional level, TFRC expression is upregulated by the production of erythropoietin (EPO) under low oxygen and the stabilization of HIF-1α during hypoxia. By inactivating or degrading Iron Regulatory Proteins (IRP1 and IRP2), low cellular iron stabilizes TFR1 mRNA post-transcriptionally, whereas high iron decreases expression. Apo-transferrin can post-translationally reduce TFR1 protein levels without changing its mRNA, albeit the exact mechanism is still unknown.Overall, TFR1 serves as a central receptor for iron uptake, with its levels carefully controlled by oxygen, EPO, and iron availability to ensure proper red blood cell development.(Richard and Verdier, 2020).

CHAPTER THREE

MATERIALS AND METHOD

The research methodology details the strategies and procedures that will be utilized to achieve the research objectives and evaluate the hypotheses. This study proposes the following methodology:

3.1. Study Population Description

The study population consisted of Drosophila melanogaster, a commonly used model organism in biomedical research. Drosophila melanogaster strains were obtained from a reputable source (Drosophila Research center, University of Ibadan) and bred in the laboratory. Flies of an age range (1-2 days old) and both sexes were selected.

3.2. Inclusion criteria

Adult Male and female fruit flies (Drosophila melanogaster) less than 17 days old were fed with Uziza (*Piper guineense*) leaf extract. Control group also included Drosophila melanogaster that are 18 to 48 hours old and fed with only a basic cornmeal diet.

3.3. Exclusion criteria

Adult male and female Drosophila melanogaster above 17 days of life and immature stages of the flies.

3.4. Study Location

The study took place in a specialized laboratory facility—BIOTOXCS Research Laboratory, part of the Central Biomedical Research unit situated at the University of Benin, Benin City, Edo State, Nigeria. This laboratory was equipped with the appropriate infrastructure and equipment for rearing and handling Drosophila melanogaster. The controlled environment of

the laboratory provided optimal conditions for maintaining the flies and conducting the experimental procedures.

3.5. Drosophila melanogaster Feeding and Handling

The flies were given the standard prepared cornmeal diet, which contained the following components: cornmeal (52 g), brewer's yeast (5 g), glucose (3.5g), agar agar(7.9 g), nipargin (1g), water (850mL) and ethanol (2mL). Distilled water was used in the preparation of the diet. Flies less than 17 days old were selected at random from vials. When counting the files, extreme caution was exercised, and a soft bristled brush was utilized. To avoid handling stress, the flies were handled with great care.

3.6. Procedure for Feed Preparation

i. 850mL of distilled water was measured
ii. 150mL of the distilled water was removed to mix cornmeal
iii. The remaining 700 mL of distilled water was boiled
iv. Little quantity of boiling water was removed to mix the yeast
v. Agar was added to the boiling water and stirred until fully dissolved.
vi. The dissolved cornmeal was added and stirred.
vii. The dissolved yeast was added and stirred.
viii. The glucose was added with continued stirring
ix. The mixture was left to boil for about 2 minutes
x. The dissolved yeast was added
xi. The Nipargain was dissolved in ethanol and added after removing the meal from heat
xii. The meal was transferred into appropriate vials and allowed to cool.

3.7. Collection and Identification of Uziza leaves

Dried Uziza leaves were sourced from New Benin Market, Benin City, Edo State. It was identified as *Piper guineense* at the herbarium of the Department of Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, Nigeria, with the voucher number UBH-P094. The leaves were separated from the stem, sorted to remove the debris, washed using clean tap water and they were both air dried at room temperature for about a month in the laboratory.

3.8. Preparation of Plant Extract

The dried leaves and stems of *Piper guineense* were further air-dried at room temperature for 7 days and then pulverized to powder level using a commercial blender. Fifty grams (50g) of the powdered leaves was stirred into 450 ml of distilled water and allowed to stand at room temperature for 48 hours. The extracts were filtered multiple times using a cheesecloth after which the residue was thrown away and the filtrate concentrated to paste level using an incubator at 37°C for 7 days to actualize the crude extracts. The extracts were later preserved in a sample container and kept in a refrigerator at 4°C until the time for usage.

3.9. Experimental Design

This study employed a randomized controlled trial design, where fruit flies *(Drosophila melanogaster)* were randomly divided into treatment groups, each exposed to specific concentrations of *Piper guineense* leaf and stem extracts. A control group was included for comparison, consisting of flies not exposed to the extracts. This experimental setup is aimed to compare the effects of different concentrations of *Piper guineense* leaf extracts on the Transferrin receptor gene one in Drosophila melanogaster.To maintain consistency, Flies were randomly chosen from vials prepared within the past 18 to 24 hours and anesthetized by

being chilled in a freezer at 4°C for 5 minutes. To reduce stress during counting, careful handling was employed, using a soft-bristled brush.To ensure consistency, a total of 35 flies in each treatment group were raised and kept under identical environmental conditions, such as temperature, humidity, and lighting. Additionally, the exposure time to *Piper guineense* leaf and stem extract was standardized across all treatment groups to guarantee comparable results.

3.10. Extract Dosing of *Piper guineense*

Group A: was the control group which received Cornmeal and distilled water

Group B: was administered 100 mg/kg *Piper guineense* leaf extract

Group C: was administered 200 mg/kg *Piper guineense* leaf extract

Group D: was administered 300 mg/kg *Piper guineense* leaf extract

Group E: was administered 400 mg/kg *Piper guineense* leaf extract

For Group B

Ig is equivalent to 1000mg

Ig of the extract is dissolved in 10mL of distilled water

Concentration of extract-1000/10 =100mg/mL

For Group C

2g is equivalent to 2000mg

2g of the extract is dissolved in 10mL of distilled water

Concentration of extract-2000/10=200mg/mL

For Group D

3g is equivalent to 3000mg

3g of the extract is dissolved in 10mL of distilled water

Concentration of extract-3000/10=300mg/mL

For Group E

4g is equivalent to 4000mg

4g of the extract is dissolved in 10mL of distilled water

Concentration of extract-4000/10=400mg/mL

3.11. Experiment 1: Survival Assay

For the survival assay, flies (both gender) of 1-2 days old were divided into five groups, with each group having three vials each. Each vial contained 35 flies each. Three groups had varied concentrations of aqueous Uziza leaf extract while the control group which had no amount of *Piper guineense* leaf extract and only contained cornmeal and 0.2mL of distilled water.

GROUP 1 (Control): Control flies fed on a 9.8g Cornmeal diet.

GROUP 2 (100mg): Flies fed on 100mg *Piper guineense* Leaf Extract + 9.8g Commeal diet

GROUP 3 (200mg): Flies fed on 200mg *Piper guineense* Leaf Extract + 9.8g Cornmeal diet

GROUP 4 (300mg): Flies fed on 300mg *Piper guineense* Leaf Extract + 9.8g Cornmeal diet

GROUP 5 (400mg): Flies fed on 400mg *Piper guineense* Leaf Extract + 9.8g Cornmeal diet

The survival assay was performed in three duplicates of each concentration. Throughout this experiment, the diet was changed every five days. The survival rate was calculated using all

concentrations, and both live and dead flies were counted daily. By the end of the experiment (21 days), the data had been gathered and plotted as a proportion of live and dead flies. The results were then compared to the control.

3.12. Tissue Homogenate Preparation for Biochemical Assay

To assess the biochemical tests, a second group experiment was conducted. Flies (both sexes) that were one to two days old were divided into five groups for this experiment, and each group received three vials. Thirty-five (35) flies at various dosages of Uziza leaf extract were put in each treatment vial, and the experiment was monitored for five days.

GROUP 1(A): Control flies fed on normal standard 9.8g commeal diet.

GROUP 2(B): Flies fed on 100mg/kg *Piper guineense* LE + 9.8g Commeal diet.

GROUP 3(C): Flies fed on 200mg/kg Piper guncense LE + 9.8g Cornmeal diet.

GROUP 4(D): Flies fed on 300mg/kg Piper guineente LE + 9.8g Commeal diet.

GROUP 5(E): Flies fed on 400mg/kg Piper guineente LE + 9.8g Commeal diet.

At the conclusion of the treatment period,the flies were placed on ice to sedate them, weighed, and homogenised in cold 0.IM phosphate buffer with a pH of 7.0 (1:10 w/v). They were then centrifuged at 3500g for 5 minutes at 4°C (Allegra X-15R Centrifuge; Beckman Coulter, USA). After being separated into labelled Eppendorf tubes, the supernatants were utilised for the MDA biochemical test. Each of the five groups underwent three duplicates of every experiment.

3.13. Preparation of Reagent

Following that, a homogenising buffer (0.1M phosphate buffer, pH 7.0) was made. Solution A was formed by dissolving 2.76g of Na_2HPO_4 in 100ml of water. To formulate solution B,

2.839g of NaH_2PO_4 was dissolved in 100ml of purified water. The buffer, which was subsequently kept at room temperature, was made by adding 39 millilitres of solution A to 61 millilitres of solution B.

3.14 Transferrin Receptor 1 (Tfr1) mRNA Assay

3.14.1 RNA Extraction

Total RNA was extracted from pooled adult Drosophila melanogaster using the TRIzol method. For each replicate, 10–15 flies were anesthetized on ice, transferred into a pre-chilled RNase-free tube, and homogenized in 1 mL of TRIzol reagent with a sterile pestle. The homogenate was allowed to stand for 5 minutes at room temperature to ensure full dissociation of nucleoprotein complexes. Chloroform (200 µL) was added, the tube was shaken vigorously for 15 seconds, and centrifuged at 12,000 × g for 15 minutes at 4 °C. The clear aqueous phase was collected into a fresh RNase-free tube, and RNA was precipitated with an equal volume of isopropanol. The pellet was washed twice with 75% ethanol, air-dried, and resuspended in RNase-free water. To eliminate genomic DNA contamination, the RNA was treated with RNase-free DNase I.

RNA concentration and purity were measured on a NanoDrop spectrophotometer by checking absorbance at 260/280 nm. Samples with ratios between 1.8 and 2.0 were considered suitable. Integrity was further confirmed by running 1 µg RNA on a 1% agarose gel; clear 28S and 18S rRNA bands indicated good quality RNA. The purified RNA was stored at –80 °C until use.

3.14.2 cDNA Synthesis

Complementary DNA was synthesized from 1 µg of total RNA using a reverse transcription kit (Applied Biosystems, USA) with random hexamer primers. The reaction mixture was

incubated at 42 °C for 1 hour and terminated at 70 °C for 10 minutes to inactivate the enzyme. The resulting cDNA was diluted five-fold in nuclease-free water and kept at –20 °C until PCR analysis. A no-reverse transcriptase control was included to confirm absence of genomic DNA contamination.

3.14.3 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):set:> NM_078544.3 Drosophila melanogaster tsfr1, Forward primer GCAGTTCACCAGGATCTAAT Reverse primer AGCACAGGAAGTACGGAATG, > NM_080094.4 Drosophila melanogaster Glycerol-3-phosphate dehydrogenase (Gpdh), Forward primer TCGGACTGCGTAGACACTAGA Reverse primer AGCGCCATCTATGTAAGGATGT.

PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using “image J” software (Elekofehinti et al., 2020).

3.14.4 Semi-Quantitative PCR (sqPCR)

The expression of Tfr1 was analyzed by semi-quantitative PCR using gene-specific primers designed from Drosophila melanogaster sequences. Ribosomal protein L32 (RpL32) served

as the internal control. Each 25 µL PCR reaction contained 2 µL cDNA template, 12.5 µL of 2× PCR master mix, 1 µL of each primer (10 µM), and nuclease-free water.

Thermal cycling conditions were: 95 °C for 3 minutes, followed by 35 cycles of 95 °C for 30 seconds, annealing at 58–60 °C for 30 seconds, extension at 72 °C for 45 seconds, and a final extension at 72 °C for 5 minutes.

PCR products were separated on a 1.5% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. Five microliters of each product mixed with loading dye was loaded into the gel alongside a 100 bp DNA ladder. Electrophoresis was run at 100 V for 45–60 minutes. Bands were visualized under UV light and documented using a gel documentation system.

3.14.5 Data Analysis

Band intensities were measured with ImageJ software. Expression of Tfr1 was normalized to RpL32 by calculating the target-to-reference band intensity ratio. Relative expression values were compared between control and treated groups, and statistical analysis was performed using one-way ANOVA. Significance was accepted at $p < 0.05$.

CHAPTER FOUR

RESULTS

Table 4.1 shows the survival percentages of *Drosophila melanogaster* exposed to varying concentrations of *Piper guineense* extract over a 21-day period. On Day 7, the control group recorded a survival rate of (88.67 ± 3.22). Flies treated with 100 mg of the extract showed the highest survival (94.67 ± 0.88), followed by those given 400 mg (91.67 ± 2.91), 200 mg (89.33 ± 2.60), and 300 mg (85.67 ± 3.18). Statistical evaluation revealed no significant differences among the groups ($p = 0.183$). By Day 14, the control survival declined to (84.33 ± 2.33), while the 100 mg group again had the highest value (89.33 ± 2.33). This was followed by 200 mg (83.00 ± 2.52), 400 mg (81.67 ± 2.33), and 300 mg (78.33 ± 2.19). Group differences approached but did not reach statistical significance ($p = 0.075$). At Day 21, overall survival further decreased across all treatments. The control group recorded the lowest survival (71.33 ± 1.76), whereas the 100 mg group retained the highest value (80.00 ± 2.65), followed by 200 mg (75.67 ± 3.38), 400 mg (73.33 ± 2.03), and 300 mg (72.33 ± 2.33). No significant variation was observed among the groups at this stage ($p = 0.184$).

Table 4.1. Percentage of survival at different concentrations of *Piper guineense*

Time Point	Group A (Control) (%)	Group B (100 mg) (%)	Group C (200 mg) (%)	Group D (300 mg) (%)	Group E (400 mg) (%)	F	p value
Day 7	88.67 ± 3.22	94.67 ± 0.88	89.33 ± 2.60	85.67 ± 3.18	91.67 ± 2.91	1.927	0.183
Day 14	84.33 ± 2.33	89.33 ± 2.33	83.00 ± 2.52	78.33 ± 2.19	81.67 ± 2.33	2.955	0.075
Day 21	71.33 ± 1.76	80.00 ± 2.65	75.67 ± 3.38	72.33 ± 2.33	73.33 ± 2.03	1.916	0.184

Values are shown as Mean ± SEM. $p < 0.05$ is considered significant.

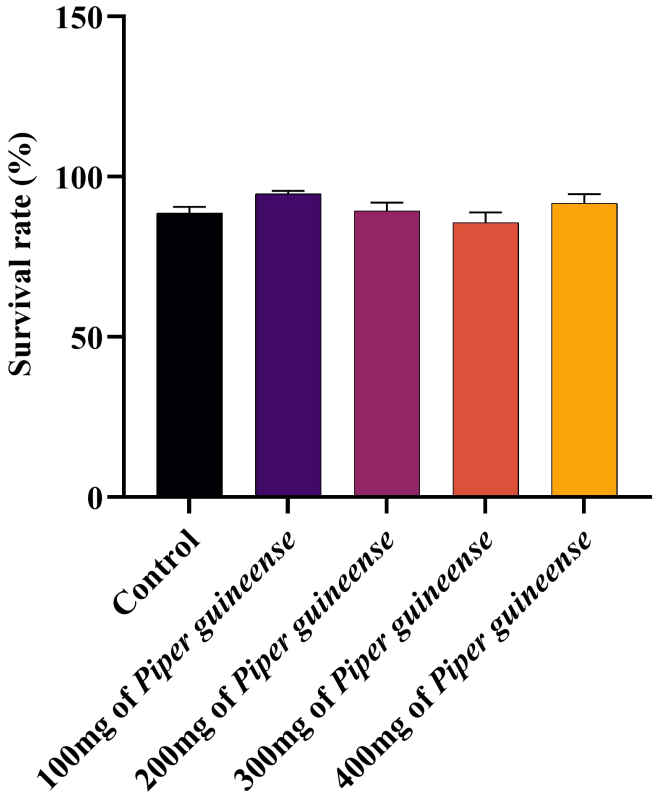

Figure 4.1: Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 7. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

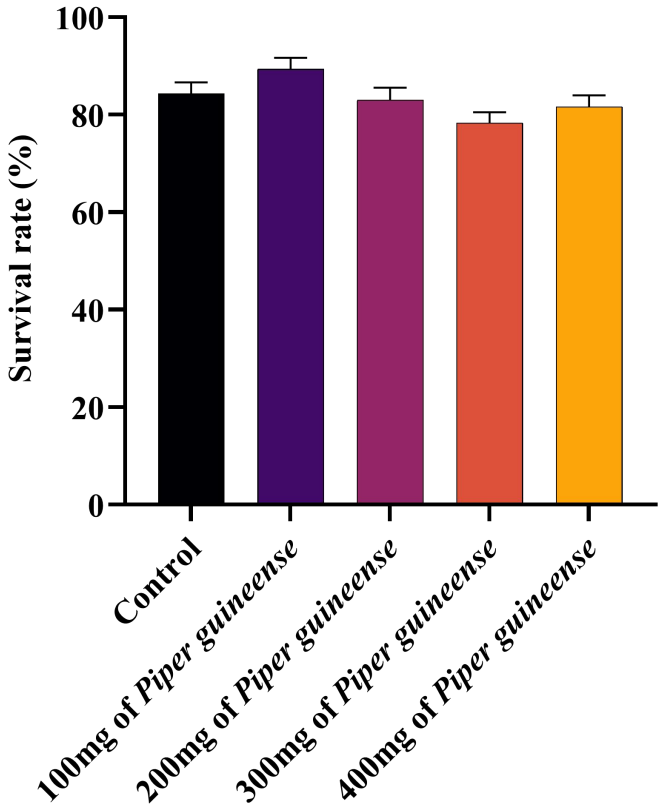

Figure 4.2: Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 14. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

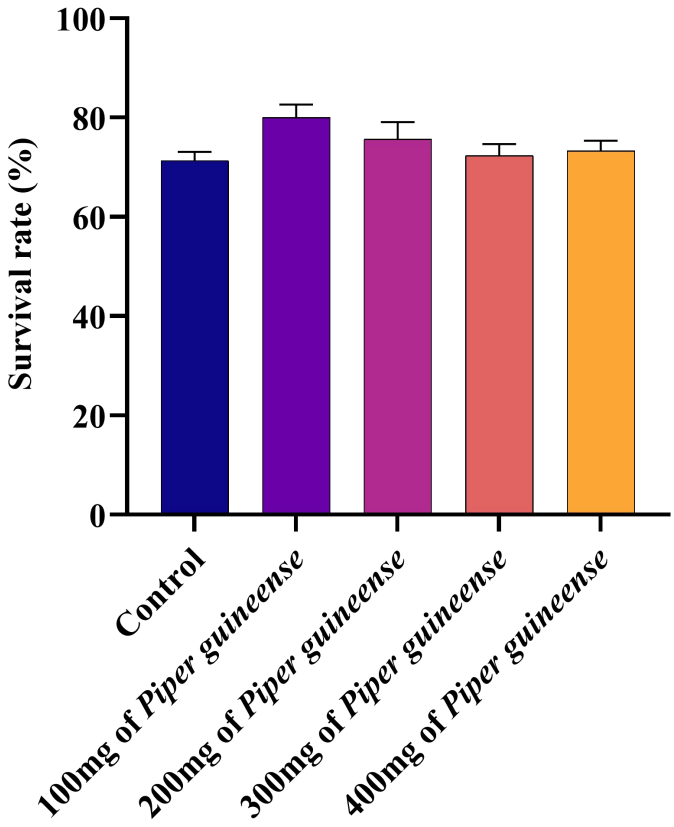

Figure 4.3: Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 21. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

Figure 4.4 shows the survival curve of *Drosophila melanogaster* over the 21-day period. Survival declined progressively across all groups, including the control and the various treatment doses. At the start of the study, all groups recorded 100% survival. Throughout the experiment, the 100 mg group consistently exhibited the highest survival, while the control, 300 mg, and 400 mg groups experienced the greatest decline. The 200 mg group maintained an intermediate survival pattern, positioned between the higher and lower performing groups.

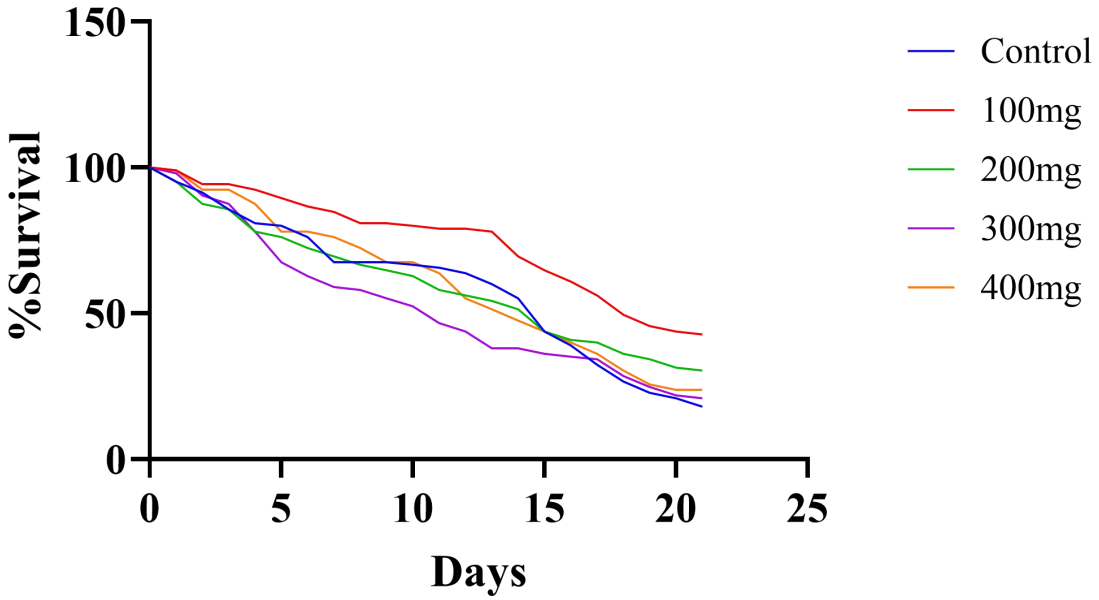

Figure 4.4: Survival curve of *Drosophila melanogaster* administered different concentration of *Piper guineense* over the 21 days.

Figure 4.5 represents the mRNA expression levels of Transferrin receptor 1 (TSFR1) across five groups (A–E): the control group and those administered 100 mg/kg, 200 mg/kg, 300 mg/kg, and 400 mg/kg of *Piper guineense* in *Drosophila melanogaster*, with each group displayed as a separate bar in the chart. TSFR1 mRNA expression was significantly lower in the group administered 400mg of *Piper guineense* (1.89±0.05) when compared to control group (2.30±0.10) ($p<0.05$). The groups administered 100mg of *Piper guineense* (2.30±0.10), 200mg of *Piper guineense* (2.20±0.10) and 300mg of *Piper guineense* (1.90±0.10) had no significant difference in the mRNA expression of TSFR1 when compared to the control ($p>0.05$). However, the group administered 100mg of *Piper guineense* had significantly higher TSFR1 expression than the group administered 400mg ($p<0.05$).

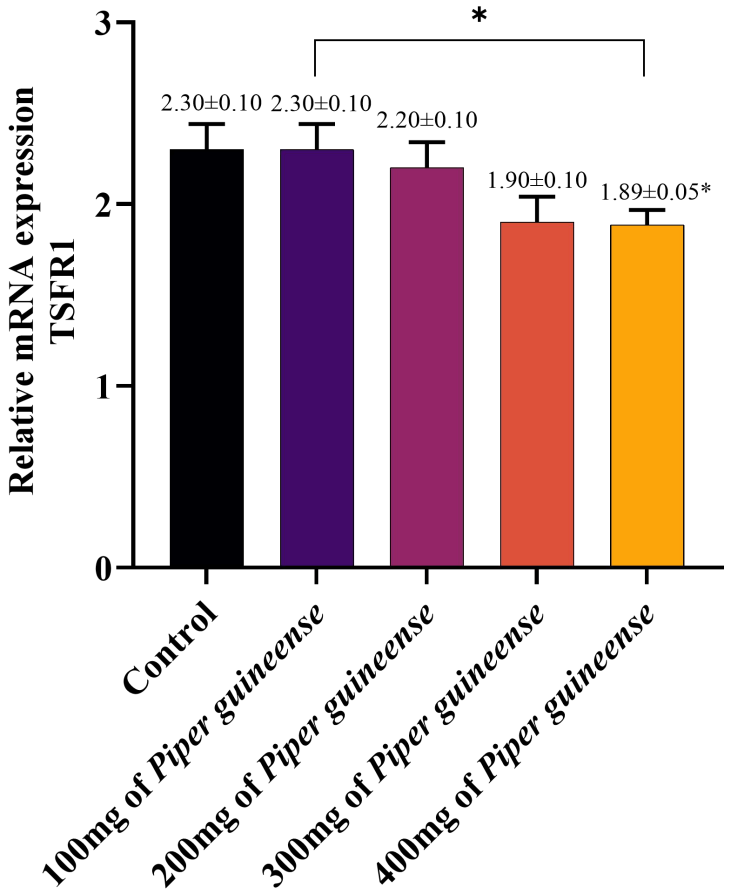

Figure 4.5: PCR of Transferrin receptor 1 (TSFR1) gene. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05).

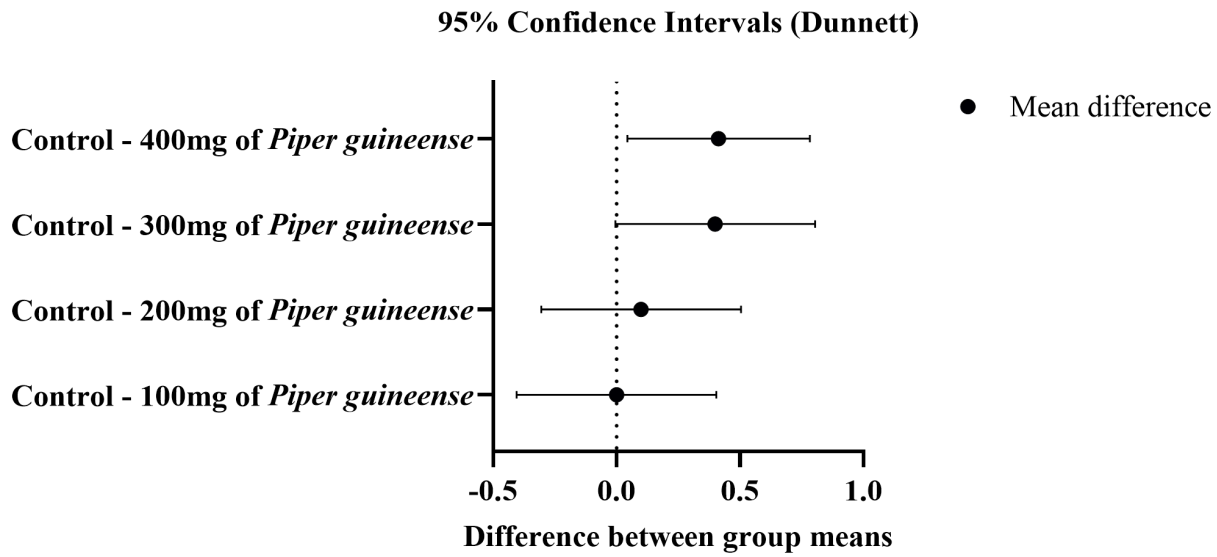

Figure 4.6: Confidence interval of the multiple comparison of control and the various groups treated with *Piper guineense*.

CHAPTER FIVE

5.1. Discussion

This study investigated the effect of *Piper guineense* leaf extract on the expression of transferrin receptor gene 1 (TSFR1) in *Drosophila melanogaster*. Transferrin receptors are main regulators of cellular iron uptake, and their expression provides information into iron homeostasis, oxidative stress, and related metabolic processes (Vogt *et al*., 2021). Given that *Piper guineense* is a medicinal plant widely recognized for its phytochemicals, including alkaloids, flavonoids, and essential oils with antioxidant and therapeutic properties (Alagbe *et al*., 2021), assessing its influence on TSFR1 expression in a model organism such as *Drosophila* offers an important perspective on its potential biological effects. By analyzing changes in TSFR1 expression across different concentrations of the extract, this study provides evidence that may support the pharmacological relevance of *Piper guineense* in modulating iron metabolism and oxidative balance.

The survival analysis of *Drosophila melanogaster* exposed to different concentrations of *Piper guineense* leaf extract over a period of 21 days revealed that at Day 7, survival rates were generally high across all groups. Flies administered 100 mg of the extract had the highest survival rate, suggesting a possible protective effect of the extract at low concentration. The survival advantage observed at 100 mg may be attributed to the presence of bioactive compounds in *Piper guineense*, such as flavonoids and alkaloids, which are known to possess antioxidant properties that can reduce oxidative stress and enhance cellular survival (Carsono *et al*., 2022). The observation that higher doses (200–400 mg) did not cause a proportional increase in survival suggests that the beneficial effect of the extract may be dependent on the dosage, with low concentrations having higher benefits. Similar findings have been reported in studies where plant extracts demonstrated hormetic effects, in which

low doses promoted survival while higher doses exerted neutral or slightly adverse effects (Calabrese and Baldwin, 2003). By Day 14, survival rates began to reduce in all groups, consistent with the expected natural attrition in *Drosophila* lifespan (Kimber and Chippindale, 2013). However, the group treated with 100 mg once again maintained the highest survival, further proving the notion that a moderate concentration of *Piper guineense* supports longevity of the organism. This finding aligns with one of the main functions of phytochemicals which is their ability to act as antioxidants, as these phytochemicals in *Piper guineense* may have helped mitigate age-related oxidative damage, which is a key determinant of survival in *Drosophila* (Forni *et al*., 2019; Belyi *et al*., 2020). Previous research has highlighted the role of plant-derived antioxidants in extending fly lifespan through attenuation of reactive oxygen species (ROS) and improvement of mitochondrial function (Peng *et al*., 2011). At Day 21, a further decrease in survival was observed across all groups, showing the progressive aging process in *Drosophila* (Tsurumi and Li, 2020). The control group had the lowest survival, whereas the 100 mg treatment group continued to show the highest survival. This sustained advantage supports the view that low-dose *Piper guineense* supplementation may confer resilience against age-associated mortality as observed at day 7 and day 14. The reduction in survival at higher doses may be linked to potential pro-oxidant effects of phytochemicals when consumed in excess, as has been documented in studies where high concentrations of polyphenols and alkaloids paradoxically increased oxidative stress (Halliwell, 2008; Fernando *et al*., 2019).

The gene expression analysis of transferrin receptor 1 (TSFR1) in *Drosophila melanogaster* following administration of *Piper guineense* leaf extract revealed dose-dependent alterations that may reflect underlying changes in iron metabolism and cellular homeostasis. TSFR1 is a key membrane protein responsible for mediating cellular iron uptake by binding transferrin-

bound iron and internalizing it through receptor-mediated endocytosis (Talukder, 2021). Its expression is tightly regulated at the post-transcriptional level by the iron regulatory proteins (IRPs), which stabilize or degrade TSFR1 mRNA in response to intracellular iron levels (Kuhn, 2015). Consequently, changes in TSFR1 expression serve as an important marker of cellular iron demand and storage status (Wang *et al*., 2019). In the present study, the control group exhibited baseline TSFR1 expression (2.30 ± 0.10), which was comparable to that of flies administered 100 mg/kg (2.30 ± 0.10) and 200 mg/kg (2.20 ± 0.10), indicating that at low doses, *Piper guineense* did not significantly alter iron uptake pathways. The maintenance of TSFR1 expression at these concentrations suggests that cellular iron demand and availability remained balanced, possibly due to the antioxidant effects of phytochemicals in *Piper guineense* mitigating oxidative stress without disturbing iron metabolism (Uhegbu *et al*., 2015). Flavonoids, for example, have been reported to modulate iron homeostasis by scavenging free radicals and chelating excess iron without interfering with iron transport proteins at physiological concentrations (Lesjak and Srai, 2019). The group administered 300 mg/kg showed a mild but not statistically significant reduction in TSFR1 expression (1.90 ± 0.10). This reduction indicates a possible shift toward reduced iron uptake, which may occur when intracellular iron availability is sufficient or mildly elevated (Anderson and Frazer, 2017). Excess iron can generate reactive oxygen species through the Fenton reaction, and a downregulation of TSFR1 could represent a protective cellular mechanism aimed at preventing iron overload (Dixon and Stockwell, 2014). Similar compensatory responses have been documented in mammalian studies where dietary polyphenols and alkaloids influenced iron absorption and utilization (Yiannikourides and Latunde-Dada, 2019). The most notable finding as regards transferrin reception 1 was observed in the 400 mg/kg group, where its expression was significantly reduced (1.89 ± 0.05) compared to the control. This decrease

suggests that high concentrations of *Piper guineense* extract either increased intracellular iron levels to a point where TSFR1 expression was actively repressed, or that bioactive components of the extract directly interfered with iron transport pathways. High doses of plant polyphenols are known to bind non-heme iron, forming insoluble complexes that reduce bioavailability (Petry, 2014; Scarano *et al*., 2023). This can mimic a state of intracellular iron sufficiency, prompting reduced TSFR1 expression despite possible extracellular sequestration of iron (Hurrell and Egli, 2010). Another possible explanation is that high-dose *Piper guineense* caused a pro-oxidant effect, leading to mechanisms that suppress iron uptake to minimize oxidative damage (Ademuyiwa *et al*., 2023). In this study, it was observed that the 100 mg/kg group had significantly higher TSFR1 expression compared to the 400 mg/kg group, which shows that the action of *Piper guineense* on TSFR1 is dependent on dosage. At low concentrations, the extract may support normal iron trafficking and cellular metabolism, whereas at high doses it disrupts these processes, possibly through iron chelation or modulation of IRP–IRE signaling.

5.2. Conclusion

This study shows that *Piper guineense* leaf extract influences survival and transferrin receptor gene expression in *Drosophila melanogaster* in a dose-dependent manner. Flies treated with 100 mg had the highest survival across 21 days, indicating a protective effect at lower concentrations. TSFR1 expression was maintained at low to moderate doses but significantly reduced at 400 mg/kg, suggesting suppression of iron uptake at higher levels. These findings highlight that while low doses of *Piper guineense* may support survival and iron balance, higher doses could disrupt transferrin receptor regulation.

5.3. Recommendations

1. Future research should look deeper into how *Piper guineense* actually influences iron regulation at the genetic and cellular level.
2. A wider range of doses would be helpful to better understand the safe and most effective levels.
3. Studies in higher animals are needed to see if these findings can be applied beyond *Drosophila*.
4. Checking the effects on other iron-related markers and stress pathways could give a fuller picture.

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APPENDIX

MATERIALS AND REAGENTS USED

MATERIALS USED

Centrifuge

Incubator

Automated Pipettes

Eppendorf tubes

Piper guineense leaf extract

Homogenising stick

Measuring cylinders

Digital weighing scale

Falcon tubes

Funnel and Whatman's filter paper

Laboratory coat and gloves

REAGENTS USED

Trizol regards

Phosphate buffer

Distilled water

Primers used were synthesized by Inqaba Biotec, South Africa.

DNA extraction kit

Agarose gel