EFFECT OF THE VARIED CONCENTRATION OF UTEZI LEAF EXRACT ON IRON METABOLISM IN *Drosophila melanogaster*

BY

ISIBOR DORCAS OLUWASEUN

BMS1702088

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY.

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A PROJECT PRESENTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE. IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF

THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE (BMLS) DEGREE.

SUPERVISOR:

MR JAMES OSAKUE

SEPTEMBER, 2023

CERIFICATION

This is to certify that this project work was carried out by **ISIBOR DORCAS OLUWASEUN** with matriculation number **BMS1702088** under the supervision of **MR. JAMES OSAKUE**, in partial fulfilment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

_____ _____

MR JAMES OSAKUE **DATE**

Supervisor

_____ _____

DR. B.I.G ADEJUMO **DATE**

Ag. Head of department

_____ _____

External Examiner **DATE**

DEDICATION

I dedicate this project work to God Almighty for his love, grace, wisdom and knowledge he bestowed upon me throughout my stay in the University of Benin.

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I thank God Almighty, who enabled me with philosophy, perception and motivation to present this work, after so many hurdles and obstacles. Without him all efforts will account to nothing.

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TABLE OF CONTENTS

Cover page	i
Title page	ii
Cerification	iii
Dedication	iv
Acknowledgments	v
Table of contents	vi
List of tables	viii
List of figures	ix
Abstract	x
CHAPTER ONE	**1**
INTRODUCION	**1**
1.2:Background to the study	1
1.2 Justification of the study	5
1.3. Aim of Study	6
1.4 Specific Objectives	6
1.5 Research Questions	6
1.6 Research Hypothesis	7
1.7 Scope of Study	7
CHAPTER TWO	**8**
LITERATURE REVIEW	**8**
2.1Utazi Leaf	8
2.1.1 Origin And Geographical Distribution	9
2.1.2 Botanical Characteristics	9
2.1.3 Ethnopharmacological Uses	11
2.1.4 Phytochemistry Of Gongronema Latifolium	12
2.1.5 Pharmacological Properties Of *Gongronema Latifolium*	13
2.2 Effect Of Utazi Leaf Extract On Iron Metabolism In *Drosophila Melanogaster*	15
2.3 Iron metabolism in *Drosophila Melanogaster*	15
2.4 Iron absorption	18
2.4.1. Iron uptake	18
2.4.2. Iron transport	19

2.5.Transferrin 21
2.6 Iron storage 23
2.7 Iron Utilization 24
2.8 Iron homeostasis 24
2.7 Iron excretion 25
2.8 Immune response 25
2.9 Drosophila melanogaster 25
CHAPTER THREE **33**
MATERIALS AND METHODS **33**
3.1 Study design 33
3.2 Study subject 33
3.3 Inclusion Criteria 33
3.4 Exclusion Criteria 33
3.5 Fruit Flies and Treatment 34
3.6 *Drosophila melanogaster* Feed Formulation and its handling 34
3.7 Source and Preparation of Aqueous Utazi leaf Extract 35
3.8 Experimental design 36
3.8.1 Experiment 1: Survival assay 37
3.8.2 Experiment 2: Tissue homogenate preparation for biochemical assay 38
3.8.3 Experiment 3: EVALUATION OF THE DIFFERENT PARAMETERS 39
3.8.4 Reagent preparation for Ferritin 40
3.8.5 Method for the evaluation of Ferritin 40
3.8.6Reagents and Concentration for TIBC 41
3.8.7 Method for the evaluation of TIBC 42
3.8.7 Reagent Concentration for Serum Iron Assay 43
3.8.8 Method for Serum Iron Assay 43
3.9 Statistical analysis 43
CHAPTER FOUR **45**
4.0 RESULTS **45**
5.1 DISCUSSION 56
5.2 Conclusion 61
APPENDIX 66

LIST OF TABLES

Table 4.1: shows the number of *Drosophila melanogaster* flies that died within a 21day period of the survival studies 46

Table 4.2: Percentage Survival of *Drosophila melanogaster* with varied concentration of Utazi Leaf Extract 47

Table 4.3 show the p value in different groups 55

LIST OF FIGURES

Figure 2.1 *Gongronema latifolium* 1 (Hannah *et al.*, 2012) 14

Figure 2.2: Comparison of iron metabolism in mammals and the fruit fly (Nichol et al., 2002) 17

Figure 2.3: Life-cycle of Drosophila (♂: male; ♀: female) with three larval instar and a pupa stages (Perveen F.K., 2018). 28

Figure 2.4: Identification of sexes in adult fruit flies. (30

Figure 4.2: Graph of Serum Iron and TIBC levels against Concentration of Plant Extract 51

Figure 4.3: Graph of Ferritin levels against Concentration of Plant extract. 52

ABSTRACT

Iron is one of the integral components of many biochemical properties which is maintained normal physiological activities for the healthy life. Insufficient iron causes different effects at the cellular level like limited oxygen supply, meager work performance and reduces immunity. The aim of this study is to determine the effect of the varied concentration of *Gongronema latifolium* leave extract on iron metabolism in *Drosophila melanogaster***.** Samples were 1-2 days old virgin male and female *Drosophila melanogaster* fed with varied concentration of Utazi leaf extract supplemented corn meal diet. The control subjects include 1-2 days old virgin male and female *Drosophila melanogaster* fed with only corn meal diet and distilled water. The survival assay was carried out in three replicates of each concentration, for the determination of biochemical assays, a second group experiment was carried out. Ferritin ELISA kit was used to determined the Ferritin level, TIBC was determine using TIBC ELISA kit, serum iron was determined using serum iron ELISA kit. Results show that the average percentage survival was observed to be the highest in Group 5 having 80 percent of flies still alive at day 21. The Survival curve showed a negative correlation curve which shows that as the days increase, survival decreases. Group 1, the control group, exhibited a Ferritin level of 0.42±0.44, while Group 2, treated with aqueous Utazi extract, showed a slightly lower Ferritin level of 0.40±0.00. However, Group 3 (given Utazi extract), Group 4 (administered 5.0 mg Utazi extract), and Group 5 (given 2.5mg and 0.1mg of Utazi extract, respectively) demonstrated significant changes ($p < 0.001$) in Ferritin levels, with Group 4 having the lowest level at 0.22±0.18. there was also highly significant difference ($F=128.969$, $p=0.000^*$) in Ferritin levels among the group. Iron levels varied significantly among the groups, with Group 3 exhibiting the highest levels (16.18±18.46) and Group 2 the lowest (4.65±0.00). TIBC levels followed a similar trend, with Group 3 having the highest value (38.29±15.43) and Group 2 the lowest (6.33±0.00). The ANOVA analysis for TIBC showed significant differences ($F=31.389$, $p=0.000^*$) among the groups. In conclusion, the study revealed a decreasing survival rate with increasing number of days that is a negative correlation between the concentration of Utazi leaf extract in the diet and on the survival of *Drosophila melanogaster*, suggesting potential toxicity at higher concentrations. Additionally, significant alterations in Ferritin, Iron, and Total Iron-Binding Capacity (TIBC) levels among experimental groups was observed, indicating that Utazi leaf extract can influence iron metabolism in fruit flies

CHAPTER ONE

INTRODUCION

1.2:Background to the study

Plants and plant extracts have long been used to treat medical conditions. Recent years have seen an increase in the popularity of this technique because it has fewer adverse effects than conventional medicine (Ajiboye *et al.,* 2019).A Growing interest has been shown in researching potential alternatives to synthetic drugs' adverse effects, which are constantly in doubt. Despite the technological advances, intuitive people in all parts of the world, particularly in developing nations, still rely on herbs to treat some illnesses. This may be because to poverty or the effectiveness of the herbal remedies (Etetim *et al.,* 2008).

Natural herbs have been used throughout human history to treat or prevent a number of illnesses. The increased understanding of diverse medicinal plants has contributed to the control of numerous ailments in both humans and animals. (Afolabi *et al.,* 2008). Due to the ban on the use of some antibiotics, which have negative side effects and are inexpensive, the use of herbal feed additives is becoming more and more important in the production of animals. It has been discovered that a range of feed additives, including probiotics, prebiotics, organic acids, and plant extracts, have positive benefits on animal productivity. It is important to take use of medicinal herbs' enhanced digestibility, antibacterial, anti-inflammatory, anti-oxidant, and immune-stimulating activities while feeding animals and creating safe food products for humans. To overcome the difficulty

of regulated amounts of herbal feed additives, more research is needed (Muneendra *et al.,* 2014).

One of these plant species, *Gongronema latifolium*, possesses leaves and other plant parts that have had amazing effects on both people and animals. The nutrient-dense plant *Gongronema latifolium* has a high protein and carbohydrate content as well as antimicrobial capabilities (Afolabi, 2007).

To the Asclepiadaceae family belongs the plant *Gongronema latifolium*. It is a plant that is edible, nutritious, and healing that is primarily found in rain forest regions in Nigeria and other tropical African nations (Olugbenga, 2015). In South Eastern and South Western Nigeria, the locals refer to it as "Utazi" and "Arokeke" respectively. Along with being present in North and South-East Asia, it is also found in South America. *Gongronema latifolium* has long been acknowledged as a traditional African treatment for a number of illnesses, including intestinal and mental diseases, hypertension, diabetes mellitus, and malaria. *Gongronema latifolium* leaves are used in a tea blend that is mostly sold to people with diabetes mellitus in the United States. *Gongronema latifolium* extracts have been shown to exhibit a number of pharmacological effects that have provided experimental support for the empirical ethno-pharmacological use of this plant in folk medicine. For instance, reports of anti-inflammatory, antifungal, laxative, and anti-diabetic properties ((Muneendra *et al.,* 2014).

Different portions of *Gongronema latifolium* have been discovered to include anthraquinones, alkaloids, saponins, sitosterol, sitostenone, lupenyl esters, pregnancy ester, glucosides, and essential oils over the past 20 years. (Al-Hindi *et al.,* 2019).

Fly species belonging to the taxonomic order Diptera and family Drosophilidae include *Drosophila melanogaster*. The species is usually known by the names fruit fly, smaller fruit fly, or less frequently "vinegar fly" or "pomace fly." (Green, 2003). Beginning with Charles W. Woodworth's suggestion to use this species as a model organism in 1901,

(Holden, 2015) for biological studies in genetics, physiology, microbial disease, and life history evolution, *Drosophila melanogaster* is still extensively employed. Six Nobel Prizes have been awarded to drosophilists as of 2017 for their work with the bug (Sang, 2011). Due to its quick life cycle, straightforward genetics (it only has four pairs of chromosomes), and huge number of progeny per generation, *Drosophila melanogaster* is frequently utilized in research. Since all non-African lineages have a common ancestor, it was originally an African species. All continents, including islands, are included in its geographical range (Markow, 2015). A frequent pest in residences, eateries, and other locations where food is served is *Drosophila melanogaster* (Ewart, 1998).

"Fruit flies" are another name for members of the Tephritidae family of flies. Especially in the Mediterranean, Australia, and South Africa, where the Mediterranean fruit fly Ceratitis capitata is a commercial pest, and this can be confusing.

Drosophila is a model organism that is essential in the many different life sciences study domains. (Akter *et al.,* 2016). Drosophila is simple to handle, affordable to maintain, reproduces and grows rapidly, produces a huge number of offspring, and its genome sequence and genetic makeup are well known. (Hoskins *et al.,* 2007).

Due to its genetic similarity to humans, the fruit fly, *Drosophila melanogaster*, is the most significant (Flint and Mackay, 2009). This fruit fly has been used to simulate various animal diseases, including a wide variety of physiological changes (Tang and Zhou, 2013). Drosophila is also thought to be useful for researching the possible harmful effects of a variety of substances, particularly metal toxins. (Hosamani and Muralidhara, 2013). It is simple to assess the effect of metal poisoning during development and adulthood because of their shorter life cycles. Additionally, this organism makes it simple to conduct experiments for neural activity, survival, and behavior (Ugur *et al.,* 2016). In Drosophila, it is simple to look for molecular processes that are mediated by toxicity. Even though Drosophila has been the subject of numerous investigations, only a very small number of studies about iron toxicity in *Drosophila melanogaster* have been published (Calap-Quintana *et al.,* 2017)

One of the essential elements of several biochemical processes that maintains healthy physiological functions is iron (Drygalski and Adamson, 2013). At the cellular level, inadequate iron has a variety of negative effects, including reduced immunity, poor work performance, and limited oxygen delivery (Bhaskaram, 2001). Anemia is the most typical symptom of iron deficiency, which lowers the size and quantity of red blood cells (Lopez, *et al.,* 2016). On the other hand, when it is present in excess, iron can be poisonous and even fatal (Corbett 1995). All meals of plant and animal origin, as well as water, include iron as a natural component. Under normal physiological settings, the body contains 3 to 5 g of iron (Anderson *et al.,* 2017). When iron is found in the environment as pollutants, it can have negative effects on people, animals, and plants. If

iron homeostasis is not properly controlled, a deviation from the normal iron range can cause tissue damage and the production of free radicals. Due to the absence of a suitable physiological mechanism to eliminate excess iron, the content of tissue iron in humans is predominantly regulated by absorption (Abbaspour *et al.,* 2014). For *Drosophila melanogaster's* physiological functions, iron absorption is crucial (Mandilaras *et al.,* 2013). One of the most important minerals for fruit flies' growth and development is Iron. A complicated process in Drosophila controls iron homeostasis in a way that is analogous to how iron is maintained in the human body (Missirlis *et al.,* 2007).

1.2 Justification of the study

It has been observed that the extracts of *Gongronema latifolium* contain phytochemical compounds including alkaloids, saponins, tannins (flavonoids), and glycosides. Some studies have shown that these phytochemicals found in *Gongronema latifolium* may influence cellular proteins with enzymic activities.

Ugochukwu *et al.,* 2003 documented the plant's use in controlling diabetes mellitus in ethnomedicine. The leaves and bark have therapeutic uses. Asthma, eczema, psoriasis, scrofula, dyspepsia, genital issues, bronchitis, coughing, intestinal colic, leishmaniasis, venereal illness, impotence, and syphilis-related skin disorders are some conditions it is used to treat. It is used as a diuretic, stimulant, aphrodisiac, and treatment for syphilis. It contains several vitamins, minerals, and other necessary nutrients. It has a significant amount of mineral salts and has up to five times the vitamin C of oranges.

Given the multiple advantages *Gongronema latifolium* offers for humans and its chemical composition, which has been detailed in numerous study publications, it is anticipated that using it as an extract or as a feed additive will benefit *Drosophila melanogaster* (Bindhu *et al.,* 2007). There are some indications of the plant's normoglycemic, hypolipidemic, and antioxidative action, but not of its uses based on any effect on *Drosophila melanogaster's* iron metabolism. Therefore, the purpose of this study is to ascertain the effect of various *Gongronema latifolium* leaf extract concentrations on *Drosophila melanogaster's* iron metabolism.

1.3. Aim of Study

The aim of this study is to determine the effect of the varied concentration of *Gongronema latifolium* leave extract on iron metabolism in *Drosophila melanogaster*

1.4 Specific Objectives

To determine the effect of *Gongronema latifolium* leave extract on Ferritin in *Drosophila melanogaster*

To determine the effect of *Gongronema latifolium* leave extract on Serum Iron in *Drosophila melanogaster*

To determine the effect of *Gongronema latifolium* leave extract on TIBC in *Drosophila melanogaster*

1.5 Research Questions

Does *Gongronema latifolium* leave extract have any effect on Ferritin level in *Drosophila melanogaster*

Does *Gongronema latifolium* leave extract have any effect on Serum Iron in *Drosophila melanogaster*

Does *Gongronema latifolium* leave extract have any effect on TIBC level in *Drosophila melanogaster*

1.6 Research Hypothesis

Null Hypothesis (H_0)

Gongronema latifolium leave extract does not have any effect on iron metabolism in *Drosophila melanogaster*

Alternate Hypothesis (H_A)

Gongronema latifolium leave extract does have effect on iron metabolism in *Drosophila melanogaster*

1.7 Scope of Study

This study was designed to cover the effect of *Gongronema latifolium* on some iron metabolism in (Ferritin, Serum Iron and TIBC), *Drosophila melanogaster.*

CHAPTER TWO

LITERATURE REVIEW

2.1 UTAZI LEAF

The tropical rain forest's diverse floral species are gifted with a vast array of organic plant products valued for their powerful medicinal and nutritional properties (Dalziel *et al.,*1937). One such plant is the Asclepiadaceae family member *Gongronema latifolium* Benth, formerly known as Marsdenia latifolia (Okafor, 1975).

Locally cultivated *Gongronema latifolium* is known by a variety of names in West Africa, including "Utasi" by the Ibibios, Quas, and Efiks, "Utazi" by the Igbo, and "Arokeke" by the Yorubas (Edim *et al.,* 2012). *Gongronema latifolium* is additionally well-known in Ghana and Senegal, where it is referred to as "Akan-Asanteaborode" and "Servergasule," respectively (Hutchinson, 1973). When eaten fresh, this nutrient-dense edible plant has a

distinct taste that is sharp, bitter, and just a touch sweet. Additionally, the plant generates white latex on the wound and has green leaves and yellow flowers (Balogun *et al.,* 2016)

Gongronema latifolium leaves have a great nutritional value due to their abundance in lipids, proteins, vitamins, minerals, and numerous necessary amino acids. (Eleyinmi, 2007). "Utazi" is frequently used as a spice in dried powder form or as a vegetable in soup and salad recipes. (Okafor, 1975) and (Morebise *et al.,* 2002). For use as a purgative against intestinal worms, colic, and stomach ache, the sliced plant is either boiled with lime juice or infected with water for at least three days (Onike 2010). The main aim of this mini-review is to provide a detailed description of the origin and geographical distribution, botanical characteristics, ethnopharmacological use, phytochemical profile, and pharmacological properties of *Gongronema latifolium.*

2.1.1 ORIGIN AND GEOGRAPHICAL DISTRIBUTION

The *Gongronema latifolium* plant was discovered in the western part of Africa. It may be easily propagated via seed or stem cuttings and is commonly planted throughout the tropical and sub-tropical nations including Nigeria, Guinea-Bissau, Western Cameroon, Ghana, Senegal, Côte d'Ivoire, and Sierra Leone. Additionally, it can be found in North and Southeast Asia. Due to its medicinal and nutritional value, *Gongronema latifolium* is found in the natural African woodland and is also grown in family farms (Owu *et al.,* 2012).

2.1.2 BOTANICAL CHARACTERISTICS

A perennial climbing shrub with the ability to twine around vertical supports is called *Gongronema latifolium*. Up to 5 meters long, it can grow horizontally on the ground. When in contact with soil, the soft woody stem creates adventitious roots. (Osuagwu *et al.,* 2013). White latex, which is discharged upon cutting or wounding, is present in the hollow, soft, and hairy stem of the plant. To offer firm support, the stem's base is woody and hard. Simple, opposite, decussate, and sporadically whorled green leaves with a long petiole and an entire edge are present. (Osuagwu *et al.,* 2013). The broad, oblong to nearly round leaf blade has a deep cordate base and an acuminate tip (Balogun *et al,* 2016).

The axillary cymes-type inflorescence of *Gongronema latifolium* has petite, fragrant, bisexual, star-shaped (actinomorphic), pale yellow flowers (Osuagwu *et al,* 2013). The calyx lobes range in shape from elliptical to rounded, and the tip is hairy. Long, tubular, and campanulate near the tip describe the corolla. Five soft, creamy lobes with a brown base make up the corona. Anthers have membrane apical appendages and are upright. Each pollinarium contains two pollinia, and the ovary is superior (Balogun *et al.,* 2016; Osuagwu *et al.,* 2013 and Mosango, 2022). The Gongronema latifolium plant blooms every year in Nigeria in July and August (Mosango, 2022).

Gongronema latifolium produces fruit that starts out green and ripens to a dark brown or black color. It is an oblong-lanceolate dehiscent seed pod known as a follicle (Osuagwu *et al.,* 2013). When the fruit reaches maturity, it splits apart lengthwise to release flat seeds that are connected to a white silky tuft (pappus), which facilitates pollinator

dissemination (Balogun *et al.,* 2016; Osuagwu *et al,* 2013).The tiny, comma-shaped seeds have a length of about 0.5 cm (Osuagwu *et al.,* 2013).The plant matures in 12 months when cultivated from stem cuttings. It typically demands a warm environment between 32° and 37°C. Insects pollinate flowers because of their appealing color and aroma. Fruits grow very slowly, and frequently, mature, aged fruits meet newly blooming plant parts. *Gongronema latifolium* seeds germinate with a 67% germination rate in 1 to 2 weeks at 27°C (Osuagwu *et al.,* 2013). According to recent anatomical characterization by (Aderiran *et al.,* 2022), *Gongronema latifolium* exhibits non-glandular, uniseriate multicellular trichomes, rosette-shaped calcium oxalate crystals, and nomocytic stomata under a microscope. Additionally, it was discovered that the stomatal number and index were 8.250.52 and 17.600.95, respectively.

2.1.3 ETHNOPHARMACOLOGICAL USES

Different ethnic groups have taken advantage of *Gongronema latifolium's* therapeutic qualities for a variety of ethnomedical purposes. The Ikales of Ondo State, Nigeria, have traditionally utilized the leaves of the *Gongronema latifolium* to alleviate anorexia, nausea, and malaria (Morebise *et al.,* 2006). Some communities in West Africa use Gongronema latifolium to treat cough, intestinal worms, diarrhea, dyspepsia, and malaria, according to the reports of (Owu *et al.,* 2012) and (Mosango *et al.,* 2022). Additionally, the people of Sierra Leone produce an oral infusion or decoction using stems of the *Gongronema latifolium* plant and lime juice to relieve colic and stomach pain (Oliver, 1986). Senegal and Ghana use the leaves of *Gongronem latifolium* for different purposes,

including applying them directly to the body joints of children to help them walk while the boiled extract of the fruit is used as a laxative.

(Mosango, 2022) describes how the Efik and Quas tribes of Nigeria's Cross River State employ leaf extract to cure conditions like diabetes, malaria, hypertension, and constipation.

In Nigeria, it is frequently used to cure coughs (Essien *et al.,* 2007). In addition, patients with asthma chew fresh leaves to help with wheezing, and oral cold-macerated preparation of *Gongronema latifolium* roots is recommended for treating asthma (Essien *et al.,* 2007). A small number of African groups also use this plant to cure bilharzia, viral hepatitis, and other microbiological illnesses (Mosango, 2022). A decoction of *Gongronema latifolium*, *Mormodica charantia*, *Veronica amygdalina*, and *Ocimum gratissimum* is one of the well-known polyherbal treatments for malaria and hepatitis.

According to Juliani *et al .,* 2009, the extract of *Gongronema latifolium* is commonly used throughout Nigeria for the management of blood glucose levels (diabetes) and as a purging remedy by Muslims during Ramadan. According to Osuagwu *et al.,* 2013, *Gongronema latifolium* fruits are eaten orally with or without seeds for stomaches, malaria, and laxative effects. Additionally, the leaves are used in dishes including soups, grits, and well-known Ibo stews like the Nkwobi (cow leg pepper soup) and Isiewu (goathead pepper soup). According to Adelaja and Fasidi, 2009, the substance imparts a bitter taste, has a sweet scent, and increases appetite.

2.1.4 PHYTOCHEMISTRY OF *Gongronema latifolium*

Numerous phytochemists were drawn to study the makeup of the herb *Gongronema latifolium* because of its ethnomedical and nutritional benefits in African cultures. Numerous research have been done on the distribution and prevalence of the main types of secondary metabolites in various plant sections. *Gongronema latifolium's* dried leaves have a high concentration of saponins (18.11%), tannins (16.23%), cyanides (14.32%), flavonoids (11.13%), and phenols (11.11%), but only a little amount of alkaloids (0.12%). Osuagwu *et al.,* 2013 showed significant alkaloid content (10%) on fresh leaves in compared to the dried sample in another phytochemical study. Additionally, Egbung found that the root extract of the plant *Gongronema latifolium* contained more flavonoids, alkaloids, hydrogen cyanide, and tannins than the stem extract did.

The active principle (s) of this plant is not fully established although Iwu *et al.,* 1998 reported flavones and sterols as the most likely active constituents. The claim was strengthened when (Morebise and Fafunso *et al.,* 1998) examined the antimicrobial activity of a methanolic extract containing saponins and flavonoids.

The presence of tannins (poly phenolic compounds) in the leaves was also confirmed by (Eze and Nwanguma, 2013), who proposed the potential of *Gongronema latifolium* extract as a food preservative.The results by Osuagwu *et al.,* 2013 showed that the fruits of *Gongronema latifolium* are more potent than leaves as believed by the local tribes due to a higher concentration of alkaloids, saponins and phenols. According to a recent comparative phytochemical research, bitterleaf, African basil, and African black pepper all contain lower amounts of alkaloids, glycosides, saponins, tannins, and reducing sugars than *Gongronema latifolium* leaf extract (Mgbeje *et al.,* 2019). Notable discovery: Iloneoside, a brand-new ditigloylated pregnane glycoside with strong antileukemic action, was found in the 80% methanolic extract of dried leaves from *Gongronema latifolium* (Gyebi *et al.,* 2017).

2.1.5 PHARMACOLOGICAL PROPERTIES OF *Gongronema latifolium*

Due to the presence of a wide range of phytochemicals, *Gongronema latifolium* demonstrates a variety of pharmacological effects. Each pharmaceutical response's precise mechanism of action is unknown.However, *Gongronema latifolium's* high ethnopharmacological significance continues to draw researchers for diverse in-vitro and in-vivo analyses of this herb.

Figure 2.1 ***Gongronema latifolium*** **1 (Hannah** ***et al.*****, 2012)**

2.2 EFFECT OF UTAZI LEAF EXTRACT ON IRON METABOLISM IN *Drosophila melanogaster*

Iron metabolism in *Drosophila melanogaster* is a combination of the following processes: Iron uptake, Iron transport, Iron storage, Iron utilization, and Iron excretion

There are similarities and differences between Iron metabolism in Mammals and *Drosophila melanogaster*.

2.3 Iron metabolism in *Drosophila Melanogaster*

Iron is essential to almost all organisms due to its multiple vital roles in biological processes. Iron does, however, have the potential to be hazardous when oxygen is present because it can catalyze reactions that result in hydroxyl radicals that can harm vital cellular components, harming cells, tissues, and organs. To ensure nutritional availability while avoiding the damaging effects of excessive or misplaced iron, cellular and systemic transportation, storage, and regulation of iron must be kept under strict control (Warburg 1925).

Although intensively studied in mammals and yeast, much less is known about the mechanism of iron homeostasis for the largest and most diverse group of organisms on earth—insects. Understanding the similarities and diversities of iron metabolism between insects and other organisms is important for unraveling the evolutionary background of iron homeostasis, and also helps to model and understand iron-related diseases in invertebrate organisms including insects and to suggest new prospects for the control of insect pests or disease vectors (Law, 2002).

This review mainly summarizes what we know about insect iron homeostasis from a systemic view. In particular, we will introduce some exciting new findings made in the fruit fly *Drosophila melanogaster*, a model organism happening to be an insect. For better understanding, when applicable, our knowledge of mammalian iron metabolism will be briefly introduced for a comparative analysis. Iron metabolism in mitochondria is very briefly touched upon here as it appears to be a highly conserved process among different organisms.

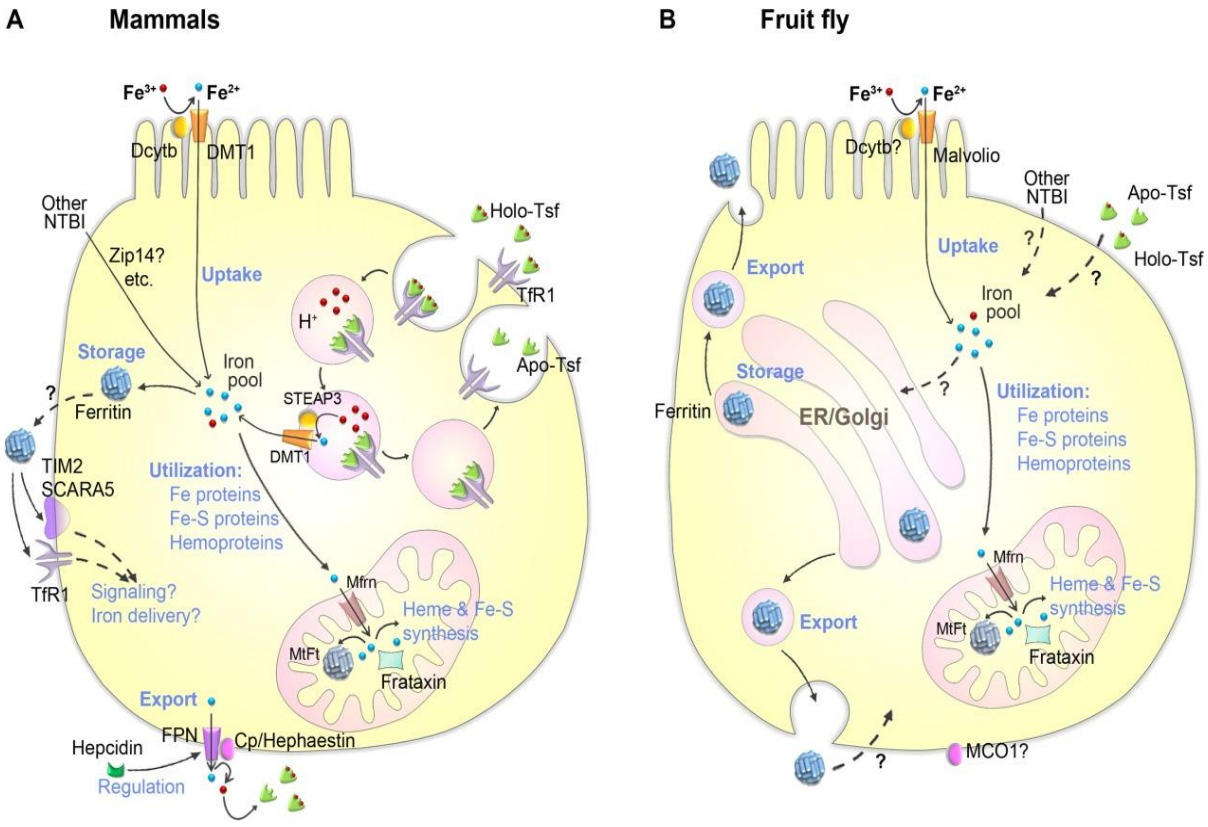

Figure 2.2: Comparison of iron metabolism in mammals and the fruit fly (Nichol *et al.*, 2002)

Main pathways of iron uptake, storage, and export are compared in a generic cell model. Diversities mainly come from different sub-cellular localization of ferritin and the lack of transferrin receptor and ferroprotein in Drosophila melanogaster.

2.4 Iron absorption

2.4.1. Iron uptake

Drosophila obtains iron from its diet, primarily through the gut epithelium. Iron can be present in various forms, such as heme-bound iron or non-heme iron.

Iron absorption by the intestinal epithelia is the first step for the system to integrate the environmental iron, and needs to be highly controlled. The whole process is initiated by uptake of dietary iron from the gut lumen across the apical membrane, and concluded in transfer of the metal across the basolateral membrane to the circulatory system.

Absorbed iron in mammalian enterocytes can be stored inside ferritin or exported to circulating serum transferrin (Tsf). Iron efflux across the basolateral membrane of enterocytes requires the transport protein ferroportin (the only known cellular iron exporter) and a multi-copper oxidase (MCO), hephaestin (ceruloplasmin homologue expressed in duodenum), to oxidize exported Fe2+ for loading into serum Tsf

Inorganic dietary iron occurs primarily as Fe3+. In mammals, Fe3+ is reduced to Fe2+ at the intestinal brush border by Dcytb, a member of the cytochrome b561 family of plasma membrane reductases. Then Fe2+ is absorbed across the apical membrane via the divalent metal transporter (DMT1 or NRAMP2) (Chavez *et al.,* 2000). NRAMP1 (Natural resistance-associated macrophage protein 1) is homologous to DMT1/NRAMP2, expressed within phagolysosomal membranes, and participates in iron import from phagocytic vesicles

Malvolio (mvl), a *Drosophila melanogaster* gene with close similarity to NRAMP1, has been proposed to be a DMT1/NRAMP2 orthologue (Warren *et al*., 2002)

2.4.2. Iron transport

Iron is carried throughout the body by proteins like transferrin after it has been absorbed by the gut. The iron-binding protein ferritin participates in the storage and transportation of iron in *Drosophila melanogaster*.

A hollow-sphere complex made up of 24 subunits called ferritin, which is present in all animals, may store up to 4,500 iron atoms in a bioavailable state (Arosio *et al.,* 2009). As a result, ferritin is regarded as an anti-oxidant protein and the main molecular location for iron storage (Theil and Elizabeth, 2012). The heavy and light chain homologous components (H and L) of mammalian holoferritin are heteropolymers that are produced by distinct genes and are referred to in many insects as ferritin 1's heavy chain homolog and ferritin 2's light chain homolog, respectively. L subunit lacks ferroxidase activity but can aid in the crystallization and stability of the H subunit, which is distinguished by a ferroxidase core required for the oxidation of ferrous iron to ferric iron.

The crystal structure of a lepidopteran secreted ferritin revealed a symmetrical arrangement of 12 H and 12 L chain subunits (Hamburger *et al.,* 2005), whereas mammalian ferritin has a tissue-specific ratio of H and L chain subunits (Harrison and Arosio, 1996), the ratio in other insects has not been established.

Endoplasmic reticulum (ER), Golgi complexes, and secretory vesicles, as well as hemolymph, are the primary sites for iron transport of ferritin in insects (Zhou *et al.,* 2007).

In insects secreted ferritin is found in hemolymph in mg/L concentrations, approximately 1,000-fold higher than blood ferritin in mammals (Ong *et al.,* 2006).

It has long been hypothesized that ferritin is involved in dietary iron absorption and transport in insects due to the secretory character of insect ferritin (Zhou *et al.,* 2007).

All nine species in six orders had an abundance of iron in their midgut epithelia, and several species had iron that was locally localized, according to Prussian blue iron staining. The iron area (or iron cell region) is a collection of specific iron-rich cells found in the middle midgut of numerous species of Drosophila (Efthimios *et al.,* 2014). Additionally, the midgut of these species serves as a major site for ferritin expression, with the iron area of Drosophila exhibiting the highest levels of ferritin expression (Mehta *et al.,* 2009). Response to more dietary iron was increased ferritin messages and proteins. Researchers found that Fe-labeled mammalian Tsf supplied in a blood meal was absorbed and that the iron was found in the yellow fever mosquito Aedes aegypti, with the iron found in the hemolymph and egg. We recently published the first genetic proof that Drosophila melanogaster secretory ferritin directly contributes to dietary iron absorption. We discovered that RNA interference (RNAi) knockdown of either ferritin subunit decreases the protein level of both subunits. Iron builds up in the gut after RNAi specifically targets ferritin in the midgut. A systemic iron shortage caused by the midgut ferritin RNAi can also be treated with iron supplements or made worse by iron depletion (Bateman *et al.,* 2008).These results underline the critical role ferritin plays in the

absorption or efflux of dietary iron. On the other hand, iron transport on the side of the apical membrane ought to be ferritin-independent and presumably involve the aforementioned DMT1 homologue (Mvl). Both subunits are necessary for life since the development of homozygous null mutants of either Drosophila ferritin subunit is halted at the first instar larval stage. We discovered that the mutants could be greatly rescued up to early maturity by over-expressing wildtype ferritin subunits in the midgut (Georgieva *et al.,* 2002). As a result, midgut ferritin in *Drosophila melanogaster* is crucial for survival, and iron shortage is the main factor in the early mortality of mutants. Given that many different insect species' midguts express secretory ferritin in large amounts, it is highly likely that this function is conserved in insects. In this regard, secretory ferritin may function both as ferroportin, which exports iron, and as cytoplasmic ferritin, which stores iron in mammals.

FERRITIN

Ferritin is produced by Drosophila in two different forms: that which is particular to the testis and is encoded by the X chromosome. The ferritin-3-Heavy-Chain-Homolog (Fer3HCH) gene, which has a minimal (or testis-specific) function in overall iron homeostasis but is not known to contribute to iron absorption (Missirlis *et al.,* 2006) , Furthermore, the primary secretory type is charge of systemic iron storage (Mehta *et al.,* 2009) and iron absorption (Tang and Zhou, 2013). Fer1HCH and Fer2LCH, two genes that are next to each other on the left arm of the third chromosome, produce Drosophila ferritin (Dunkov and Georgieva, 1999). Fer2LCH offers the iron nucleation sites necessary for the mineralization of the ferrihydrite iron core, whereas Fer1HCH possesses the ferroxidase activity needed for iron loading (Hamburger *et al.,* 2005). Twelve Fer1HCH and twelve Fer2LCH subunits make up each ferritin molecule (Hamburger *et al.,* 2005). The majority of ingested iron concentrates in Drosophila ferritin, according to radioactive tracing (Missirlis *et al.,* 2006) . Fer1HCH and Fer2LCH single insertion mutants were examined, and it was discovered that disruption of either gene product lowers total ferritin levels in whole flies and causes death in the embryo or early larva (Missirlis *et al.,* 2007). Iron accumulated in the intestine as a result of midgut-specific RNAi of ferritin, but systemic iron shortage was still present (Tang and Zhou, 2013).

2.5.TRANSFERRIN

A glycoprotein called Transferrin (Tsf) is present in multicellular creatures and distinguished by having a very high affinity for binding iron. The antibiotic protein lactoferrin, which is expressed in milk and other extracellular fluids, and a GPI-anchored melanotransferrin, which has high expression in several disease states, particularly malignant melanoma, are other closely related superfamily members in mammals in addition to the serum Tsf. The genomes of other insect species also contained multiple copies of the Tsf genes. Tsf1, which is prevalent in hemolymph, is the Tsf gene that resembles mammalian Tsf the most; the other members are listed sequentially. Insect Tsf gene diversity, gene structure, iron binding characteristics, post-translational modification, expression patterns, and other factors were examined (Jaakkola *et al., 2001*)..

The fat body is the predominant region of Tsf1 expression, according to studies in Aedes, Drosophila, *Bombus ignitus* (Hymenoptera; bumble bee), *Apriona germari* (Coleoptera; mulberry longhorn beetle), and *Choristoneura fumiferana* (Lepidoptera; spruce budworm). Tsf1 is not found in the midgut, ovaries, or male reproductive accessory glands in Drosophila, while AaTsf1 is expressed in these tissues in A. aegypti (Donovan *et al.,* 2000). On the other hand, iron entering as heme from a blood meal or by injection upregulates Tsf1, which is proposed to be a reflection of increased oxidative burden (Warburg, 1925). There is a growing body of evidence that suggests that inorganic iron-supplemented diet decreases whole animal Tsf1 levels in insects. various roles . Insect Tsf1 has been associated with a variety of functions, including iron transport, the delivery of iron to eggs in some insects, function as an antibiotic, and stress response.

According to Evstatiev and Gasche, 2010, the majority of other insect Tsf-like proteins are discovered through sequence analysis, and little is known about their functions. According to Evstatiev and Gasche, 2010, the protein sequences of some insects all have putative GPI-anchor domains, making them resemble melanotransferrin. Melanotransferrin studies in transgenic and knockout mice show that the protein is not necessary for iron homeostasis or metabolism. Iron-bound Tsf2 has recently been found to be crucial for the formation of epithelial septal junctions in *Drosophila melanogaster*. Aedes Tsf2 differs from Aedes Tsf1 in that it is generally insensitive to blood meal treatment in larval epithelial cells (Vanoaica *et al.,* 2010). Further research is needed to determine the role of melanotransferrin in iron metabolism.

Because no equivalent of the mammalian Tsf receptor has yet been found in the published genome sequences of insects, Tsf delivery of iron via a receptor has not been demonstrated in any insects, despite the fact that it is abundant in hemolymph and that it responds to iron 3 (Law, 2002). This may indicate that insects have a distinct class of Tsf receptors or that a different transport mechanism is used to absorb iron (Law, 2002). Ferritin and/or the DMT1 homologue Malvolio are potential options.

2.6 Iron storage

Iron overload is prevented by ferritin, which stores extra iron in a non-toxic form. Complexes of Drosophila ferritin are made up of heavy and light chains.

Ferritin is produced by Drosophila in two different forms: that which is particular to the testis and is encoded by the X chromosome. The predominant secretory type responsible for systemic iron storage is ferritin-3-Heavy-Chain-Homolog (Fer3HCH), which plays a minimal (or testis-specific) function in overall iron homeostasis and is not known to contribute to iron absorption (Missirlis *et al.,* 2007)) and iron absorption. Fer1HCH and Fer2LCH, two genes that are close to one another on the left arm of the third chromosome, produce ferritin in Drosophila. According to Missirlis *et al.,* 2007, Fer1HCH has the ferroxidase activity needed for iron loading, while Fer2LCH supplies the iron nucleation sites needed for the mineralization of the ferrihydrite iron core. According to Missirlis *et al.,* 2007, each ferritin molecule is made up of 12 Fer1HCH and 12 Fer2LCH subunits. Radioactive tracing showed that most ingested iron accumulates in Drosophila ferritin (Missirlis *et al.,* 2007).The disruption of each gene product lowers total ferritin levels in whole flies and causes embryonic or early larval death, according to analysis of single insertion mutants that disrupt Fer1HCH and Fer2LCH, respectively (Missirlis *et al.,* 2007). Iron accumulated in the intestine but there was a systemic iron deficiency as a result of midgut-specific RNAi of ferritin.

2.7 Iron Utilization

Iron is necessary for several biological functions, including DNA synthesis, enzymatic activities, and energy production (as a component of cytochromes). Particularly crucial in these processes are proteins with iron-sulfur clusters.

2.8 Iron homeostasis

Drosophila controls the absorption, storage, and release of iron to maintain iron homeostasis. This involves the regulation of iron metabolism-related genes, frequently through the action of a transcription factor known as iron response protein (IRP).

Hepcidin, a hormone produced in the liver, and ferroportin, an iron exporter, are essential for systemic control.

The post-transcriptional regulatory mechanism IRP/IRE remains conserved, despite the absence of reports of hepcidin and ferroportin in insect species. Several insect species, including A. aegypti, A. gambiae, *Drosophila melanogaster*, and M. sexta, have been found to contain proteins with IRE-binding ability that are more comparable to human IRP1 than IRP2 (Evstatiev and Gasche, 2012). Two IRP1 homologues (IRP1A and IRP1B) were discovered in *Drosophila melanogaster*. Despite the fact that both of them have aconitase activity, only IRP1A can bind IREs (Jafari *et al.,* 2006).

2.7 Iron excretion

Iron-containing granules are accumulated and secreted into the midgut lumen by Drosophila and other dipteran larvae (Pantopoulos *et al.,* 2012). These studies imply that, despite the absence of cell turnover in gut epithelia, the midgut's exocytosis of iron-loaded ferritin may be the primary mechanism by which iron is lost by insects (Law, 2002). Gut ferritin plays a significant role in iron excretion, whether by excretion in insects or cell sloughing in mammals (Law, 2002).

2.8 Immune response

Iron is utilized by Drosophila as a component of its immune system. The immune system of the fly has the ability to sequester iron away from pathogens during infection, which inhibits their growth (Levy *et al.,* 2004).

Total Iron Binding Capacity

Determining total Iron Binding Capacity is an indirect means of assessing transferring levels.

A TIBC (total iron-binding capacity) test is one of a few tests healthcare providers use to diagnose iron-related conditions, like anemia and hemochromatosis. They often compare the results to iron and ferritin blood test results.

2.9 Drosophila melanogaster

Drosophila melanogaster, a species of fly in the Drosophilidae family, is a commonly used model organism for biomedical research and experiments (Jafari *et al*., 2006), it is also a developing model to demonstrate adverse drug responses (Avanesian *et al*., 2009). Due of its ease of laboratory rearing, little space needs, and low dietary requirements, *Drosophila melanogaster* is frequently employed in research. At room temperature, the reproductive cycle may be completed in approximately 12 days, which enables speedy examination of test crosses. Fruit flies breed in large numbers, making it possible to gather adequate information. Examining and compiling data on fruit flies is further made simple by their ability to be quickly and easily immobilized. Low-power magnification may also be used to detect a variety of genetic abnormalities. Four pairs of chromosomes

make up the majority of the genome in Drosophila, which has fewer chromosomes than the human complement of 23 pairs. Human genes have many similarities with Drosophila genes, which are studied to better understand the functions of these proteins in humans.

The Drosophila lifecycle consists of beautiful developmental stages made up of the first, second, and third larval instars, pupae, and adults. In response to dietary, environmental, and hormonal factors, transcriptional control regulates each stage to a substantially different extent (Kozlova and Thummel, 2000). The fruit fly is thought to be a good model system for assessing medication toxicity due to the extensively conserved pathways between fruit fly and mammalian reproductive systems (Avanesian *et al*., 2009). Specific and measurable traits of particular note are the reproductive phenotypes, such as fertility, egg production, and mating behavior. Female fecundity, which is defined as the production of eggs, is a difficult but well-established trait to assess toxicity and has been used to assess the harmful effects of chemotherapy drugs like methotrexate on reproduction (Kislukhin *et al*., 2013). Since it has been shown that a variety of parameters, including mating behavior, hormones, testes development, reproductive morphology, and spermatogenesis, have an impact on male fertility in Drosophila, which is defined as the generation of viable offspring, it is also regarded a phenotype to assess drug-induced toxicity (Tiwari *et al*., 2011).

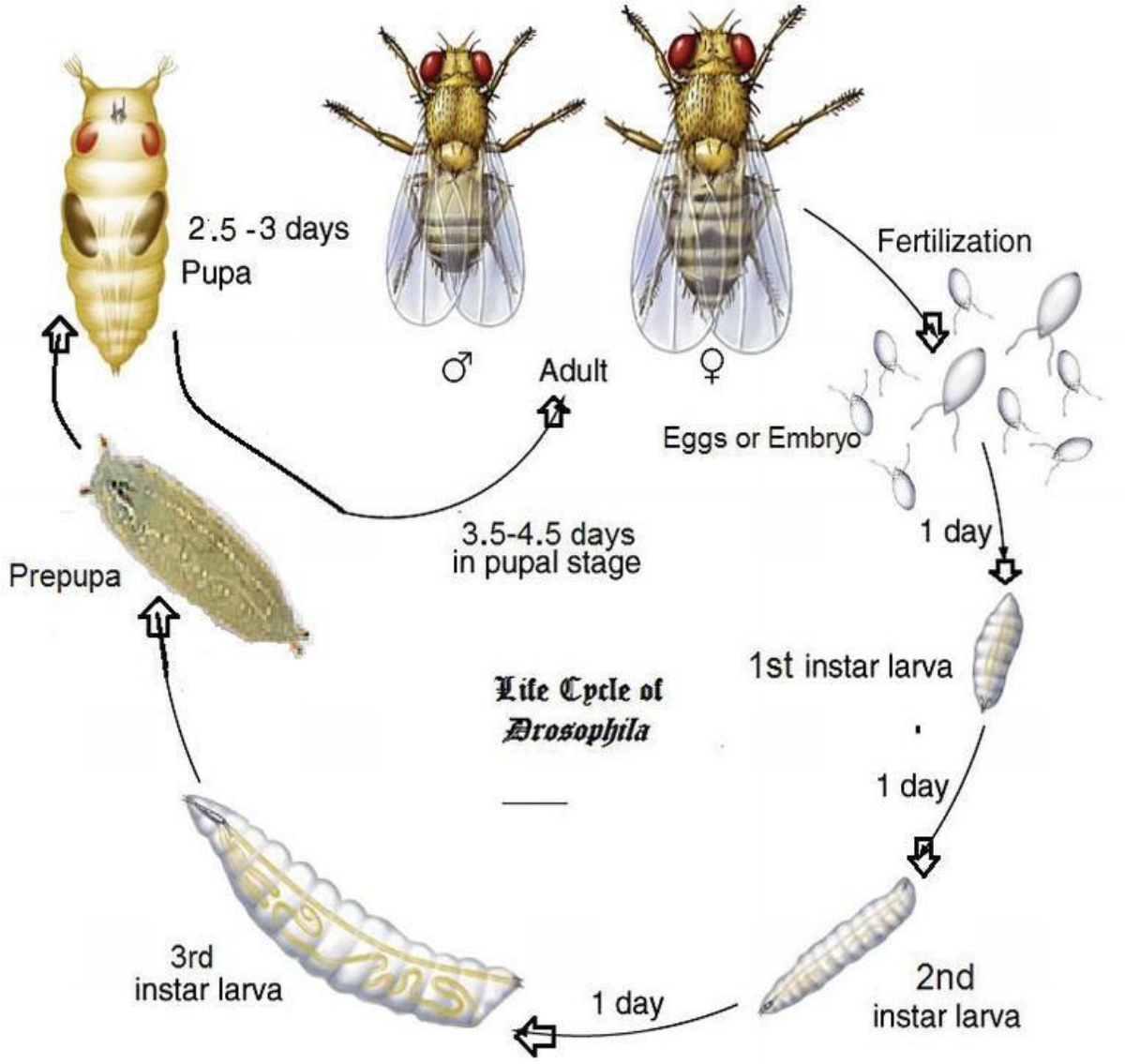

Figure 2.3: Life-cycle of Drosophila (♂: male; ♀: female) with three larval instar and a pupa stages (Perveen F.K., 2018).

Cultures of Drosophila should be maintained at room temperature, where the temperature does not range below 20˚C or above 25˚C. They are grown on a fermenting medium and contains yeast extract, dextrose, sugar, and cornmeal. The common breeding ratio is 1:3

(male: female), Fungi, mites, and bacteria are some of the typical culture contaminants. Following ether anesthesia, the male and female are distinguished (under the microscope) based on their size, marks on their belly, and existence of sex combs (Blum *et al*., 2013).

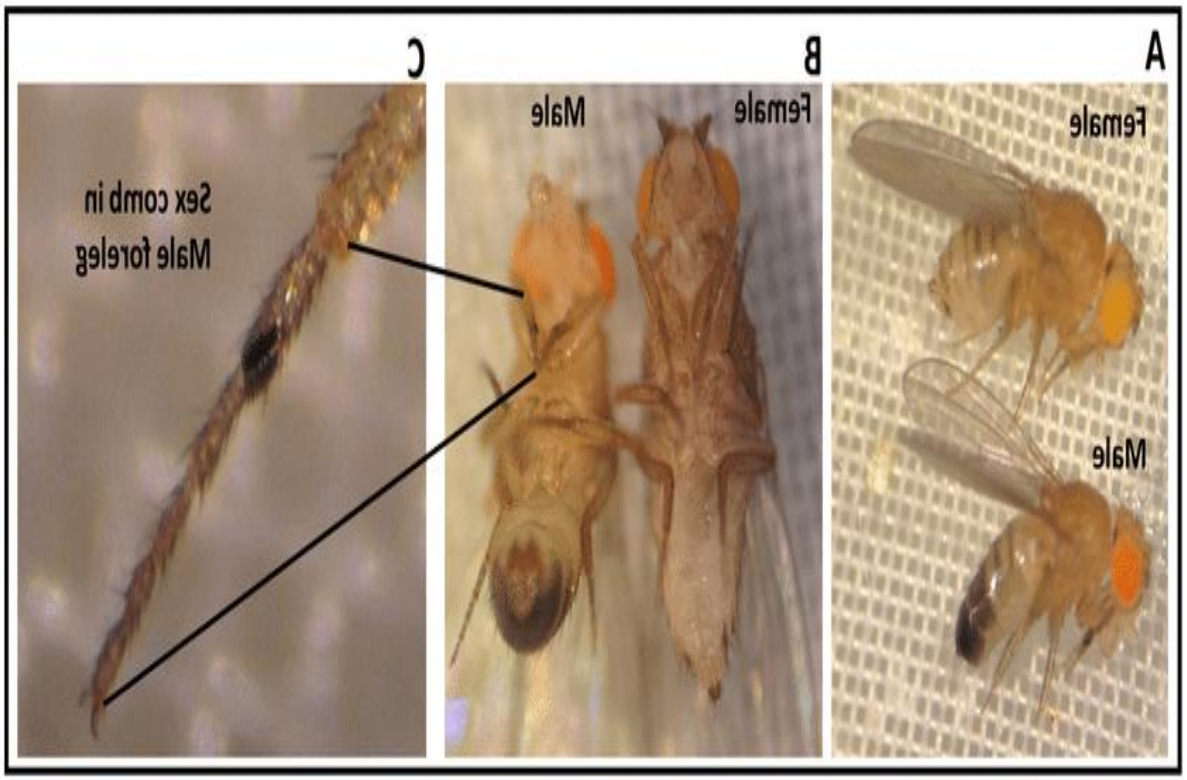

Figure 2.4: Identification of sexes in adult fruit flies. (A) Morphological characteristics and sexual dimorphism of adult *Drosophila melanogaster* (lateral view). Adult female fly (top) has a light colored abdomen region; however, adult male fly (bottom) has a dark posterior abdomen region. (B) Morphological differences between male and female flies (ventral view). (C) Magnified view of the male fly foreleg shows male specific sex comb structure (Majeed et al., 2017).

The lifetime of the *Drosophila melanogaster* is around 50 days from egg to death when growing circumstances are ideal at 25°C like many ectothermic species, the length of the developmental phase changes with temperature in *Drosophila melanogaster*. The egg develops in the least amount of time —7 days—at a temperature of 28°C. Due to heat stress, development takes longer at higher temperatures (11 days at 30˚C). Under optimal circumstances, development takes 8.5 days at 25°C, 19 days at 18°C, and more than 50 days at 12°C. In rotting fruit or other appropriate material, such as decomposing mushrooms and sap fluxes, females lay roughly 400 eggs, five at a time. After 12 to 15 hours, the about 0.5mm-long eggs begin to hatch (at 25˚C). The resultant larvae develop for around 4 days while going through two molts (becoming second and third instar larvae), respectively, at about 24 and 48 hours after hatching. The larvae then encapsulate in the puparium and go through a 4-day metamorphosis after which the adults emerge (Ashburner *et al*., 2005). In congested environments, development time lengthens and the newly emerged flies are smaller (Perveen F.K., 2018). They feed on the microorganisms that break down the fruit or food during this period, as well as the sugar in the meal (Blum *et al*., 2013).

The wings of adult flies are not completely extended, and they are delicate and pale in color. Fly color initially appears to be relatively light upon emergence, but it quickly darkens over the course of the first few hours and develops the typical adult fly look. This criteria makes it feasible to separate newly emerging flies from older ones that are present in the same culture bottle (Parvathi *et al*., 2009). They live for a month or more before passing away. After emerging from the pupa, a female waits 10 to 12 hours before mating. She keeps a sizable amount of sperm in receptacles after mating, fertilizing her eggs as

she lays them. Therefore, it is essential to employ virgin females in order to achieve a regulated mating. 'Virgin female' is the term used for these flies that have not mated (Wolfner, 2011).

Drosophila melanogaster

Scientific classification:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Drosophilidae

Genus: Drosophila

Subgenus: Sophophora

Species group: melanogaster

Species: D. *melanogaster*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This is an experimental and observational study design aimed at determining how the different concentrations of Utazi leaf extract affect the survival and behavior of *Drosophila melanogaster*

3.2 Study subject

The subjects involve 1-2 days old virgin male and female *Drosophila melanogaster* fed with varied concentration of Utazi leaf extract supplemented corn meal diet. The control subjects include 1-2 days old virgin male and female *Drosophila melanogaster* fed with only corn meal diet and distilled water.

3.3 Inclusion Criteria

Male and female fruit flies hatched within 48 hrs or less and fed with varied concentration of Utazi leaf extract. Control group will be 24-48hrs old virgin male and female *Drosophila melanogaster* flies fed with only basic cornmeal diet

3.4 Exclusion Criteria

All Male and Female flies above 48 hrs

3.5 Fruit Flies and Treatment

Male and female *Drosophila melanogaster* flies of 1-2 days old were cultured in the *Drosophila Melanogaster* laboratory section, BIOTOXICS Research Laboratory, Central Biomedical Research Laboratory, University of Benin, Benin City, Nigeria. The flies were obtained from the Federal University of Santa Maria, Brazil. The flies were originally obtained from the National Drosophila Species Stock Centre, Bowling Green Oklahoma, United States of America. The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosized into young adult fruit flies under a natural photoperiod of about 12 hours light and 12 hours dark daily for the period of the administration of the varied concentration of Utazi leaf extract. Flies were collected and separated into five experimental control groups with five vials of 36 flies in each group and the flies were treated as stated below in the experimental design.

3.6 *Drosophila melanogaster* Feed Formulation and its handling

The flies were fed with the standard formulated diet corn meal medium, which contained brewer's yeast (5g), glucose (3.5g), agar (7.5g), nipagin (1g), corn meal (52g) and ethanol (1ml). The meal was prepared using distilled water (850ml). The prepared corn meal was transferred into vials and left to solidify. Flies were transferred from vials containing 1-2 days old *Drosophila melanogaster*. Caution was taken when counting the flies into treatment tubes. This was done with the aid of a soft brush with soft bristles. Much care was taken in transferring the flies to prevent them dying from "handling stress"

Procedure for preparing standard meal:

850ml of distilled water was measured with a cylindrical flask

150ml of distilled water was removed to mix the corn meal

The remaining 700ml of water was boiled in a pot

Small quantity of boiling water was removed to mix the yeast

Agar was added to the boiling water and stirred until fully dissolved

The dissolved cornmeal was added and stirred until fully dissolved

Glucose was added and the entire content in the pot stirred

The mixture was allowed to boil for 2 minutes

Dissolved Yeast was added

Nipagin was dissolved in ethanol and added to the mixture in the pot

The meal was removed from heat source and transfer into appropriate vials and allowed to cool

3.7 Source and Preparation of Aqueous Utazi leaf Extract

Fresh Utazi leaves (*Gongronema latifolium*) were purchased from New Benin Market, Benin City, Edo State, Nigeria and then taken for leaf identification carried out by Professor MacDonald Idu at Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, Nigeria with the voucher number: **UBH-G301**. The Fresh leaves air dried for 2 weeks at room temperature of 30 degree Celsius in Central

Laboratory. It was separated from the stem, sorted to remove debris and was washed using clean tap water and then grinded to powder at a laboratory near Anatomy gate using a grinding machine. 50g of grinded Utazi leaves was stirred in 450ml of distilled water and was allowed to stand for 24 hours at room temperature . The extract was filtered using Whatman's (Nitro cellulose 45; 0.45 micrometer pore size) filter paper and the residue thrown away with the filtrate concentrated to paste level using a water bath at 100 degrees Celsius to actualize the Solid extract. The extract was preserved in a sample bottle and kept in a refrigerator at 4 degree Celsius until the time for usage.

3.8 Experimental design

The experimental design used for this study was a randomized control trial. *Drosophila melanogaster* flies were randomly assigned to different treatment groups, each exposed to a specific concentration of Utazi leaf extract. A control group consisting of flies not exposed to the extract was included. This design allowed for a comparison between the effects of the different concentrations of *Gongronema latifolium* leaf extract on the iron metabolism of *Drosophila melanogaster.* To maintain consistency, flies within each treatment group were reared and maintained under the same environmental conditions, including temperature, humidity, and lighting. The duration of exposure to the Utazi leaf extract was standardized across all treatment groups to ensure comparability of results. Throughout the experiment, appropriate replication was employed to enhance the statistical robustness of the findings. By employing a randomized controlled trial design, this study minimized confounding variables and allowed for the assessment of the

specific effects of varied concentrations of *Gongronema latifolium* leaf extract on the iron metabolism of *Drosophila melanogaster.*

3.8.1 Experiment 1: Survival assay

The survival assay was carried out in three replicates of each concentration. The diet was changed every five days, during the period of the experiment. The survival rate was determined with all the concentrations and dead flies were recorded daily. By the end of this experiment (21 days), the data obtained were accumulated and plotted as percentage of dead flies. The result was then compared to that of control (1). For the survival assay, flies (both genders) of 1-2 days old were divided into 5 groups, with each group having 3 treatment tubes each. Each treatment tube contained 35-40 flies each. All 5 groups had varied concentration of Utazi leaf extract.

GROUP 1 (1): Control flies fed on 9.8g of Corn meal diet / 0.2ml of water

Group 2 (2) : Flies fed on 10mg/ml *Gongronema latifolium* aqueous Leaf Extract / 9.8g Cornmeal diet

Group 3 (3) : Flies fed on 5mg/ml *Gongronema latifolium* aqueous Leaf extract / 9.8g Cornmeal diet/0.2ml of water

Group 4 (4) : Flies fed on 2.5mg/ml *Gongronema latifolium* aqueous Leaf extract /9.8g Cornmeal diet /0.2ml of water

Group 5 (5): Flies fed on 1mg/ml *Gongronema latifolium aqueous* Leaf extract/9.8g Cornmeal diet

The survival assay was carried out in three replicates of each concentration. The diet was changed every five days, during the period of the experiment. The survival rate was determined with all the concentrations and dead flies were recorded daily. By the end of this experiment (21 days), the data obtained were accumulated and plotted as percentage of dead flies. The result was then compared to that of control (1).

3.8.2 Experiment 2: Tissue homogenate preparation for biochemical assay

For the determination of biochemical assays, a second group experiment was carried out. In this experiment, flies (both genders) of 1-3 days old were divided into 5 groups with each group having three vials each. Each vial contained 35-40 flies with varied concentration of Utazi leaf extract in each treatment vial. It was monitored for 6 days.

GROUP 1 (1): Control flies fed on 9.8g of Corn meal diet / 0.2ml of water

Group 2 (2) : Flies fed on 10mg/ml *Gongronema latifolium* aqueous Leaf Extract / 9.8g Cornmeal diet

Group 3 (3) : Flies fed on 5mg/ml *Gongronema latifolium* aqueous Leaf extract / 9.8g Cornmeal diet/0.2ml of water

Group 4 (4) : Flies fed on 2.5mg/ml *Gongronema latifolium* aqueous Leaf extract /9.8g Cornmeal diet /0.2ml of water

Group 5 (5): Flies fed on 1mg/ml *Gongronema latifolium aqueous* Leaf extract/9.8g Cornmeal diet

At the end of the treatment period, flies were anaesthetized in ice, weighed, and homogenized in cold 0.1M phosphate buffer, PH 7.0 (1:10 w/v), and centrifuged at 4000

x g for 5minutes (Allegra X-15R Centrifuge, Beckman Coulter, USA). Then the supernatants were separated into labelled Eppendorf tubes, and used for the various biochemical assays. All the assays were carried out in three replicates for the 5 groups and relative absorbance, analyzed using a microplate reader using Elisa method

Homogenizing buffer (0.1M Phosphate buffer, Ph 7.4)

5.05g of Na2HPO4 and 1.69g of NaH2P04 were dissolved in 200ml of distilled water and made up to 250L at room temperature

3.8.3 Experiment 3: EVALUATION OF THE DIFFERENT PARAMETERS

SERUM FERRITIN

The Ferritin ELISA Kit is a solid phase sandwich assay method, based on a Streptavidin-biotin principle. The standards, serum samples and the biotinylated Anti-Ferritin antibody reagent are added into designated wells, coated with Streptavidin. Endogenous Ferritin in the patient's serum binds to the antigenic site of the biotinylated Anti-Ferritin antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by the buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-Ferritin Antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies labelled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon addition of substrate, the intensity of color developed is directly proportional to the concentration of Ferritin in the samples.

The Ferritin ELISA kit purchased from Calbiotech was used. The Kit contained the following reagents: **Materials provided**

Microwells, coated with Streptavidin**,** Ferritin Standards: 6 vials ready to use, Anti-Ferritin biotin Reagent (ready to use)**,** Anti-Ferritin enzyme reagent (ready to use)**,** TMB (3 3,5 5-Tetramethylbenzidine) (Ready to use)**,** Stop solution (1 bottle)**,** 20x Wash concentrate: 1 bottle

Materials not provided

Distilled or deionized water**.** Precision pipettes or automated pipettes**,** Disposable pipette tips**,** ELISA reader capable of reading absorbance at 450nm**,** Absorbance paper or paper towel

3.8.4 Reagent preparation for Ferritin

Prepare 1x Wash buffer by adding the contents of the bottles (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20–25-degree Celsius)

3.8.5 Method for the evaluation of Ferritin

Before assay procedure, the reagents were allowed to stand at room temperature (20-25 degree Celsius) and reagents gently mixed before use. The desired number of coated strips were placed into the holder. 25 micro liters of Ferritin standards, control, and samples were measured into appropriate wells. 100 micro liters of Biotin were added into each well. The plate was shaken for 20 seconds. The plate was incubated for 30 minutes at room temperature (20-25 degree Celsius). Liquid was removed from all wells and

wells washed three times with 300 micro liters of 1x wash buffer. The washed well was blotted on absorbance paper or paper towel.

Calculations for Ferritin

Automated Instruments calculate the absorbance (nm) and concentration (ng/ml) upon completion of reaction.

EVALUATION OF TOTAL IRON BINDING CAPACITY

Total Iron Binding Capacity (TIBC) is a measure of the maximum concentration of iron that the serum proteins can bind. TIBC is used for the diagnosis of Anemia and other disorders of iron metabolism. Serum TIBC is increased in Iron deficiency and decreased in anemia

Materials provided:

Microtitre wells, Calibrator or calibration serum, Neutral buffer, Acidic reagent

Materials not provided:

Distilled or deionized water, Precision pipettes or automated pipettes, Disposable pipette tips, ELISA reader capable of reading absorbance at 660nm

3.8.6Reagents and Concentration for TIBC

Acidic Reagent: Chromazural B (165mmol/l), Cetromide (735 micromol/l), Ferric Chloride (16micromol/l), Acetate Buffer (PH 4.55). Neutral buffer reagent which has a concentration , Sodium bicarbonate (338mol/l) and Preservatives (0.0023)

3.8.7 Method for the evaluation of TIBC

The wells were made clean and dried using an incubator. 20ul of calibrator serum was measured into one well and 200 micro liters of acidic reagent other wells following the arrangement of column by column. 20 micro liters of 13 samples each were measured into the wells containing the acidic reagent. The well containing the calibrator serum was left out. The mixture was well mixed and allowed to incubate for 5 minutes at assay temperature**,** 60 micro liters of neutral buffer was added to the wells containing the calibrator serum and wells containing the acidic reagent and serum sample. A green coloration was observed. Absorbance 1 was measured immediately and Absorbance 2 measured after incubation of the microtiter wells for 7.5 minutes at assay temperature.

Calculation for TIBC

Automated Instruments calculate the absorbance upon completion of reaction. The concentration (micro mole/ L) is calculated using the formula below:

$$\text{TIBC (micromole/L)} = \frac{\text{Absorbance 2} - \text{Absorbance 1}}{\text{Absorbance 2- Absorbance 1 of Standard}} \times 41.61\text{mmol/L}$$

EVALUATION OF SERUM IRON

Iron is liberated from transferrin under acidic conditions. Ascorbate reduces the released ferritin to ferrous ions. These ions react with Ferrozine to form a colored complex which is then measured at 544nm. The color intensity thus formed is directly proportional to the concentration of iron in the serum plasma.

3.8.7 Reagent Concentration for Serum Iron Assay

Buffer: Acetate Buffer (50mmol/l), Detergent, Ferrozine: Ferrozine (5mmol/l), Standard: Iron (35.8 micromole/l)

3.8.8 Method for Serum Iron Assay

4 parts of the buffer was mixed with 1 part of Ferrozine , 20 micro liters of serum sample, 20 micro liters of Distilled Water and 100 micro liters of standard were pipetted into microtiter wells , 200 micro liters of buffer was added to the microtiter wells containing 20 micro liters of the sample blank. 200 micro liters of working reagent were added to wells containing the standard and the reagent blank. The mixture was mixed and incubated for 5 minutes of assay temperature (37 degree Celsius).

Absorbance was measured using a Microplate Reader

Calculations for Serum Iron Assay

$$\text{Iron concentration in micromole/l} = \frac{\text{Sample} - \text{Sample Blank}}{\text{Difference in the absorbance of Standard}} \times 35.8 \text{ mmol/l (or 0.2mg/dl)}$$

3.9 Statistical analysis

Graph Pad Prism version. 5.01 (Graph Pad Software Inc. San Diego, CA, USA) was used to analyze the data obtained. Normally distributed data were analyzed with one-way analysis of variance (ANOVA) and Turkey's Post-test. A *P*-value of less than 0.05 was

considered statistically significant. Bar charts were used to compare data and show the relationship between them.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 shows the number of *Drosophila melanogaster* flies that died within a 21day period of the survival studies.

Table 4.2 shows the percentage survival rate of flies at different concentrations of Utazi leaf extract incorporated into the *Drosophila melanogaster* diet (1- Control Group, 2= 10mg/ml group, 3= 5mg/ml group , 4=2.5mg/ml group, 5=1mg/ml group). The average percentage survival was observed to be the highest in Group 5 having 80 percent of flies still alive at day 21.

Table 4.1: shows the number of *Drosophila melanogaster* flies that died within a 21day period of the survival studies

GROUPS	Day 0	Day 1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Control 1	0	1	2	2	2	2	2	2	2	3	3	5	5	5	5	7	7	7	8	9	9	10
Control 2	1	2	2	2	2	3	3	5	5	5	7	8	8	8	8	10	13	14	14	15	16	17
Control 3	0	0	0	2	2	2	2	3	3	3	3	3	4	5	5	5	5	5	7	7	7	8
Extract 1	0	0	1	1	1	1	1	1	1	1	1	1	1	2	2	2	4	4	7	8	8	9
Extract 2	0	0	0	0	1	2	2	2	2	3	3	3	3	3	4	5	5	7	7	7	7	8
Extract 3	0	0	0	0	0	2	2	2	2	2	3	3	3	3	3	4	4	5	6	7	8	9
5mg solution 1	0	1	2	2	2	2	2	2	2	3	3	3	3	5	6	7	8	9	10	11	12	13
5mg solution 2	0	1	1	3	3	3	3	3	3	4	4	4	4	4	4	5	5	5	6	7	9	10
5mg solution 3	0	1	1	1	1	1	1	1	1	1	2	2	3	4	4	4	5	6	7	8	8	8
2.5mg solution 1	0	0	0	1	1	1	1	1	1	1	1	1	2	3	4	5	5	5	5	5	6	7
2.5mg soultion 2	0	0	0	0	0	1	1	1	1	1	2	2	2	2	3	3	7	7	7	7	8	9
2.5mg solution 3	0	0	0	2	0	2	2	4	4	4	4	4	4	5	5	5	5	5	6	7	8	9
0.1mg solution 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	3	5	7
0.1mg solution 2	0	0	0	0	0	1	1	1	1	2	3	3	3	4	4	4	5	5	6	7	8	9
0.1mg solution 3	0	0	0	0	0	1	1	2	3	3	4	4	5	5	5	5	5	6	6	6	7	8

Table 4.2: Percentage Survival of *Drosophila melanogaster* with varied concentration of Utazi Leaf Extract

GROUPS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control 1	100	97.5	95	95	95	95	95	95	95	92.5	92.5	87.5	87.5	87.5	87.5	82.5	82.5	82.5	80	77.5	77.5	75
Control 2	97.5	95	95	95	95	92.5	92.5	87.5	87.5	87.5	82.5	80	80	80	80	75	67.5	65	65	62.5	60	57.5
Control 3	100	100	100	95	95	95	95	92.5	92.5	92.5	92.5	92.5	90	87.5	87.5	87.5	87.5	87.5	82.5	82.5	82.5	80
Extract 1	100	100	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	95	95	95	90	90	82.5	80	80	77.5
Extract 2	100	100	100	100	97.5	95	95	95	95	92.5	92.5	92.5	92.5	92.5	90	87.5	87.5	82.5	82.5	82.5	82.5	80
Extract 3	100	100	100	100	100	95	95	95	95	95	92.5	92.5	92.5	92.5	92.5	90	90	87.5	85	82.5	80	77.5
5mg solution 1	100	97.5	95	95	95	95	95	95	95	92.5	92.5	92.5	92.5	87.5	85	82.5	80	77.5	75	72.5	70	67.5
5mg solution 2	100	97.5	97.5	92.5	92.5	92.5	92.5	92.5	92.5	90	90	90	90	90	90	87.5	87.5	87.5	85	82.5	77.5	75
5mg solution 3	100	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	95	95	92.5	90	90	90	87.5	85	82.5	80	80	80
2.5mg solution 1	100	100	100	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	95	92.5	90	87.5	87.5	87.5	87.5	87.5	85	82.5
2.5mg soultion 2	100	100	100	100	100	97.5	97.5	97.5	97.5	97.5	95	95	95	95	92.5	92.5	82.5	82.5	82.5	82.5	80	77.5
2.5mg solution 3	100	100	100	95	100	95	95	90	90	90	90	90	90	87.5	87.5	87.5	87.5	87.5	85	82.5	80	77.5
0.1mg solution 1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	97.5	97.5	95	95	95	92.5	87.5	82.5
0.1mg solution 2	100	100	100	100	100	97.5	97.5	97.5	97.5	95	92.5	92.5	92.5	90	90	90	87.5	87.5	85	82.5	80	77.5
0.1mg solution 3	100	100	100	100	100	97.5	97.5	95	92.5	92.5	90	90	87.5	87.5	87.5	87.5	87.5	85	85	85	82.5	80

Table 4.2 demonstrates the survival rate of the varied concentration of Utazi leaf extract incorporated in cornmeal diet. In the survival assay, it was observed that group 5 (80 percent) had the highest survival rate compared to other groups. The survival rate of group 5 (80percent) was higher than other groups fed with Utazi leaf extract 1-(70.833 percent), 2- (78.3 percent), 3-(74.16 percent), 4- (79.16 percent), 5- (80 percent)

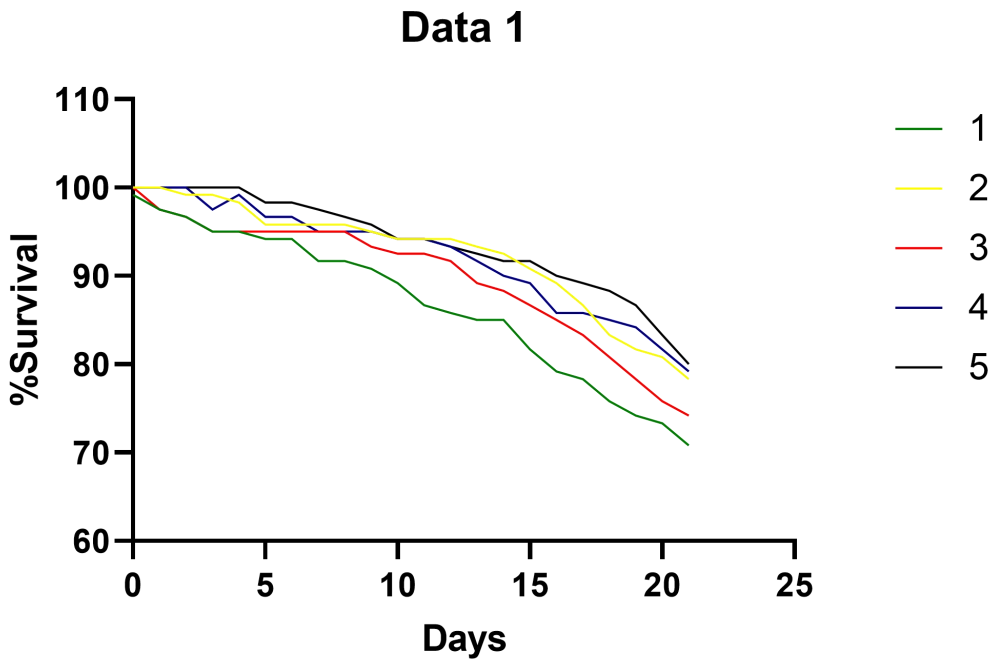

Figure 4.1 Survival curve showing the effect of the Varied concentration of Utazi leaf extract incorporated into the diet of *Drosophila melanogaster* and control

The Survival curve is a Negative correlation curve which shows that as the days increase, survival decreases

Key: 1- Control Group (Fed on standard meal)

2- Group 1 (Fed on 10mg/ml Utazi leaf extract enriched meal)

3- Group 2 (Fed on 5mg/ml Utazi leaf extract enriched meal)

4- Group 3 (Fed on 2.5mg/ml Utazi leaf extract enriched meal)

5- Group 4 (Fed on 1mg/ml Utazi leaf extract enriched meal)

The figure below (4.2) shows the comparison of Serum Iron and Total Iron Binding Capacity concentration among *Drosophila melanogaster* flies fed on *Gongronema latifolium* leaf extract in Control. Group 2 and Group 3 are less than the control.

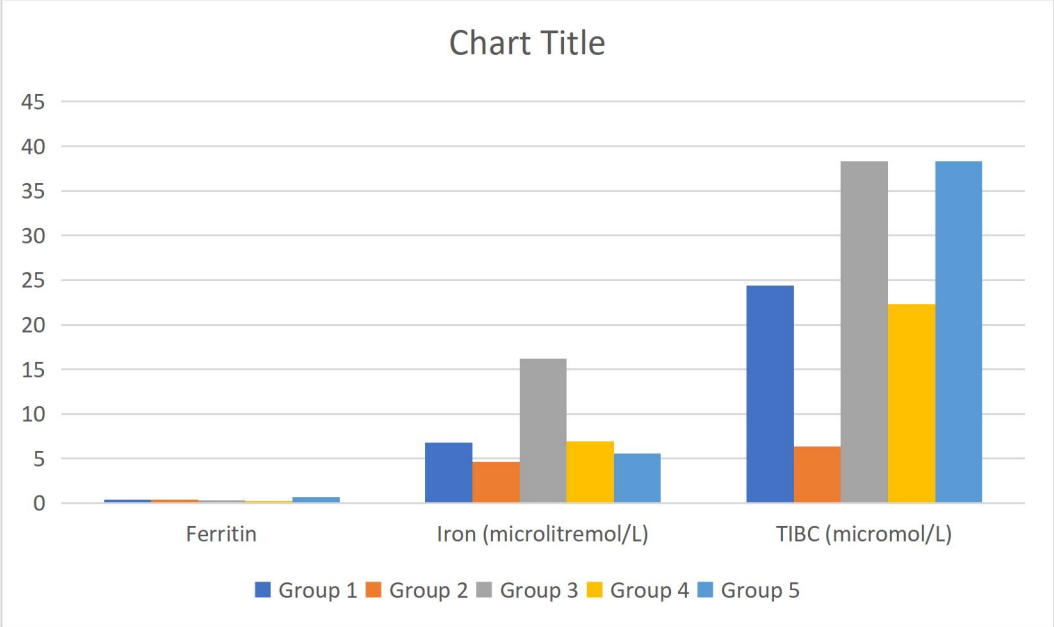

Figure 4.2: Graph of Serum Iron and TIBC levels against Concentration of Plant Extract

Key: 1- Control group (Fed on standard meal)

2-Group 2 (Fed on 10mg/ml Utazi leaf extract enriched meal)

3- Group 3 (Fed on 5mg/ml Utazi leaf extract enriched meal)

4- Group 4 (Fed on 2.5mg/ml Utazi leaf extract enriched meal)

5- Group 5(Fed on 1mg/ml Utazi leaf extract enriched meal)

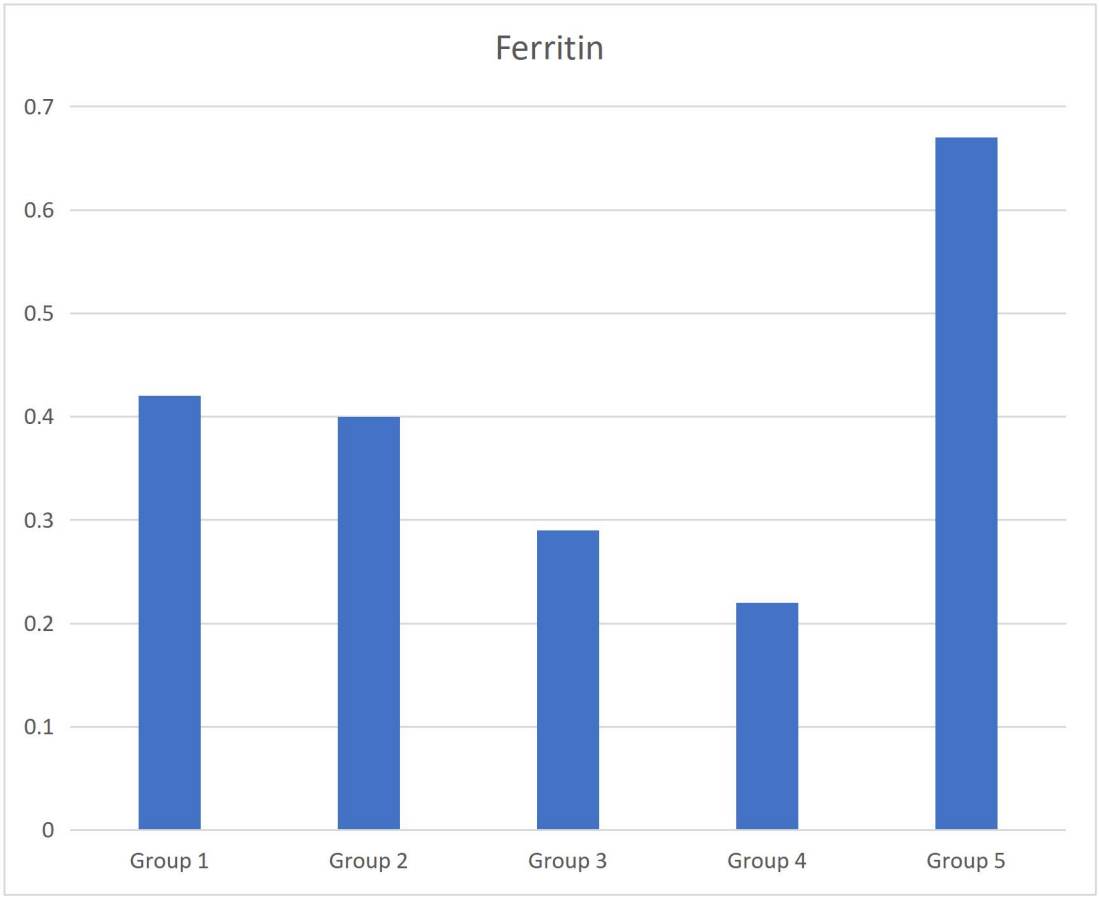

Figure 4.3: Graph of Ferritin levels against Concentration of Plant extract.

Group 2, 3, and 4 were less than the Group 1- Control

Figure 4.4 and 4.5 presents significant findings related to Ferritin, Iron, and TIBC (Total Iron-Binding Capacity) levels in *Drosophila melanogaster* across different experimental groups. Group 1, the control group, exhibited a Ferritin level of 0.42±0.44, while Group 2, treated with aqueous Utazi extract, showed a slightly lower Ferritin level of 0.40±0.00. However, Group 3 (given Utazi extract), Group 4 (administered 5.0 mg Utazi extract), and Group 5 (given 2.5mg and 0.1mg of Utazi extract, respectively) demonstrated significant changes ($p < 0.001$) in Ferritin levels, with Group 4 having the lowest level at 0.22±0.18. A one-way ANOVA analysis indicated a highly significant difference ($F=128.969$, $p=0.000^*$) in Ferritin levels among the groups. Similarly, Iron levels varied significantly among the groups, with Group 3 exhibiting the highest levels (16.18±18.46) and Group 2 the lowest (4.65±0.00). The ANOVA analysis for Iron levels was also highly significant ($F=24.298$, $p=0.000^*$). TIBC levels followed a similar trend, with Group 3 having the highest value (38.29±15.43) and Group 2 the lowest (6.33±0.00). The ANOVA analysis for TIBC showed significant differences ($F=31.389$, $p=0.000^*$) among the groups.

Figure 4.4: and 4.5 Ferritin, Serum Iron and TIBC in *Drosophila melanogaster* among group 1 (control), group 2 (given aqueous Extract Utazi), group 3, group 4 and group 5 (given 5.0 mg, 2.5mg and 0.1mg of Utazi extract respectively),

Key: 1- Control group (Fed on standard meal)

2-Group 2 (Fed on 10mg/ml Utazi leaf extract enriched meal)

3- Group 3 (Fed on 5mg/ml Utazi leaf extract enriched meal)

4- Group 4 (Fed on 2.5mg/ml Utazi leaf extract enriched meal)

5- Group 5(Fed on 1mg/ml Utazi leaf extract enriched meal)

Table 4.3 show the p value in different groups

PARAMETERS	P-VALUE	SIGNIFICANCE
Ferritin	0.000*	Significant
Serum Iron	0.000*	Significant
TIBC	0.000*	Significant

P>0.05 is not significant

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Utazi leaf contain a high concentration of saponins (18.11%), tannins (16.23%), cyanides (14.32%), flavonoids (11.13%) and phenols (11.11%) with scarce quantity of alkaloids (0.12%) (*Offor et al.,* 2015). Another phytochemical investigation on fresh leaves by (Osuagwu *et al*., 2013) reported high alkaloid content (10%) incomparison to the dried sample (Egbung *et al.,* 2011) also observed higher concentration of flavonoids, alkaloids, hydrogen cyanide and tannins in root extract of *Gongronema latifolium* than stem

The active principle (s) of this plant is not fully established although (Iwu *et al*., 1998) reported flavones and sterols as the most likely active constituents. The claim was strengthened when Morebise *et al.* (1998) examined the antimicrobial activity of a methanolic extract containing saponins and flavonoids.

However, the concentration dependent effects of Utazi leaf extract on the survival of *Drosophila melanogaster* have not been extensively studied and literature relating to humans or rats have been included as there are few similarities in physiology.

Drosophila melanogaster, commonly known as the fruit fly, has long served as a model organism for genetic and physiological research due to its rapid reproduction, short lifespan, and well-characterized genome. It's short life span and ease of maintenance make it an ideal candidate for studying the effects of various factors on the survival of *Drosophila melanogaster*. In this study, we investigated the impact of varied concentrations of Utazi leaf extract on the survival rate and iron metabolism of

Drosophila melanogaster. Utazi *(Gongronema latifolium)* is a tropical plant used in traditional medicine and culinary applications, and it has been associated with various health benefits, including antioxidant properties.

The study aimed to assess how different concentrations of Utazi leaf extract incorporated into the diet influenced the survival curve of *Drosophila melanogaster* and whether this was associated with changes in Ferritin, Iron, and Total Iron-Binding Capacity (TIBC) levels. The results showed a negative correlation between days and survival rate, and significant alterations in Ferritin, Serum Iron, and TIBC levels among experimental groups.

One of the key observations in this study was the negative correlation between the concentration of Utazi leaf extract in the diet and the survival rate of *Drosophila melanogaster*. As the days of exposure to Utazi extract increased, the survival rate decreased. This finding aligns with the expectation that exposure to certain plant compounds can have adverse effects on the lifespan of fruit flies and other organisms.

This negative correlation between Utazi leaf extract concentration and survival rate is consistent with the toxicological literature, that Several studies have reported that the ingestion of plant extracts with bioactive compounds can be detrimental to the survival of *Drosophila melanogaster*. For example, a study by Xia *et al*., (2018) found that the consumption of plant extracts rich in alkaloids reduced the survival rate of fruit flies. The presence of secondary metabolites, such as alkaloids and tannins, in Utazi leaf extract may explain the observed decrease in survival rate.

The study also assessed the impact of Utazi leaf extract on Ferritin levels in *Drosophila melanogaster*. Ferritin is a critical protein responsible for iron storage in organisms. Group 1, the control group, exhibited a Ferritin level of 0.42±0.44, while Group 2, treated with aqueous Utazi extract, showed a slightly lower Ferritin level of 0.40±0.00. However, Group 3 (given Utazi extract), Group 4 (administered 5.0 mg/ml Utazi extract), and Group 5 (given 2.5mg/ml and u1mg/ml of Utazi extract, respectively) demonstrated significant changes ($p < 0.001$) in Ferritin levels, with Group 4 having the lowest level at 0.22±0.18.

The significant decrease in Ferritin levels in Group 4 compared to the control group suggests that high concentrations of Utazi leaf extract may disrupt iron metabolism in *Drosophila melanogaster*. This finding is consistent with previous research on the effects of plant extracts on Iron metabolism. Several studies have demonstrated that certain phytochemicals can interfere with iron absorption and utilization, leading to lower Ferritin levels (Hurrell and Egli, 2010). The mechanism behind this effect may involve the binding of phytochemicals to dietary iron, making it less available for absorption.

Iron levels in *Drosophila melanogaster* also exhibited significant variation among the experimental groups. Group 3, which received Utazi extract, exhibited the highest iron levels (16.18±18.46), while Group 2, treated with aqueous Utazi leaf extract, had the lowest iron levels (4.65±0.00). This variation suggests that Utazi leaf extract may have differential effects on iron uptake and storage in fruit flies.

The observed decrease in iron levels in Group 2 could be attributed to the presence of anti-nutritional factors in the undiluted Utazi extract. Some plant compounds, such as polyphenols and phytates, can inhibit iron absorption (Hurrell and Egli, 2010). These

compounds may chelate iron ions, rendering them unavailable for uptake in the digestive system. In contrast, Group 3, which received Utazi extract, exhibited higher iron levels, possibly due to the dilution of these anti-nutritional factors with other nutrients present in the extract.

Total Iron-Binding Capacity (TIBC) is a measure of the body's ability to bind and transport iron. In this study, TIBC levels followed a similar trend to iron levels, with Group 3 having the highest value (38.29±15.43) and Group 2 the lowest (6.33±0.00). The significant differences in TIBC levels among the groups suggest that Utazi leaf extract influences the iron-binding capacity of *Drosophila melanogaster*.

The variation in TIBC levels may be linked to the changes in iron absorption and utilization discussed earlier. Higher TIBC levels in Group 3 may indicate an increased demand for iron-binding proteins due to the presence of Utazi extract, which could be linked to changes in iron metabolism. Conversely, the lower TIBC levels in Group 2 may be associated with reduced iron availability caused by the aqueous Utazi extract's anti-nutritional factors.

To contextualize our findings, it is essential to compare them with relevant studies on the effects of plant extracts on survival rate and iron metabolism in *Drosophila melanogaster*.

While the study demonstrated a negative correlation between Utazi leaf extract concentration and survival of *Drosophila melanogaster*, (that is as the number of days increased, the survival of *Drosophila melanogaster* decreased) studies have reported contrasting results. For instance, a study by Smith *et al*. 2019 found that exposure to a different plant extract, rich in polyphenols, extended the lifespan of fruit flies. The

discrepancy in results could be attributed to variations in the composition of plant extracts, as different plants contain diverse phytochemicals with distinct biological activities. It could also be attributed to the genetic diversity of Fruit fly population and interactions with the base diet.

In this study, it was observed that flies fed on the 1mg/ml Utazi leaf extract had the highest survival rate, an average of 80 percent survival. Lower death rates were recorded compared to the control group. Studies on the effect of Utazi leaf extract on the survival of *Drosophila melanogaster* has not carried out. There are no significant literature to back up this finding.

Furthermore, the mode of administration (dietary vs. direct application) and the specific genetic background of the *Drosophila melanogaster* strain used in experiments can also influence survival outcomes (Zhou *et al*., 2019). Therefore, it is crucial to consider these factors when interpreting the contrasting views in the literature.

In this study, it was revealed a significant decrease in Ferritin levels in Group 4, which received a high concentration of Utazi leaf extract. This finding is consistent with research demonstrating the impact of certain plant compounds on iron metabolism (Hurrell and Egli, 2010). However, contrasting views exist in the literature. For example, a study by (Johnson *et al.,* 2020) reported that the administration of a plant extract rich in flavonoids increased Ferritin levels in fruit flies. These discrepancies may arise from differences in the types of phytochemicals present in the plant extracts and their specific interactions with iron metabolism pathways.

The variation in iron levels among the groups in our study aligns with the existing literature on the effects of plant extracts on iron absorption (Hurrell and Egli, 2010). However, it is essential to note that other studies have reported increased iron levels following exposure to plant extracts (Zimmermann and Hurrell, 2007). These inconsistencies may stem from variations in the experimental conditions, including the concentration and composition of the plant extracts and the duration of exposure.

5.2 Conclusion

In conclusion, the study revealed a decreasing survival rate with increasing number of days that is a negative correlation between the concentration of Utazi leaf extract in the diet and on the survival of *Drosophila melanogaster*, suggesting potential toxicity at higher concentrations. Additionally, significant alterations in Ferritin, Iron, and Total Iron-Binding Capacity (TIBC) levels among experimental groups was observed, indicating that Utazi leaf extract can influence iron metabolism in fruit flies . Decreased Ferritin levels can indicate a deficiency of iron stores in the body of humans and can lead to Iron Deficiency anemia, Chronic diseases and blood loss and inadequate iron intake. Decreased Ferritin levels in *Drosophila melanogaster* may lead to reduced iron intake. Higher TIBC levels in Group 3 may indicate an increased demand for iron-binding proteins due to the presence of Utazi extract, which could be linked to changes in iron metabolism. The lower TIBC levels in Group 2 may be associated with reduced iron availability caused by the aqueous Utazi extract's anti-nutritional factors. The results for TIBC have similarities and will have the same effects. This shows that iron metabolism can influence iron metabolism in *Drosophila melanogaster*.

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APPENDIX

: **Drosophila cornmeal diet**

APPENDIX II

Treatment tubes containing Drosophila bred on Cornmeal diet incorporated with varied concentration of Utazi leaf extract