AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

EFFECT OF YOUNG AND OLD *Moringa oleifera* LEAF EXTRACT ON HAEMATOLOGICAL, RENAL AND LIVER INDICES IN *Rattus novergicus*

JOSHUA DWOMOH

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BY

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A thesis submitted to the School of Graduate Studies, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development in partial fulfillment of the requirements for the award of a Master of Philosophy degree in Biology

DECEMBER, 2023

DECLARATION

Candidate's Declaration

I hereby declare that this dissertation is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Joshua Dwomoh

Signature: ……. **Date: ……..**

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with guidelines on supervision of dissertation laid down by the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development.

Dr. Duodu Addison (Principal Supervisor)

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Dr. Fritz R. K. Bonsu (Co-supervisor)

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ABSTRACT

The study aimed to ascertain the effects of ethanolic leaf extract of young and old *Moringa oleifera* on haematological, renal, and liver indices in Wistar rats. The study was carried out at the Animal Science Department of AAMUSTED. A completely randomized design was used for the study. A total of twenty male Wistar rats of age eight weeks old were used for the study. The animals were kept in aluminum cages under a 12-hours light and 12-hours dark cycle. The rats were divided into four groups; G1T1 received 1 mL of normal saline, G2T2, G3T3 and G4T4 received 100 mg/kg/bw of iron (III) hydroxide polymaltose, 100 mg/kg/bw of young *M. oleifera* extract (YMoE) and 100 mg/kg /bw of old *M. oleifera* extract (OMoE) as treatments respectively for four weeks Data from the study was expressed in terms of mean and standard error of the mean. Parameters in groups were compared by one-way ANOVA. Phytochemical screening revealed the presence of triterpenoid, glycosides, flavonoid, and saponins in both young and old *M. oleifera* leaves, however, alkaloids and tannins were found only in young leaves of *M. oleifera*. Both extract (YMoE and OMoE) significantly ($P < 0.05$) influenced rat's feed intake and body weight. An insignificant ($P > 0.05$) effect of the treatment on haematological parameters was observed. However, there was a significant ($P < 0.05$) effect of YMoE and OMoE treatments on haemoglobin which mirrored the effect of iron (III) hydroxide polymaltose. The study found no significant effect ($P > 0.05$) of YMoE and OMoE treatments on liver enzymes, lipid profile and Blood urea nitrogen. Creatines levels showed elevation in the group that received iron (III) hydroxide polymaltose, while those administered with Moringa extract had similar effect to the normal saline group. Histopathological examinations showed normal kidney and liver architecture in normal saline and *M. oleifera*

treatments. Mild renal epithelium degeneration was observed in the iron (III) hydroxide poly-maltose treatment. The findings from this study suggest that both young and old *M. oleifera* leaves, may effectively manage anaemiaj without causing kidney or liver damage.

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DEDICATION

I dedicate this work to my family for their relentless support, advice, and encouragement throughout my education and research period. I further dedicate this work to all my loved ones who, in one way or another, have contributed to the success of this research piece.

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ABBREVIATIONS

ACD	-	Anaemia of Chronic Diseases
AK	-	Adenylate
ALP	-	Alkaline Phosphatase
ALT	-	Alanine Transaminase
AST	-	Aspartate transaminase
BPGM	-	Diphosphoglycerate mutase
BUN	-	Blood Urea Nitrogen
EDTA	-	Ethylenediaminetetraacetic acid
ENO	-	Enolase
G6PD	-	Glucose -6-phosphate dehydrogenase
GAPDH	-	Glyceraldehyde-3-phostate dehydrogenase
GBD	-	Global Burden of Diseases
GGT	-	Gamma-glutamyl trasferase
GR	-	Glutathione reductase
H&E	-	Eosin and Haematoxylin
Hbg	-	Haemoglobin
HCT	-	Haematocrit
HDL	-	High density lipoprotein
HFD	-	High-fat diet
HMB	-	Heavy Menstrual Bleeding
HMG CoA-3	-	Hydroxy-3-Methyl-Glutaryl-Coenzyme A
HSt	-	Hereditary stomatocytosis
LDL	-	Low density lipoprotei

MCH - Mean Corpuscular Haemoglobin

MCHC - Mean Corpuscular Haemoglobin Concentration

MCV - Mean Corpuscular Volume

MDA - Malondialdehyde

MOE - *Moringa oleifera* extract

MSG - Monosodium Glutamate

MVP - Mean Platelet Volume

NAFLD - Non-alcoholic fatty liver disease

NIH - National Institute of Health

OECD - Organization of Economic Co-operation and Development

OMoE - Old *Moringa Oleifera* extract

OMoL - Old *Moringa oleifera* leaves

P5N - Pyrimidine 5'-nucleotidase

PA - Pernicious anaemia

PGK - Phosphoglycerate Kinase

PK - Pyruvate Kinase

PLT - Platelets

RBC - Red Blood Cell

SCD - Sickle Cell Disease

SEM - Standard Error of the mean

TAG - Triglyceride

TC - Total cholesterol

TDN - Titanium Dioxide Nanopatricles

VLDL - Very Low-density lipoprotein

WBC - White Blood cells

WHO	**-**	World Health Organization
YMoE	-	Young *Moringa oleifera* extract
YMoL	**-**	Young *Moring oleifera* leaves

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Blood is an essential homeostatic fluid in the human body (Ghersi-Egea *et al.,* 2018). Its physiological functions include the transport of nutrients and respiratory gases to cells, and the transport of waste products of metabolism away from cells (Jacob *et al.,* 2016). The volume of blood accounts for about 10% of the general body weight of an adult human. Reduction in blood haemoglobin concentration as a fallout of genetic, nutritional, or environmental factors leads to a condition termed anaemia (Gupta *et al.,* 2017). Iron deficiency anaemia (IDA) has remained a global public health challenge, especially in developing countries. Its prevalence has been attributed to worm infestation, malaria infection, bacterial and viral infections, pregnancy, and malnutrition; mostly food that is deficient in iron (Kumar and Thakur, 2022). Iron deficiency anemia has been reported to have a high prevalence among pregnant women and children under age five.

The effects of IDA on the latter include disrupted physical and mental development leading to low intellectual ability and weakened immunity. In pregnant women, it results in an increased risk of labor complications (Aryeetey *et al.,* 2022). Efforts have been made to curb the effects of anaemia, yet we have not reached the point of satiation. However, the cost of conventional prognosis and treatment is highly expensive, and blood transfusion, the commonest treatment may also result in blood transfusion-transmitted infections (TTIs) such as hepatitis A, B, C, and E, chikungunya virus, Dengue fever, Human Immunodeficiency Virus, Human T-cell Lymphotropic Virus

(HTLV), Variant Creutzfeldt-Jakob Disease (vCJD), among others if blood is not screened properly (CDC, 2022).

Free access and proximity of herbal plants for the treatment of IDA has left the destitute populace in developing countries to depend heavily on medicinal plants for the treatment and management of IDA. Personal communications with local herbal practitioners within five localities in Asante Mampong have shown that different maturity (old and young) type of *M. oleifera* leaves are used in herbal formation for the treatment and management of IDA. Given these reasons, a knowledge of the maturity type of *M. oleifera* will enhance efficiency in its use as a haematinic and provide evidence on how the extracts affects renal and liver function.

M. oleifera, is a short, slender, and perennial tree belonging to the *Moringaceae* family. It is widely cultivated and naturalized in tropical India, Africa, tropical America, Sri lanka, Mexico, Malaysia and the Phillipine Islands (*Chivapat et al.,* 2011). The plant has been used since antiquity for the treatment and management of diseases such as hypertension, diabetes, anti-inflammatory, anti-cancer cardiovascular diseases, and infections has been a subject of exploration when it comes to finding a potent treatment for iron deficient anaemia (Azlan *et al.,* 2022; Maina Hassan *et al.,* 2020). Since the 1970s, a great deal of in-depth research has been done on the phytochemical and nutraceutical constituents of *M. oleifera* and the results have been astounding.

Studies have reported that *M. oleifera* plant is well-endowed with essential nutrients and distinct phytochemical components that are valuable medically (Azlan *et al.,* 2022). On top of that, the discovery of standardized extraction procedures has also been a game

changer as the bioactive compound in *M. oleifera* can be extracted without distorting the original composition and structure. However, regardless of the extensive studies carried out on the efficacy of *M. oleifera* in treating IDA, there is limited literature on the leave type of *M. oleifera* that possesses the haematinic effect and the toxicity levels of young and old *M. oleifera* leaves extract on liver and renal indices as the plant is used in managing IDA.

1.2 Problem Statement

Iron deficiency anaemia (IDA) has become a public health concern for both low and middle-income countries around the globe (Gonete *et al.,* 2018). The World Health Organization has stated that IDA affects approximately one-fourth of the global population with Africa being the most affected continent (Halli & Biradar, 2020). In Ghana, IDA affects 66% of children under the age of five, while it affects 42% of pregnant women, as reported by the 2014 Ghana Demographic and Health Survey. Over 90% of cases of anaemia in Ghana are caused by iron deficiency, making it the most prevalent type of anaemia. The high occurrence of IDA in many parts of Ghana is a significant threat to public health. Ghana has different levels of anaemia prevalence depending on the region and population. In general, rural areas and low-income populations have higher prevalence rates. In 2014, a study conducted by the Ghana Demographic and Health Survey showed that the Upper East (84%), Northern (81%), and Upper West (80%) are the regions with the highest prevalence of anaemia among children under the age of five. Greater Accra (35%), Eastern (43%), and Western (47%) had the lowest prevalence rates by region.

In Ghana, efforts are being made to reduce anaemia by increasing access to foods high in iron, encouraging iron supplementation for at-risk groups, promoting proper nutrition education, and enhancing the healthcare system to ensure early identification and treatment of anaemia. The National Micronutrient Fortification Program and the Ghana School Feeding Program are just two of the anaemia-related initiatives that the Ghanaian government has put in place (Ghana Health Service, 2016). Despite these efforts, iron deficiency anaemia remains a significant public health issue in Ghana, and continued research and resources are needed to address this problem.

Previous studies have explored the hematological effects of *M. oleifera* extracts, such as the investigation by Ekpo *et al.* (2019) on alcohol-induced haematotoxicity in Wistar rats, which reported a significant reduction in hematological parameters. Similarly, Airaodion *et al.* (2019) found a significant increase in hematological indices in rats fed a crude oil-treated diet and induced with an ethanolic leaf extract of *M. oleifera.* However, these studies did not specify the maturity type of *M. oleifera* leaves that caused these hematological changes. Moreover, they did not assess the effect of the extract on feed intake, body weight, renal, and liver functions.

This gap in the literature indicates a need for comprehensive research to determine which maturity stage of *M. oleifera* leave (young or old) possesses the most potent hematinic effects. Additionally, understanding the toxicological implications of these extracts on body weight, feed intake, renal, and liver functions is also important. Identifying the specific maturity type that is most effective as a hematinic can enhance the therapeutic use of *M. oleifera* and provide insights into its safety profile concerning renal and liver functions.

1.3 Significance of the Study

This study on the effects of young and old *M. oleifera* leaves extract on hematological, renal, and liver indices is significant as it will provide critical understandings into the nutritional and medicinal differences between the leaves based on their age, informing dietary recommendations and therapeutic applications. By evaluating the effect on blood parameters, renal, and liver function, the study will elucidate the safety and efficacy of the respective leave extracts, guiding their usage in managing IDA, and highlight the toxicological effect of the extracts on kidney, and liver function. Additionally, understanding the age-dependent phytochemical composition will optimize harvesting practices, leading to better quality supplements and personalized health interventions. The findings will influence agricultural practices, enhance market development for age-specific Moringa products, and contribute to public health improvements through informed health policies and education. Ultimately, this study will lay the groundwork for further research in phytomedicine, broadening the understanding of age-related variations in plants and their health impacts.

1.4 Research Questions

The germane questions that will guide this study include;

1. What is the phytochemical diversity in young and old *M. oleifera* leaves*?*
2. To what extent could young and old *M. oleifera* extract affect feed intake and body weight?
3. To what extent could young and old *M. oleifera* leaves extract affect haematological parameters in *Rattus novergicus?*
4. What will be the effects of the extract on the liver, and renal indices (LFT, KFT, and Lipid profile) in *Rattus novergicus*?

5. To what extent could the extract affect the renal and liver tissues of the *Rattus novergicus*?

1.5.1 Objectives of the Study

The main objective of this current study sought to assess the effects of young and old *M. oleifera* leaves extract on haematological, renal, and liver indices in *Rattus novergicus.*

The specific objectives of this study sought to assess the;

- phytochemical diversity of young and old *M. oleifera* leaves.
- the effect of the extract on body weight and feed intake
- effect of the extract on blood parameters (WBCs, RBCs, MCHC, Hematocrit, Hbg, MCV, MCH, MPV and Platelets).
- effect of the extract on the liver and kidney biochemical parameters (LFT, KFT, and Lipid profile).
- histopathological effect of the extract on renal and liver tissues of the Wistar rats.

1.6 Hypothesis

H_O: Administration of young and old *M. oleifera* leaf extract will have effect on haematological, renal, and liver parameters in *Rattus novergicus*.

H_A: Administration of young and old *M. oleifera* leaf extract will not have effect on haematological, renal, and liver Parameters in *Rattus novergicus*.

1.7 Limitations of the Study

The small sample size of only twenty male Wistar rats may affect the generalizability of the findings to a larger population. The study's duration of four weeks offers initial insights, albeit with limitations in capturing the long-term effects over an extended timeframe.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Overview of Anaemia

Humans benefit from optimal haematological parameters; however, their deviations have a negative impact on their health. The World Health Organization, for example, identifies anaemia as a regional and international health issue (WHO, 2008; Jung *et al.,* 2019). Nutritional deficiencies, haemoglobinopathies, and pathogenic disorders such as malaria, TB, HIV, and parasitic infections are common causes of anaemia. Anaemia remains one of the major global health issues that is difficult to eradicate. It has far-reaching effects on human health and socioeconomic development and impacts both developed and developing countries. Anaemia is characterized by a decrease in the body's ability to transport oxygen to meet the physiological demands of the tissues due to a decrease in haemoglobin (HGB) content or red blood cell (RBC) count below a reference range that has been established at 12 g/dL in non-pregnant women and 13 g/dL in men (Elysium, 2011; Gamit & Talwelkar, 2017).

Comparatively, the Global Burden for Diseases in 2019 also defined anaemia as a decrease in blood haemoglobin concentration regardless of the underlying aetiology, red blood cell shape, or red blood cell function (Abbafati *et al.,* 2020). It is worth noting that an individual's physiology and environment may alter the threshold of haemoglobin concentration. To ensure that anaemia is accurately diagnosed, and its harmful effects are prevented, it is decisive to establish suitable Hb thresholds to define anaemia. In addition, developing effective interventions that address the context-specific causes of anaemia, and understanding the varied and complex aetiology of

anaemia is also essential for evaluating the success of anaemia control programs (Chaparr & Suchdev, 2019). Anaemia has been ascribed to be the most significant micronutrient deficiency in the world, which is predominant in low socioeconomic populations as a result of poverty. A third of the world's population suffers from iron deficiency anaemia, the most prevalent type of anaemia. The number of deaths attributed to iron deficiency was estimated to be 183,000 in 2013, down from 213,000 in 1990 (Ranjan *et al.,* 2022; Safari *et al.,* 2021). More so, it affects pregnant women more than men, along with young children and the elderly (Ranjan *et al.,* 2022). Nutritional deficiencies and chronic diseases are typically the most frequent etiologies of anaemia in children and adults, respectively. Anaemia has a variety of correlates based on its underlying pathophysiology (Varghese *et al.,* 2022). Depending on the types of anaemia and its degrees of severity, the clinical manifestations and complications of anaemia vary. The notable effects of iron deficiency anaemia can include impaired neurocognitive function, immune system dysfunction, digestive tract disturbances, and impaired thermoregulation. It can also be a risk factor or predictor for the prognosis of other life-threatening conditions such as heart failure and tuberculosis (Gamit & Talwelkar, 2017). The adverse effects of anaemia have made it a condition of public health concern.

2.2 Types of Anaemia

As established earlier, anaemia can occur due to various abnormalities in red blood cells (RBCs). These abnormalities include impaired production, as seen in aplastic anaemia, impaired maturation deficit in megaloblastic anaemia, errors in haemoglobin synthesis leading to iron deficiency anaemia, genetic defects in haemoglobin maturation seen in thalassemia, synthesis of abnormal haemoglobin found in haemoglobinopathies, sickle

cell anaemia, and thalassemia, as well as loss of weight in RBCs, as seen in hemolytic anaemia (Ranjan *et al.,* 2022). Knowing the various classifications can aid in identifying the signs and preventing anaemia altogether. Therefore, this section of the chapter focuses on the various classifications of anaemia.

2.2.1 Iron Deficiency Anaemia

As abundant as iron is on earth, severe iron deficiency is one of the most prevalent causes of anaemia in humans, which is the most prevalent cause of anaemia worldwide. It is well known that iron plays a major role in the proper functioning of several physiological processes within the human body, including the production of haemoglobin. A condition known as iron deficiency anaemia occurs when the body's blood iron levels are below normal. This type of anaemia is more prevalent in teenagers and women before menopause (Mirza *et al.,* 2018). This condition can be attributed to blood loss from heavy periods, internal bleeding from the gastrointestinal tract, or persistent blood donation (Miller *et al.,* 2013; Soundarya, 2017). Anaemia due to a lack of iron in the body can arise from a number of different causes, as stated by Gamit and Talweker (2017).

Pregnancy or childhood growth spurts, heavy menstrual cycles, poor iron absorption, intestinal bleeding, dietary variables (an iron-poor or limited diet), medication (aspirin, ibuprofen, naproxen, and diclofenac), Red blood cell abnormalities, renal hemorrhage, hookworm infection, vitamin deficiencies (such as folic acid and vitamin B12), heavy periods, and bone marrow issues are the causes of iron-deficiency anaemia (Gamit & Talwelkar, 2017; Soundarya, 2017). Some of the notable symptoms associated with iron deficiency anaemia include tiredness, lethargy, headaches, irregular heartbeats

(palpitations), altered taste, sore mouth, and tinnitus. Iron deficiency anaemia in pregnancy increases the risk of complications in both mother and baby such as low birth weight, preterm (premature) delivery, and postnatal depression. Low iron reserves in the baby may also lead to anaemia in the newborn baby (*Di Renzo et al.,* 2015).

2.2.2 Pernicious Anaemia (PA)

PA is an autoimmune disease that is characterized by chronic atrophic gastritis (CAG) as well as a deficiency of cobalamin (CD). Pernicious anaemia is the leading cause of cobalamin deficiency throughout the world. The prevalence is hugely affected by age, ranging from 0.1% in the general population to 1.9% in the elderly population above the age of 60 years. PA accounts for about 20% -50% of the etiology of CD in adults. Although the incidence of PA rises with age and varies geographically. It is often described as being very common in Scandinavian countries (Htut *et al.,* 2021). Furthermore, pernicious anaemia is the most common cause of vitamin B_{12} deficiency. Vitamin B12 is essential to life. It is required to produce new cells in the body; it is mainly found in meat, fish, eggs, and milk. Anaemia and other conditions can result from a vitamin B_{12} deficiency. Anaemia can result from a lack of vitamin B_{12}.

Pernicious anaemia commonly develops among individuals over fifty years of age; men are less likely to be afflicted than women, and it frequently runs in families. It happens more frequently in those with other autoimmune illnesses (Mohamed *et al.,* 2020; Soundarya, 2017). Moreover, several drugs may prevent the absorption of vitamin B_{12}. Metformin, colchicine, neomycin, and several anticonvulsants used to treat epilepsy are the most archetypal examples. Nervous difficulties, including numbness, pins and needles, visual abnormalities, and unsteadiness, as well as psychological issues like

melancholy, disorientation, memory loss, or even dementia, might manifest. Therefore, persistent, or severe vitamin B_{12} deficiency may result in long-term brain or nerve damage (Mohamed *et al.,* 2020).

2.2.3 Haemolytic Anaemia

The condition known as hemolytic anaemia is characterized by the destruction of red blood cells by the body before they have completed their normal lifespan and are removed from the bloodstream. It is worth noting that hemolytic anaemia can affect people of all ages, races, and genders (Rees *et al.,* 2018; Thomas, 2017). The effects of hemolytic anaemia include fatigue, pain, arrhythmias, enlarged hearts, and the risk of heart failure if the condition is not treated in time. Classically, there are two classes of hemolytic anaemia: hereditary or genetic disorders affecting the erythrocyte cytoskeleton, which can manifest as a range of diseases with varying genetic and phenotypic characteristics (Rees *et al.,* 2018). These conditions can range from asymptomatic states that may only become apparent during a hemolytic or aplastic crisis triggered by a viral infection to severe congenital hemolytic anaemias that require regular blood transfusions. Diseases caused by defects in the erythrocyte cytoskeleton can vary substantially in both genetics and phenotype, from being completely asymptomatic until a hemolytic or aplastic crisis is triggered by a viral infection to being fatally congenital and requiring lifelong transfusions (Briglia *et al.,* 2017). Sickle cell anaemia, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, stomatocytosis (HSt), glucose-6 phosphate dehydrogenase (G6PD), and pyruvate kinase deficiency are the most prevalent erythrocyte cytoskeleton diseases (Soundarya, 2017). Acquired hemolytic anaemias include immune hemolytic anaemia, autoimmune hemolytic anaemia, alloimmune hemolytic anaemia, and drug-induced hemolytic

anaemia. Mechanical hemolytic anaemias, paroxysmal nocturnal haemoglobinuria, certain infections, and substances can also damage red blood cells and lead to hemolytic anaemia (Vaschenko & Vil'yaninov, 2019). Fatigue is the primary symptom of hemolytic anaemia, which is caused by a lack of oxygen. Other symptoms include jaundice, pain in the upper abdomen, leg ulcers and soreness, and a strong reaction to a blood transfusion. Treatments for hemolytic anaemia include blood transfusions, medicines, plasmapheresis, surgery, blood and marrow stem cell transplants, and lifestyle changes (Rees *et al.,* 2018).

2.2.4 Sickle Cell Anaemia

Sickle cell anaemia is a genetic condition that occurs as a result of haemoglobin disorders. Disorders of haemoglobin are genetic blood disorders that are inherited from mutant strains of haemoglobin genes from both parents (Wonkam & Makani, 2019). Nwabulo and others have also asserted that sickle cell disease (SCD) is an autosomal recessive genetic disorder of red blood cells that is transferable from parent carriers an AS father and an AS mother to their offspring. It is a common haemoglobinopathy of public health importance. SCD is predominantly attributed to a single nucleotide mutation that affects the synthesis of glutamic acid.

The aforementioned mutation results in the replacement of glutamic acid with valine at the sixth position of the amino acid sequence in beta-globin. In the de-oxygenated state, the sickle red blood cell becomes sticky and loses the physiological properties of an ideal red blood cell, leading to a cascade of problems that may give rise to sickle cell crises and complications (Nwabuko *et al.,* 2022). Globally, about 300,000 newborns are reported to have acquired severe haemoglobin disorders each year (Asare *et al.,*

2018; Nwabuko *et al.,* 2022). Sub-Saharan Africa contributes about 75% to the global burden of SCD. In Ghana, 2% (about 15,000) of newborns have SCD, with 55% of them having the homozygous form of both parents (Asare *et al.,* 2018) while Nigeria accounts for 100,000-150,000 newborns living with SCD annually (33% of the global burden of SCD) (Wonkam & Makani, 2019). Comparatively, Ghana's SDC burden is higher than Nigeria's in %age terms (Asare *et al.,* 2018; Wonkam & Makani, 2019). Sickle cell anaemia is an inherited lifelong disease, and the disease is reported to be highly prevalent in people of African, South, or Central America, the Caribbean islands, the Mediterranean, India, and Saudi Arabian descent. Clinical symptoms of SCD include acute pain episodes, anaemia, recurrent infections, and chronic end-organ damage (Asare *et al.,* 2018; Soundarya, 2017).

2.2.5 Thalassemia

An inherited blood condition known as thalassemia results in the body producing fewer healthy red blood cells and less haemoglobin. According to Kadhin *et al.* (2017) studies, thalassemia is a group of congenital anaemias that share a common characteristic of insufficient synthesis of one or more subunits of normal human haemoglobin (Hb). Typically, the main problem is related to the number of normal globin chains, with reduced or absent synthesis being the primary defect (Kadhim *et al.,* 2017). Thalassemia is classified into two major types: alpha-thalassemia and beta-thalassemia. The most severe manifestation of alpha-thalassemia is referred to as alpha-thalassemia major or hydrops fetalis. On the other hand, the severe form of beta-thalassemia is known as thalassemia major or Cooley's anaemia. These conditions affect both males and females and are more commonly observed in individuals of Italian, Greek, Middle Eastern, Asian, and African descent *(Kadhim et al.,* 2017; Soundarya, 2017). A study

indicated that red blood cells contain two types of protein chains, namely alpha globin and beta globin, which make up haemoglobin. When there is insufficient production of these protein chains in the body, it leads to improper formation of red blood cells and inadequate oxygen-carrying capacity. The production of these protein chains is regulated by genes. Thalassaemia is inherited from parents to their children through genetic transmission (Taher *et al.,* 2018). Furthermore, a study also commented on the prevalence of thalassemia: it affects males and females equally, and its occurrence is approximately 4.4 per 10,000 live births. The annual global incidence of symptomatic individuals is estimated at 1 per 100,000, while it is 1 per 10,000 people of European descent. However, there is a dearth of precise data regarding carrier rates in numerous populations, particularly in regions of the globe that are recognized or anticipated to experience significant impact. (Kadhim *et al.,* 2017). Thalassemia symptoms are brought on by a deficiency of oxygen in the blood. The degree of the illness affects how severe the symptoms are.

Mild anaemia can occur in people with alpha or beta thalassemia, and mild to severe anaemia can occur in those with intermediate beta-thalassemia (Baird *et al.,* 2022). In addition, they could suffer from bone issues and an enlarged spleen. Sluggish development, and delayed puberty. Severe thalassemia and other serious health issues are present in those who have beta thalassemia major or haemoglobin H illness that is drab and lifeless, sluggish appetite symptoms include jaundice, slow development, delayed puberty, dark urine, enlarged spleen, liver, and heart bone conditions (Helmi *et al.,* 2017). Blood transfusions, iron chelation therapy, and folic acid supplements are the three "standard therapies" used to treat moderate and severe types of thalassemia (Sa *et al.,* 2013).

2.2.6 Aplastic Anaemia

Aplastic anaemia is a blood disorder in which the body's physiological mechanism does not allow the bone marrow to produce enough new blood cells. This condition may result in a number of health-related problems including arrhythmias, cardiomegaly, heart failure, infections, and bleeding. Impairment to the bone marrow's stem cells causes aplastic anaemia (Rees *et al.,* 2018; Soundarya, 2017). A number of acquired diseases, conditions, and factors contribute to the cause of aplastic anaemia, including toxins, such as pesticides, arsenic, and benzene, radiation, and chemotherapy, medicines such as chloramphenicol, infectious diseases such as hepatitis, and Epstein-Barr virus, cytomegalovirus, parvovirus B19, and HIV, autoimmune disorders such as lupus and rheumatoid arthritis (Soundarya, 2017).

Inherited conditions such as Fanconi anaemia, Schwachman-Diamond syndrome, dyskeratosis, and Diamond-Blackfan anaemia may also cause aplastic anaemia (Thomas, 2017). Results reported by Escalante *et al.* (2019) suggested that the most common symptoms of aplastic anaemia are fatigue, breathing weakness, dizziness, headaches, cold hands and feet, soft skin, mouth, and nails, and chest pain. Treatment of aplastic anaemia includes blood transfusion, blood and bone marrow stem cell transplantation, and medications. These therapies can prevent or limit complications, alleviate symptoms, and improve the quality of life. Transplantation of blood and bone stem cells can cure aplastic anaemia disorders.

2.3 Major Causes of Anaemia

2.3.1 Genetic

Anaemia can be brought on by genetic red blood cell diseases that affect the generation, structure, or function of red blood cells. According to Kassebaum and GBD 2013 Anaemia Collaborators (2016), genetic red blood cells disorder such as thalassemia and the thalassemia trait, sickle cell disorders and the sickle cell trait, glucose-6-phosphate deficiency, other haemoglobinopathies and haemolytic anaemias, and Krüppel-like factor 1 variant are responsible for about 11% of anaemia globally (Kulczynska *et al.,*2019). Even though all populations have inherited red blood cell abnormalities, the frequency of anaemia varies substantially within and between various countries, even across small geographical distances (Kassebaum *et al.* 2014; Williams and Weatherall 2012).

Populations in or originating from Africa, the Middle East, and Asia have the greatest incidences. Sickle cell disease, haemolytic anaemias, and G6PD deficiency all enhance the destruction of red blood cells through various mechanisms, whereas thalassemias result in the production of red blood cells that are inefficient and have a shorter lifespan (Unissa *et al.,* 2018). Genetic red blood cell abnormalities are immutable risk factors for anaemia, yet thalassemia prevention and treatment are relatively well-developed in many Asian nations (Fucharoen and Weatherall 2016). Although the knowledge and resources for managing hereditary red blood cell abnormalities are severely lacking in many nations, collaborations are being formed to enhance management and therapy (Fucharoen and Weatherall 2016).

2.3.2 Environmental

Exposure to air pollutants, such as NO_2 and $PM_{2.5}$ (fine particulate matter) is one possible factor in the development of anaemia. Systemic inflammation has been demonstrated to rise in response to exposure to $PM_{2.5}$ and NO_2 (Cliff *et al.,* 2016; Honda *et al.,* 2017) and affect bone marrow stimulation, particularly in those individuals who have conditions associated with chronic inflammation, such as diabetes or obesity (Thomson *et al.,* 2016). Many of these studies have reported on the short-term changes in circulating inflammatory marker levels, but new research suggests that exposure to air pollutants over an extended period may also result in chronic and self-perpetuating systemic inflammation (Honda *et al.,* 2017). These results suggest that air pollution, through its effects on systemic inflammation, may cause a cascade of events including downregulation of erythropoietin production, exacerbation of hematopoietic precursors' refractoriness to endogenous erythropoietin, and chronic and sustained upregulation of hepcidin, an iron regulatory protein, each of which can result in decreased haemoglobin and anaemia.

Similar trends have been reported by Das & Chatterjee (2014) in their work on the assessment of the haematological profiles of adult male athletes from two different air pollutant zones in West Bengal. They indicated that an individual's reaction to air pollutants depends on the type of pollutant to which an individual is exposed, the degree of exposure, and the concentration of the chemicals (Das & Chatterjee, 2014). Prolonged exposure to air pollution at low concentrations may induce anaemia in people (Elbarbary *et al.,* 2020). The study further indicated that many components of airborne pollutants have the propensity to reach the blood rapidly without previously being bio-transformed, moreover, the hematopoietic system is extremely vulnerable to

air pollutants since its cells undergo constant restructuring. Anaemia may result from red blood cell destruction from toxic airborne pollutants (Elbarbary *et al.,* 2020). The biological processes through which air pollution may limit the formation of red blood cells are evident in the synthesis of heme, the formation of red blood cells, and their lifespan (Das & Chatterjee, 2014; Honda *et al.,* 2017).

2.3.3 Physiological Causes of Anaemia

2.3.3.1 Heavy Menstrual Bleeding (HMB)

Studies have reported that there is a relationship between heavy menstrual bleeding and iron deficiency anaemia (Munro *et al.,* 2023; Munro, 2023). Heavy menstrual bleeding is a contributing factor to significant blood loss during the menstrual period, which in turn can cause the body's levels of iron to be depleted as indicated by Munro *et al.,* 2023. Iron is necessary to produce red blood cells, and when these levels are below the normal range, it can result in an individual being diagnosed with iron deficiency anaemia (Sriprasert *et al.,* 2017). In a study by Kocaoz *et al*. (2019), they objectively defined heavy menstrual bleeding as the loss of 80 ml or more blood during every menstrual cycle. In contrast, Sripraset *et al.* (2017) have reported that the National Institute for Health and Care Excellence in the United Kingdom has given a more refined definition, which defines HMB as excessive menstrual blood loss that physically, emotionally, socially, and financially affects the quality of life of women and can be seen by itself or with other accompanying symptoms (Fraser *et al.,* 2015).

2.3.3.2 Hereditary Red Blood Cell Enzymopathies

Hereditary non-spherocytic hemolytic anaemia (HNSHA) is a group of rare disorders that affect the red blood cells. These disorders are caused by mutations in genes that

encode enzymes involved in the metabolism of red blood cells. The enzymes help to maintain the integrity and function of the red blood cells, and their deficiency leads to premature destruction of the cells (Koralkova *et al.,* 2014). This results in anaemia, which is a condition of low red blood cell count or haemoglobin level. In comparison to other hereditary conditions affecting red blood cells, such as membrane disorders or haemoglobinopathies, the shape of the red blood cell does not exhibit any distinct abnormalities. The diagnosis relies on detecting reduced activity of specific enzymes and characterizing the abnormality at the DNA level. Deficiencies in glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) are the most frequent enzyme diseases. Hereditary non-spherocytic hemolytic anaemia can also be caused by a number of different enzyme abnormalities, albeit these are often far less well-publicized.

Some examples of these rare enzyme conditions are deficiencies of hexokinase, phosphofructokinase, triosephosphate isomerase, aldolase A, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), diphosphoglycerate mutase (BPGM), enolase (ENO), pyrimidine 5'-nucleotidase (P5N), adenylate kinase (AK), and glutathione reductase (GR). The clinical manifestations, inheritance patterns, and treatment options may vary depending on the type and severity of the enzyme defect. Some patients may have mild or asymptomatic anaemia, while others may experience severe hemolysis, jaundice, gallstones, splenomegaly, and chronic transfusion dependency (Koralkova *et al.,* 2014).

2.3.4 Nutritional Causes of Anaemia

The deficiency of nutrients and insufficient bioavailability of hemopoietic nutrients for a sufficient Hb concentration as well as for erythrocyte synthesis can result in nutritional anaemia. The bioavailability of hemopoietic nutrients such as iron, vitamin B_{12}, folic acid, and ascorbic acid gets affected in contact with heat and light (Andlid *et al.,* 2018). The bioavailability of elemental non-haem iron can be reduced by a number of different variables, including polyphenols, cinnamon, the phytates in whole grains and legumes, and calcium. One of the main causes of anaemia is nutritional deficiency, which means the inability of the body to get the required nutrients in a diet. Some of the commonly known nutrients that are needed for the production and function of red blood cells are iron, vitamin B12, and folate (Shubham *et al.,* 2020).Iron deficiency anaemia is the most common type of anaemia worldwide. Iron is an essential component of haemoglobin, the protein that carries oxygen in the blood. When there is not enough iron in the body, haemoglobin production is reduced and the red blood cells become small and pale.

Iron deficiency can result from inadequate intake of iron-rich foods, such as meat, eggs, and leafy green vegetables, or increased loss of iron due to bleeding, menstruation, pregnancy, or parasitic infections (Al-Naseem *et al.,* 2021; Balk *et al.,* 2019). Vitamin B_{12} deficiency anaemia is also known as pernicious anaemia. Vitamin B_{12} is a cofactor for several enzymes that are involved in the synthesis of DNA and the maturation of red blood cells. Vitamin B_{12} is primarily found in animal products, such as meat, eggs, and dairy products. Therefore, vegans and vegetarians are at risk of developing this type of anaemia if they do not supplement their diet with vitamin B_{12} or consume fortified foods. Vitamin B_{12} deficiency can also occur due to malabsorption of the vitamin in the

digestive tract, which can be caused by diseases such as celiac disease, Crohn's disease, or atrophic gastritis (Sharourou *et al.,* 2018). Folate deficiency anaemia has similarities to vitamin B_{12} deficiency anaemia in relation to its impact on the production of red blood cells and synthesis of DNA. Folate is another B vitamin that is essential for the formation and growth of red blood cells. Folate is found in various plant foods, such as fruits, vegetables, legumes, and nuts. Folate deficiency can result from inadequate intake of these foods, or increased demand for folate due to pregnancy, lactation, or rapid growth. Folate deficiency can also be caused by certain medications that interfere with folate metabolisms, such as methotrexate, sulfasalazine, or phenytoin (Kumar *et al.,* 2022; Santoyo-sánchez, 2016).

However, Shubham *et al.* (2020) shares the view that nutritional causes of anaemia can be prevented and treated by consuming a balanced diet that includes foods rich in iron, vitamin B_{12}, and folate, or by taking supplements as prescribed by a physician. Nutritional anaemia can have serious consequences on health and quality of life if left untreated. Therefore, it is important to recognize the signs and symptoms of anaemia and seek medical attention if they occur (Shubham *et al.,* 2020).

2.3.5 Anaemia Caused by Chronic Diseases

De las Cuevas Allende *and* colleagues*,* hold the view that anaemia of chronic disease (ACD) or anaemia of inflammation develops during an inflammatory process, which triggers the immune system's activation, the release of cytokines, and an increase in hepcidin. They further explained that this process causes a drop in plasma iron levels and the suppression of erythropoiesis, which leads to anaemia of chronic disease (ACD) or anaemia of inflammation (de las Cuevas Allende *et al.,* 2021). This form of anaemia

is often characterized as either normocytic, normochromic, or hypo-proliferative, with a haemoglobin level between 8 and 12 g/dL. ACD is widely recognized as the second most widespread anaemia, following iron deficiency anaemia, and it is particularly prevalent among the elderly population. Approximately one-third of the elderly population who exhibit symptoms of anaemia are diagnosed with anaemia of chronic disease (ACD). According to Weiss *et al.* (2019), this type of anaemia is commonly observed in hospitalized patients and individuals with chronic conditions. The global prevalence of anaemia is believed to be as high as 40%, with a significant proportion attributed to Anaemia of Chronic Disease (ACD) either as a standalone condition or in conjunction with other forms of anaemia. The role of ACD in this overall prevalence is of considerable significance (de las Cuevas Allende *et al.,* 2021). Rheumatoid arthritis, systemic lupus erythematosus, vasculitis, sarcoidosis, inflammatory bowel disease, neoplastic diseases, chronic kidney disease (CKD), acute or chronic bacterial infections, fungal, viral, and parasitic diseases, as well as chronic rejection of organ transplants, respiratory failure, heart failure, obesity, and other chronic processes, are all potential generators of an ACD.(de las Cuevas Allende *et al.,* 2021; Weiss *et al.,* 2019). Elevated hepcidin levels in infections represent a host defense mechanism against infections because it limits the availability of iron to the microorganisms (Ganz, 2019).

2.4.1 Regional Prevalence of Anaemia among Children Aged 6 to 59 Months

According to a study conducted by Stevens *et al.* (2022), it was found that in the year 2019, a significant proportion of children between the ages of 6 and 59 months worldwide experienced varying degrees of anaemia. Specifically, 21% of children were identified as having mild anaemia, 18% were classified as having moderate anaemia,

and 1% were diagnosed with severe anaemia. The occurrence of anaemia within this particular group exhibited regional disparities, with West and Central Africa demonstrating the highest incidence, whereas high-income countries consistently exhibited the lowest prevalence throughout the duration of the investigation. Regions characterized by a greater frequency of anaemia in children aged 6–59 months also exhibited a higher proportion of individuals with anaemia who experienced moderate or severe anaemia (Stevens *et al.,* 2012, 2022).

Furthermore, it was noted that in the year 2000, the occurrence of anaemia among children aged 6-59 months surpassed 80% in nine nations located in West and Central Africa as well as Yemen. Additionally, the prevalence of anaemia exceeded 70% in 19 more countries situated in East, West, and Central Africa. Upon further examination of the existing data, it was shown that in the year 2019, no nation exhibited an anaemia prevalence surpassing 80% among children aged 6-59 months. However, the prevalence of anaemia did exceed 70% in children within the same age group in eleven nations, notably Yemen and 10 countries located in west and central Africa. On the opposite end of the spectrum, the prevalence of anaemia in the United States of America was determined to be approximately 6% between the years 2000 and 2019. In addition, it should be noted that other high-income nations similarly exhibited a low prevalence of anaemia. However, it is important to acknowledge that the availability of data pertaining to anaemia in these countries was constrained (Stevens *et al.,* 2012, 2022).

The findings of the research conducted by Steven *et al.* (2012;2022) demonstrate that there has been a global decrease in the incidence of anaemia among children aged 6 to 59 months between 2000 and 2019. This fall was observed in most regions, with the

prevalence decreasing from 48% to 40%. The regions that experienced the most significant declines in anaemia prevalence were Latin America and the Caribbean, East and Southeast Asia, Central Asia, the Middle East, North Africa, East Africa, and south Asia. These regions observed relative reductions of 12-20% each decade. The prevalence of anaemia among children aged 6 to 59 months in the year 2000 varied across different regions. In Latin America and the Caribbean, as well as in East and Southeast Asia, the prevalence was less than 35%. On the other hand, in South Asia and East Africa, the prevalence exceeded 65%. These findings indicate that reductions in anaemia were observed throughout regions, despite the initial prevalence rates varying significantly (Kassebaum & GBD 2013 Anaemia Collaborators, 2016; Stevens *et al.,* 2022).

Several countries in different regions have had significant reductions in the prevalence of anaemia among children aged 6 to 59 months. These countries include Guatemala, the Philippines, Uzbekistan, Brazil, Kazakhstan, Panama, and Azerbaijan. The prevalence of anaemia decreased in a total of 141 nations, while the remaining 56 countries were projected to potentially experience an increase in prevalence. On a global scale, the prevalence of moderate and severe anaemia exhibited a more pronounced fall compared to overall anaemia, with relative reductions of 17% per decade for moderate anaemia and 37% per decade for severe anaemia. The prevalence of mild anaemia in children aged 6-59 months at a worldwide level exhibited stability, with regional and country-specific trends showing minor increases or decreases. In the regions of East, West, and Central Africa, as well as South Asia, there has been a potential rise in the prevalence of mild anaemia, but the occurrence of moderate and severe anaemia appears to have declined (Pasricha, 2014; Stevens *et al.,* 2022).

2.4.2 Regional Prevalence of Anaemia among Non-Pregnant Women and Pregnant Women Aged 15 - 45 Year

In the year 2019, on a global scale, around 30 % of women aged 15 - 49 who were not pregnant, and 36 % of pregnant women aged 15 - 49 were found to be affected by anaemia. These figures indicate a slight reduction in anaemia prevalence among pregnant women aged 15 -49 since the year 2000, while the rates among non-pregnant women aged 15 - 49 have remained relatively stable. In the year 2019, it was observed that 30% of women aged 15-49 years who were not pregnant, and 36% of women aged 15-49 years who were pregnant, experienced anaemia in the United States. As a result of the rise in the world's population, the number of women between the ages of 15 and 49 who suffer from anaemia rose from 493 million in the year 2000 to 571 million in 2019 (Abbafati *et al.,* 2020; Kassebaum & GBD 2013, Anaemia Collaborators, 2016; Stevens *et al.,* 2022). The patterns and trends in anaemia by severity in all women aged 15 - 49 years followed the same pattern as in non-pregnant women aged 15 - 49 years. This pattern was observed in both pregnant and non-pregnant women aged 15 - 49 years.

The occurrence of moderate and severe anaemia among women aged 15 - 49 years who are pregnant has shown significant decreases, whereas the incidence of severe anaemia among non-pregnant women aged 15-49 years has also witnessed the most substantial decrease in relative terms (Baldi *et al.,* 2022). Trends in mild anaemia were stable in some regions, slightly increased in other regions, and declined in other regions, with little change in the incidence of mild anaemia occurring globally as reported by the World Health Organization (WHO) (WHO, 2017; Stevens *et al.,* 2022)**.** More than half

of women aged 15 - 49 experienced anaemia in 2000 in Yemen, India, Cambodia, Haiti, and 20 nations in West and Central Africa, according to the study's evaluation.

The prevalence was less than 50% in most of the world in 2019, but it was more than half in ten countries: Yemen, India, the Maldives, and seven nations in west and central Africa (in descending order: Mali, Benin, Nigeria, Senegal, Burkina Faso, Gabon, and Côte d'Ivoire) (WHO, 2017; Stevens *et al.,* 2022). Anaemia was more common in pregnant women aged 15 - 49 years than in non-pregnant women aged 15 - 49 years in most countries and in most years (171 of 197 countries in 2019), but regional patterns in anaemia prevalence were similar (Costa & de Paula Ayre-Silva, 2023). This was the case despite the fact that the occurrence of anaemia was higher in pregnant women aged 15 - 49 years than in non-pregnant women aged 15 - 49 years (Stevens *et al.,* 2022). In a few nations, the prevalence of anaemia in non-pregnant women between the ages of 15 and 49 was higher than the prevalence of anaemia in pregnant women between the same ages. These countries included those with the highest overall anaemia prevalence. In 2019, the absolute difference in total anaemia prevalence between pregnant and non-pregnant women aged 15-49 years old was 3 % or higher in Yemen, India, the Maldives, Jordan, and Afghanistan. This was the case in all five of these countries. Furthermore, Steve *et al.* (2022) have emphasized that when examining the prevalence of anaemia among women aged 15 - 49 years in different regions, similar or slightly smaller trends were observed for children aged 6-59 months, with the exception of South Asia. Specifically, anaemia in children aged 6-59 months decreased by 12 % per decade, whereas anaemia in women aged 15 - 49 years decreased by 2 % per decade. It is worth noting that in both 2000 and 2019, the highest prevalence of anaemia was observed in West and Central Africa and South Asia, while the lowest prevalence was found in high-

income countries. Additionally, there were insignificant changes in anaemia prevalence across all three regions.

In contrast, the occurrence of anaemia shown a decline of 2 % per decade in Latin America and the Caribbean, 12 % per decade in East and Southeast Asia, and 12 % per decade in East Africa. When examining the years 2010-2019 in contrast to the period from 2000-2009, it is possible to observe a comparatively decelerated pace of progress in specific areas both at a regional level and on a global scale Stevens *et al.,* 2012, 2022).

2.5 Trends of Anaemia among Risk Groups in Ghana

In spite of the efforts of several different programs, the rate of anaemia in Ghana is only slowly declining. According to the National Survey conducted in 2015, a study conducted by Abu *et al*. (2021) found that anaemia affects 66 % of children in Ghana who are between the ages of 6 and 59 months. According to the findings of the survey, infections and iron deficiency caused by insufficient dietary iron intake are the primary contributors to nutritional anaemia in Ghana (Abu *et al.,* 2021). This is consistent even though several factors might induce anaemia. According to the most recent statistics conducted in 2018, 35.6% of preschoolers suffered from anaemia, 21.5% were iron-deficient, and 12.2% had iron deficiency anaemia. In Ghana, helminthic infections, which are caused by parasitic worms, are responsible for 45 % of cases of anaemia and affect 26.7 % of children (Buttner *et al.,* 2020). Maternal anaemia is a risk factor for anaemia in children aged 6 to 59 months in Malawi Mozambique, Namibia, Zimbabwe (Ntenda *et al.,* 2018), and Ghana (Egbi *et al.,* 2022), according to different research by Steven *et al.* on global and regional analyses of anaemia prevalence(Stevens *et al.,*

2022). Between 2008 and 2014, the frequency of anaemia among children was much greater in low-income homes, rural households, and children of less educated mothers, according to a survey done by the Ghana Health Services on Ghana's landscape analysis of anaemia and anaemia programming.

According to the data, while the frequency of anaemia among children somewhat increased between 2003 and 2008 (from 76 to 78 %), it marginally decreased to 66 % in 2014. However, it should be noted that less fortunate groups tended to gain disproportionately from the decline. Between 2008 and 2014, anaemia prevalence among children from the highest and lowest wealth quintiles decreased by 23 and 9 %, respectively; in 2008, the highest quintile's anaemia prevalence was 26 %age points lower than that of children from the lowest quintile (Health Service, 2016). A study by Egbi *et al.* (2022) on anaemia prevalence and associated factors among school-age children in Accra and Kumasi metropolis in Ghana reported a 20.4% prevalence of anaemia which denotes anaemia as a moderate public health problem in the area where the research was conducted according to the WHO classification of anaemia as a public health concern. Thus, this makes the anaemia situation of schoolchildren in urban settings in Ghana critical for any national policy on anaemia control among schoolchildren. Interestingly, the study found that school-aged children in Greater Accra were more likely to be anemic compared to those in Greater Kumasi. When compared to Accra, the city of Kumasi is situated in a forest belt, which means that meat, fruits, and vegetables are easily accessible and relatively inexpensive there (Egbi *et al.,* 2022). As a result, residents of Kumasi may consume a diet that is richer in iron, vitamin A, pro-vitamin A (beta-carotene), folic acid, vitamin C, and zinc, which helps to reduce the incidence of nutritional anaemia. Accra, on the other hand, is a coastal

urban setting that features higher prices for meat, fruits, and vegetables. This makes it difficult for school-aged children from low-income homes to afford these food items (Egbi *et al.,* 2022). The susceptibility to anaemia among women in the reproductive age group exhibits a distinct pattern.

According to a study conducted on the landscape analysis of anaemia in Ghana, there was a notable decline in the overall prevalence of anaemia among women of reproductive age. Specifically, the prevalence abridged from 59 % in 2008 to 42 % in 2014. (Ghana Health Service, 2016). Significantly, the decrease was observed in both urban and rural regions, as well as among various demographic groupings based on geography and wealth. There was a comparable decline in proportion for women belonging to both the top and lowest wealth quintiles, with reductions of 30 % and 28 %, respectively. The prevalence rates exhibited a consistent fall across all regions, with reductions ranging from 16 to 47 %. From 2008 to 2014, there was a substantial decrease in the prevalence of anaemia among pregnant women, amounting to a reduction of 36 %. Additionally, a significant reduction of 27 % was observed among breastfeeding and non-pregnant women. The progress achieved in reducing anaemia can be attributed to the adherence to the implementation of iron supplements provided to pregnant women and adolescent girls during their menstrual age (Ghana Health Service, 2016). Even though national surveys have reported a drop in anaemia prevalence among pregnant women does that correspond with other studies, another study has also reported that iron deficiency anaemia among Ghanaian pregnant women found that the pervasiveness of iron deficiency anaemia increased as pregnancy progresses to the 3rd trimester (Pobee *et al.,* 2021). The observed rates of anaemia prevalence among pregnant women based on the gestation period were reported to be

16%, 20%, and 38% in the 1st, 2nd, and 3rd trimesters, respectively (Pobee *et al.,* 2021). Similarly, a study by Mockenhaupt et al. (2000) has also reported an incidence rate of iron deficiency in pregnant women in Ghana ranging from 5 - 46%. Also, another study documented anaemia prevalence of 11% for women at 24 weeks and 20% for women at 36 weeks of pregnancy, the total rate was reported to be 16% during pregnancy (Engmann *et al.,* 2012)**.**

2.6 Origin, Geographical Distribution, and Botanical Description of *Moringa oleifera*

Moringa is the generic name for species of medicinal plants that are significant in both ethnobotanical and pharmacological contexts. Since the beginning of time, it has been utilized in many traditional medical practices to treat a variety of ailments. There are 13 different species in this genus, all of which have been found widespread (Rani *et al.,* 2018).

The study further indicated that the optimum temperature for growing is between 18 and 28 ◦C, and it grows in any soil type, waterlogged and with heavy clay (pH between 4.5 and 8), at an altitude of up to 2000 m. *M. oleifera* plant possesses culinary properties, as all of its parts such as leaves, roots, fruits, flowers, and nutritious pods are used as food. Consequently, this plant has gained significant popularity and is extensively utilized in numerous places worldwide. (Azlan *et al.,* 2022).

It has been given the names "nature's gift" and "wonder tree" as a result of the extensive research that has been done on its potential and properties. There are a total of 13 species in this family, and they may be found widely distributed across the entire India.

M. oleifera is the most well-known species of moringa due to the abundance of nutrients it contains and the myriad of positive effects it has on one's health. This is mostly attributable to the Ayurvedic tradition that it is associated with (Ifeyinwa *et al.,* 2017). According to a botanical description of the *M. oleifera* plant, the tree can typically grow to a height of 10 - 15 meters, with an approximate diameter of 45 centimeters and an abnormal-looking bole that is forked from the roots.

In addition, the tree has been known to produce fruit that has a high oil content. The bark of the trees has a smooth, even texture and a good hue. The shading is subdued, and the color is a light yellow. The crown is frequently characterized as having the shape of an umbrella, with an opening that is stretched out, and branches and shoots that are short and bristly. The softwood has compound leaves that can reach a maximum length of 90 centimeters and is arranged in alternate and opposing pinnae in inverse sets beginning 5 centimeters above the stalk (Udikala *et al.,* 2017). In addition to this, the *M. oleifera* flower exudes an enticing smell and possesses five asymmetrical petals that are somewhat longer than the sepals (Adusei *et al.,* 2022).

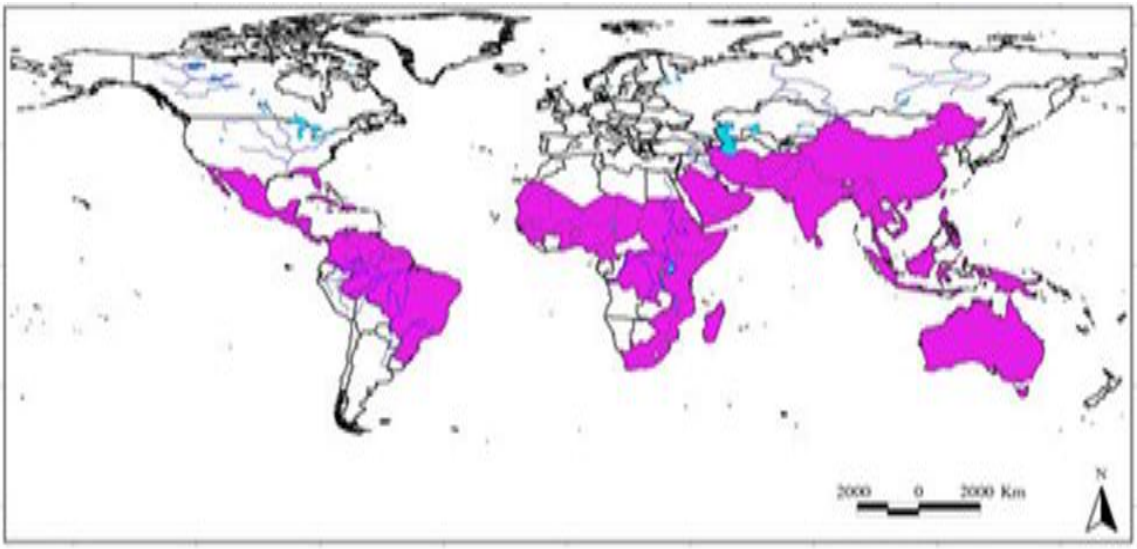

Plate 2.1: Distribution of *M. oleifera* in countries where it has been reported as native or naturalized. The image was adapted from Navie and Csurhes (2010)

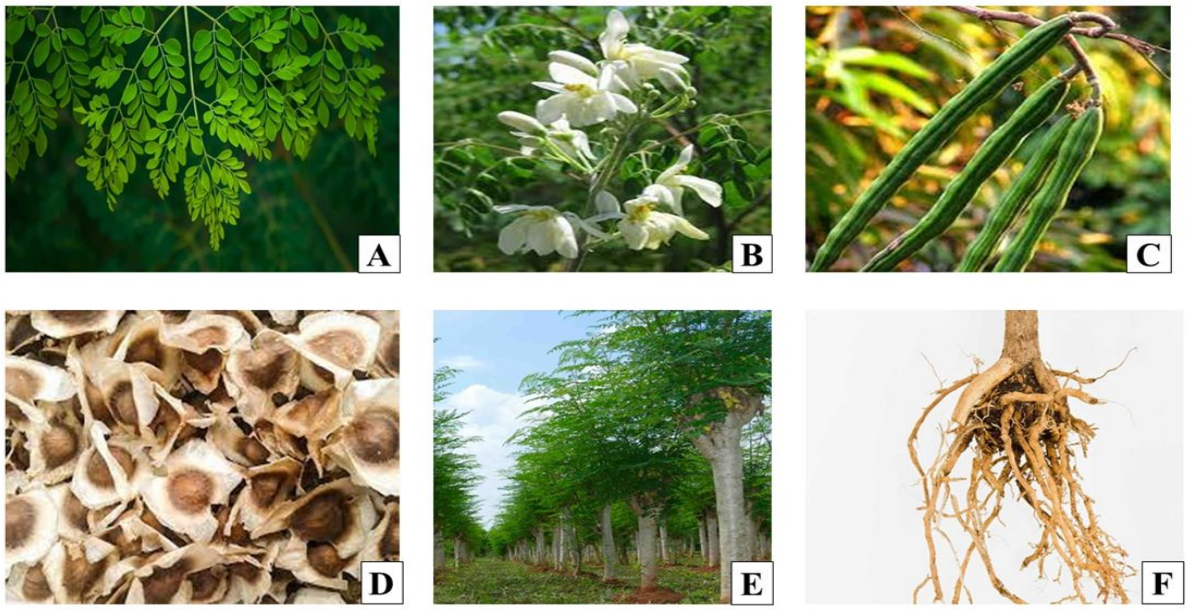

Plate 2.2: Parts of *M. oleifera*; tree, Leaves (A); Flowers (B); Fruit (C); Seed (D); Whole *M. oleifera* tree (E); Roots (F) Source: https://www.theplantlist.org

2.7 Absorption, Metabolism and Excretion of *Moringa oleifera*

M. oleifera is widely recognized as an astounding tree sometimes referred to as the "tree of life" due to its significant nutritional advantages. Hence, the investigation of the plant's bioavailability has been examined as a pivotal determinant in harnessing its nutritional advantages. Despite having a worthy content of iron, several studies have found that Moringa oleifera plants have low iron bioavailability (Gallaher *et al.,* 2017; Idohou-Dossou *et al.,* 2011). The presence of a significant amount of polyphenols in *M. oleifera* plant can potentially inhibit the absorption of iron due to the formation of polyphenol-iron complexes that are not easily absorbed by the body. Moreover, a scholarly investigation has examined the correlation between polyphenol compounds and the formation of inhibitory complexes, which afterwards hinder the absorption of iron in the human body (Azlan *et al.,* 2022b; Idohou-Dossou *et al.,* 2011). In addition, another study has suggested that the low iron bioavailability is caused by the presence of high phytic acid content in the *M. oleifera* sample, and the removal of phytic acid during its processing may improve the bioavailability (Gallaher *et al.,* 2017).

Similar to the iron bioavailability, dried *M. oleifera* leaves contain an abundant calcium content, but the existence of a substantial amount of oxalic acid in the leaves has caused interference in calcium absorption (Azlan *et al.,* 2022). In a comparative study, the researchers examined the effects of a calcium-rich meal derived from *M. oleifera* leaves and a milk diet on experimental rats. The results indicated that the milk diet exhibited superior absorption and calcium retention in comparison to the *M. oleifera* diet. Although *M. oleifera* leaves possess equivalent levels of calcium, the presence of oxalate in these leaves has been observed, potentially indicating a plausible explanation for the decreased bioavailability of calcium. Nevertheless, a significant proportion of

calcium, up to 73%, derived from the *M. oleifera* diet was observed to be absorbed, and around 59% of this absorbed calcium was maintained inside the body. These findings suggest that the *M.oleifera* diet could serve as a suitable substitute for milk in situations when access to milk is limited (Allen *et al.,* 2014; Azlan *et al.,* 2022). Akter *et al.* (2020), have reported that the conflicting bioavailability effects caused by the presence of phytic and oxalic acid in dietary consumption have paradoxically labeled them as anti-nutritional factors. Moreover, vitamins A and B are among the significant reported nutrients of *M. oleifera* leaves and one of the most abundant natural sources of β-carotene and provitamin A carotenoid (Lynos *et al.,* 2015). *In vivo* and *in vitro* studies found that the natural vitamin sources of *M. oleifera* are highly bioavailable (Nambiar & Seshadri, 2001; Pullakhandam & Failla 2007 cited by Azlan *et al.,* 2022). The in vivo investigation revealed that the rats fed with *M. oleifera* exhibited considerable increases in food intake and weight gain. Additionally, the levels of vitamin A detected in these rats were much greater compared to the control group. These findings suggest that the rats that were provided with the *M. oleifera* diet exhibited favorable assimilation of essential nutrients.

In addition, *M. oleifera* possesses nutritional advantages, since it contains all the important amino acids required for the synthesis of proteins essential for body nourishment. A randomized controlled trial conducted in Senegal examined the effects of consuming *M. oleifera* on breastfeeding women. The study revealed that lactating women who followed the *M. oleifera* diet experienced no significant changes in their body mass index (BMI) when compared to the control group. The findings of this study indicate that the group fed with *M. oleifera* exhibited a decreased rate of weight loss, the amount of digestible protein in the powder could suggest that the consumption of

Moringa was beneficial by preventing weight loss (Idohou-Dossou *et al.,* 2011). According to the findings of other studies, most of the amino acids and proteins present in *M. oleifera* are very easy to digest, which directly correlates to the plant's bioavailability (Alain Mune Mune *et al.,* 2016; Thurber & Fahey, 2009).

2.8 Phytochemicals in *Moringa oleifera*

Chemical compounds that originate from plants are referred to as phytochemicals. They are compounds found in plants that do not contribute to the plant's nutrition but have protective or disease-preventing effects (Adusei *et al.,* 2022). These constituents have been shown to have an effect on human health in addition to contributing to the taste, texture, odor, and color of plants. *M. oleifera* is rich in a combination of phytochemicals such as terpenes, quercetin, β-sitosterol, caffeoylquinic acid, kaempferol, kaempferitrin, isoquercitrin, rhamnetin, rhamnose, and a fairly unique group of compounds called the glucosinolates and isothiocyanates. Different parts of the *M. oleifera* contain numerous phytochemical compounds (Amaglo *et al.,* 2010). Coppin *et al.* (2013) reported that the stem bark contains two alkaloids, moringine, and moringinine, with vanillin, β-sitosterol, 4-hydroxymellin, and octacosanoic acid identified in the stem of the plan.

Also, the flowers of *M. oleifera* have been established to contain quercetin and kaempferol. Studies have also shown the presence of polyphenols, niazimicin, benzyl isothiocyanate, 3-caffeoylquinic, and 5-caffeoylquinic acid in the leaves of *M. oleifera* (Muhammad *et al.,* 2016). A study by Kasolo *et al*. (2010) identified the presence of protective phytochemicals, including gallic tannins, catechol tannins, steroids and

triterpenoids, saponins, anthraquinones, alkaloids, and reducing sugars in ether, ethanol, and aqueous extracts of the roots.

A study by Rani *et al.* (2018) also reported that moringa species contain various Phytochemical constituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The diversity of these phytochemicals in the genus contributes to its numerous pharmacological uses. Azlan *et al. (*2022) have indicated that *M. oleifera* has been reported to possess numerous phytochemical constituents that are beneficial and significant medically, which are mostly found on the leaves and seeds. Numerous vitamins, minerals, and other bioactive components, including potassium, calcium, phosphorus, iron, protein, vitamins, carotenoids, polyphenols, isothiocyanates, and tannins, have been identified in the leaves. Hematinic and hypotensive effects, anti-cancer, antioxidant, antibacterial, anti-ulcer, and anti-inflammatory characteristics, renal and hepatoprotection; these and other medical benefits are all attributable to these bioactive substances (Azlan *et al.,* 2022; Rani *et al.,* 2018).

2.9 Effect of *Moringa oleifera* on Body Weight

Many studies have reported on the anti-obesity effect of *M. oleifera*. However, limited research has also indicated that *M. oleifera* may have the capacity to enhance the body mass of individuals suffering from anaemia. Importantly, *M. oleifera* exhibits significant pharmacological properties in terms of its potential as an anti-obesity agent. Various investigations encompassing in-vitro, in-vivo, and clinical studies have been undertaken to investigate the anti-obesity properties of Moringa oleifera extracts or isolated compounds derived from *M. oleifera*. Compounds such as quercetin, iso-

quercetin, quercetin-3-O-malonylglucoside, and astragalin have been identified in *M. oleifera* extracts showing anti-obesity activity. One noteworthy mechanism by which *M. oleifera* reduces obesity is through the modification of an individual's lipid profile. This is typically characterized by a decrease in levels of total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein, as well as an increase in levels of high-density lipoprotein cholesterol (Ali Redha *et al.,* 2021). According to a study, male C57BL/6J mice were subjected to a High Fat Diet (HFD) supplemented with 0.1% *M. oleifera* leaf powder for a duration of 7 weeks. The results indicated a decrease in the elevated levels of total cholesterol, triglycerides, and low-density lipoprotein resulting from the HFD. Additionally, the inclusion of *M. oleifera* leaf powder prevented the development of hypercholesterolemia and the accumulation of fat in the mice (Kim & Kim, 2019).

Furthermore, a separate investigation conducted by Xie *et al.* (2018) examined the impact of petroleum ether and *M. oleifera* extract on the lipid profile of mice who were fed with a high-fat diet (HFD). The lipid profile of male C57BL/6J mice that were fed with a high-fat diet (HDF) was evaluated after administration of *M. oleifera* extract at doses of 0.125, 0.25, or 0.5 g/kg. The administration of *M. oleifera* extract resulted in significant reductions in body weight, relative weights of epididymal, perirenal, and mesenteric fat, as well as reductions in fat tissue size. Additionally, hepatic fat buildup and levels of total cholesterol, low-density lipoprotein, and aspartate aminotransferase (AST) were also decreased in the animals subjected to the extract (Xie *et al.,* 2018). The findings from this study firms that *M. oleifera* may be effective in the management of obesity and provide renal and liver protection.

Whiles Kim & Kim (2019); Xie et al.(2018) found that administration of *M oleifera* extract helps to reduce basal body weight and the levels of total cholesterol, low-density lipoprotein, triglycerides, Aya *et al*.(2015) who studied the effects of *Moringa oleifera* leaves on the body weight and haematological parameters of rabbit infected with *Trypanosoma congolence*, have also indicated that *M. oleifera* leaves have a potential for use in diets to increase the weight of rabbits without any deleterious effects on haematological parameters. This conclusion was reached as a result of the findings of their study. This contradicts the findings of Kim & Kim (2019); Xie et al. (2018).

2.10 Effect of *Moringa oleifera* on Feed Intake

Ifeyinwa *et al*. (2017) who researched on the effect of moringa leaf powder supplement on some biochemical indices of rats reported that high feed intake of a moringa-based diet indicated that the taste of the Moringa leaf supplement was not objectionable to the animals. The significantly higher increase in the body weight of rats fed moringa-supplemented diets might be attributed partly to higher feed intake and also to the fact that Moringa oleifera is rich in amino acids, vitamins, and minerals, particularly iron, and phytochemicals. Furthermore, Ifeyinwa *et al.* (2017) reported that moringa leaf powder contained higher amounts of all the essential amino acids than the amino acid pattern of the FAO reference protein and comparable to those in soybeans. These phytochemicals and nutrients promote health and growth. In a study using rats, Nambiar & Seshadri (2001) as cited by Ifeyinwa *et al.* (2017) observed a remarkable feed intake and weight gain in vitamin A deficient rats fed with dehydrated moringa leaves compared to synthetic vitamin A.

2.11 Effect of *Moringa oleifera* on Haematological Parameters

Blood studies are significant in the evaluation of the clinical and physiological condition of both animals and humans. According to research conducted by Oyedemi, Bradley, and Afolayan (2010), the assessment of haematological variables offers significant understanding into the potential adverse impacts of phytochemicals on the blood composition of different organisms. It is imperative to comprehend the implications of these chemicals on general health. Furthermore, according to Unung *et al.,* (2019), blood investigations are utilized to reveal the physiological and functional adaptations of organisms in response to their surrounding environmental circumstances.

The monitoring of haematological parameters serves as a valuable tool in detecting variations from established norms, which may signify underlying health conditions or reactions to external stimuli. Consequently, this practice plays a crucial role in facilitating prompt diagnosis and intervention.

Various studies have been carried out to ascertain the hematinic potential of moringa on blood parameters. In this section, a highlight is thrown on the effect of *M. oleifera* on haematological indices as documented by literature. In a study by Ye *et al*. (2019), on the effect of graded levels of *M. oleifera* leaf-meal in albino rat diet on some haematological parameters found that administration of *M. oleifera* meal containing 37.5 mg, 56 mg, and 75 mg of moringa to groups B, group C, and group D respectively for 21 days, showed significant differences in haemoglobin count, red blood cells, and packed cell volume in the group that was administered with 37.5 mg while there was a significant difference in lymphocyte count of animals in group C (56 mg) and D (75 mg), although all the groups were within normal range while no significant difference

was shown in the white blood cell count when compared to the control group. The conclusion drawn from the study was that minimal absorption of ≤ 3.75 mg of *M. oleifera* resulted in better haematinic effect, this was indicated by its effects on RBC, PCV, haemoglobin, and lymphocyte of the animals in the group that was administered with 37.5mg.

Adias *et al.* (2013), carried out a similar study in Madonna University Elele Campus Rivers State. The study explored the effects of *M. oleifera* leaf extract on haematological parameters of phenylhydrazine anaemia induced Wistar rats. A control group and two treatment groups were given 200 and 300mg per body weight of *M. oleifera* leaf extract respectively for a period of 28 days. Results from the study showed that there was a significant ($P < 0.05$) increase in red blood cell count, haemoglobin count, packed cell volume, and white blood cell count. Findings from the study support the claim that *M. oleifera* extract may facilitate iron absorption, as an adequate amount of this element is necessary for haemoglobin synthesis and for the animal tissues such as the kidneys and bones to take part in the synthesis of RBCs. However, the findings disagreed with the findings of Ye *et al.* (2019), who reported that minimal absorption of ≤ 3.75mg of *M. oleifera* leaves have a better hematinic effect. Adias *et al*. (2013) hold the view that the effect of oral administration of *M. oleifera* leaf extract irrespective of the dose (200 and 300mg) tends to increase blood parameters such as WBC, RBC, Hb, and PCV in anemic rats and could have the same effect in humans.

Furthermore, the effect of *M. oleifera* leaves on the haematological profile of rats affected by fluorosis was investigated by Pagadala *et al.* (2022). The control group was provided unrestricted access to water during a duration of 30 days. Group II was

subjected to the administration of sodium fluoride in the drinking water at a dosage of 50 mg/kg body weight for a duration of 30 days. Group III was administered a dosage of 50 mg/kg of fluoride in their drinking water, which was then supplemented with 200 mg/kg of *M. oleifera* orally. This supplementation was achieved by mixing *M. oleifera* with water and administering it to the subjects using an oral gavage bent needle. The duration of this treatment regimen was 30 days. The animals in Group IV were provided with unrestricted access to both food and water and were supplemented with *M. oleifera*. The supplementation was administered by combining *M. oleifera* with water and administering it to the animals through an oral gavage.

The results of the study revealed that there was a significant ($p < 0.05$) decrease in the RBC count, Hb% and increase in plasma fluoride content in fluorosis-affected rats. This reduction was not seen in the *M. oleifera* leaves with fluoride-supplemented group compared to controls. Though all the haematological parameters were reduced in the Fluorosis group as compared with Controls, only Hb% and RBC count were statistically significant. The study further concluded that the dietary supplement of *M. oleifera* may have the capacity to reverse anaemia within a short period of time after administration. This was due to the fact that it is known to include alkaloids, flavonoids, phytosterols, and saponin, all of which are known to have hemopoietic activity. In addition to these bioactive compounds that are found in the leaves of the *M. oleifera* plant, it is also claimed to be an excellent source of vitamins A, B, and C, as well as proteins and minerals like iron, all of which may be factors that contribute to the benefits that it has been shown to have on red blood cells (Pagadala *et al.,* 2022). Shinde *et al*. (2021) have also reported on the effect of *M. oleifera* on human haematological parameters. In the study, 10 girls of the ages between 17 - 21 years were selected and Oral administration

of *M. oleifera* extract (10g/ day) was given over a month to the participants. Haematological parameters were measured before and after the administration of the extract. Results from the study indicated that after the treatment with moringa extract, the haematological parameters such as WBC, RBC, Hb, platelets, and differential counts were recorded to be in the normal range. It was significantly observed that the Hb of the girls was increased after the treatment with the moringa extract. Shinde *et al.* (2021) further explained that the significant change in haemoglobin concentration in the group that received *M. oleifera* extract (10 g / day) could be attributed to the diverse phytochemical constituents in *M. oleifera*. They concluded that the administration of *M. oleifera* leaf extract regularly helps to maintain blood parameters such as WBC, RBC, and platelets in the normal range.

A study has been carried out in Kisarawe District, Tanzania by Shija *et al.* (20219) which investigated the effect of *M. oleifera* leaf powder supplementation on reducing anaemia in children below two years. The study included 95 anaemic children who were grouped into two; the intervention communities received *M. oleifera* lea*f* powder and nutrition education, while the control communities only received nutrition education for six months. At the end of the experimental period, the mean Hb concentrations of the control and intervention groups were 7.9 g/dl and 8.3 g/dl respectively. After 6 months, anaemia prevalence significantly decreased in the intervention group by 53.6% compared to 13.6% in the control community. The mean Hb was 10.9 g/ dl for intervention and 9.4 g/dl for control. The effect was also observed in the reduction of the prevalence of moderate and severe anaemia in the intervention communities by 68.2% and 77.9%, respectively, and by 23.3% and 56.9%, respectively, in the control communities. The study revealed that increasing the amount and time of using *M.*

oleifera supplementation resulted in a significant reduction in anaemia cases, therefore, can be used as a complementary solution in addressing anaemia among children, especially when the use of infant formulas and fortified food products is very poor (Shija *et al.,* 2019).

2.12 Effect of *Moringa oleifera* on Liver Biochemical Indices

Kalra *et al.* (2020), in a review stated that, the liver is a vital organ in the human body, performing a pivotal role in several physiological processes that contribute to metabolism, immunity, digestion, detoxification, and vitamin storage, among other significant roles. The adult body weight is composed of approximately 2%. The liver possesses a distinctive anatomical characteristic in that it receives blood from two separate sources: the portal vein, which accounts for roughly 75% of the blood supply, and the hepatic artery, which contributes approximately 25% of the blood supply. Physiological and morphological anomalies in the liver pose a significant threat to the function of the human system.

Several studies animal and human studies have been carried out to ascertain the potential effect of *M. oleifera* leaves on liver biochemical parameters such as alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, total protein, and albumin. This review explored what literature has documented concerning the effect of *M. oleifera* on hepatoprotection. A study by Prasetio *et al*. (2022), studied the effect of Moringa leaf extract on liver function markers in rats with hyperglycemia. The study comprised of four distinct groups: a normal control group (K0), and three groups of hyperglycemic rats induced with alloxan at a dosage of 125 mg/kg bw. These three groups were labeled

as K1, T2, and T3, and were subjected to treatment with moringa leaf extract at dosages of 0 mg, 200 mg, and 400 mg/kg bw accordingly. The treatment period lasted for a duration of 21 days. The findings of Prasetio *et al*. (2022) showed that the levels of AST and ALT were highest in the K1 group which then decreased significantly in the group that received Moringa leaves extract (T1 and T2). They attributed the significant reduction of the liver enzymes in T1 and T2 to the fact that Moringa leaf extract showed early improvement in the cellular membrane integrity of the liver cells which is evident of its anti-hepatoxic effect. Recovery of these enzymes to normal levels indicates the return of normal liver function after administration of Moringa leaf extract.

This suggests the revival of insulin secretion and regenerative activity of pancreatic islets of Langerhans cells this assumption also correlated with a study by El-bakry *et al.* (2016) and Woldekidan *et al.* (2021), who also reported a similar finding in their study on the hepatoprotective effect of *M. oleifera* leaves extract against carbon tetrachloride-induced liver damage in rats and evaluation of antihyperglycemic effect of aqueous leaves extract of moringa on alloxan-induced diabetic rats respectively. The results of the study showed that the total phenolic and flavonoid active components, including sitosterol, quercetin, and kaempferol, were responsible for the antioxidant and hepatoprotective properties of *M. oleifera* leaf extract. These active components also contributed to the reduction of the AST and ALT liver function parameters in the hyperglycemic rats. In an experiment to ascertain the ameliorative effects of *M. oleifera* leaf extract on hepatic enzymes in rats, a study was conducted by Farid & Hegazy (2020), the study explored the effect of Levofloxacin (40 mg/kg body weight (b.wt.) daily for two weeks) on rat liver function and oxidative stress markers as well as evaluated the potential hepatoprotective effects of *M. oleifera* leaf extract as a known

antioxidant herb. According to Farid & Hegazy (2020), *M. oleifera* leaf extract was discovered to alleviate the hepatic dysfunction brought on by Levofloxacin by restoring liver enzymatic activity (ALT, AST, and GGT) to normal levels. The extract exhibited a reversal of the antioxidant imbalance, as evidenced by the observed changes in catalase and superoxide dismutase activity, as well as reduced glutathione and malondialdehyde levels. Furthermore, it was also reported that the administration of *M. oleifera* leaf extract resulted in the induction of anti-inflammatory effects through the enhancement of interleukin production. Additionally, its presence decreased the downregulation of interleukin induced by Levofloxacin alone from hepatic tissues (Aly *et al.,* 2020). According to Farid and Hegazy (2020), *M. oleifera* extract can lessen the negative effect brought on by using Levofloxacin. The finding from this study was also consistent with a similar study by Aly *et al.,* (2020) who examined liver biochemical and histological effects of *M. oleifera* extract on acetaminophen-induced liver fibrosis in albino rats and it was observed that both the treated and preventive groups had a significant reduction in liver enzymes in comparison to the acetaminophen group.

The hepatoprotective effect of *M. oleifera* extract was confirmed by the findings of the biochemical and histological analyses of the liver, which were consistent with each other. The study concluded that the preventive action of the ethanolic extract of *M. oleifera* has a significant potential to prevent and treat liver damage. A review by Vergara-Jimenez *et al.* (2017), has also reported that methanol extract of *M. oleifera* leaves has a hepatoprotective effect, which might be due to the presence of quercetin. *M. oleifera* leaves had substantial effects on the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), in addition to reductions in lipids and lipid peroxidation levels in the liver of rats (Halaby *et al.,* 2015).

M. oleifera leaves have been shown to reduce plasma ALT, AST, ALP, and creatinine and to ameliorate hepatic and kidney damage induced by drugs (Ouédraogo *et al.,* 2013). In rats, co-treated with *M. oleifera* leaves and NiSO4, to induce nephrotoxicity, similar findings were observed (Adeyemi & Elebiyo, 2014). Also, the administration of the extract of *M. oleifera* leaves in mice was followed by decreases in serum ALT, AST, ALP, and creatinine (Adeyemi & Elebiyo, 2014). Almatrafi *et al*. (2017) has reported that non-alcoholic fatty liver disease (NAFLD) was averted in guinea pigs given with *M. oleifera* leaves in a model of hepatic steatosis. This was demonstrated by decreased amounts of hepatic cholesterol and triglycerides in animals treated with *M. oleifera* compared to controls. This lowering of hepatic lipids was associated with lower inflammation and expression of genes involved in lipid uptake and inflammation. The *M. oleifera*-treated guinea pigs had lower concentrations of plasma ASP (Almatrafi *et al.,* 2017).

The findings from the studies reported above suggest that administration of both young and old *M. oleifera* as a haematinic will not adversely affect renal and liver function as earlier studies have shown that moringa improve renal and liver function without causing deteriorating effect to these vital organs.

2.13 Effect of *Moringa oleifera* on Renal Indices

A crucial organ called the kidney controls the blood's levels of water, solutes, and electrolytes to control the plasma osmolarity. It also creates erythropoietin, which increases the production of red blood cells, and it maintains long-term acid-base equilibrium. In addition to this, it regulates blood pressure by producing renin and is responsible for the transformation of vitamin D into its active form (Ogobuiro & Tuma,

2023). It is one of the organs that are prone to diseases, in the quest to protect and improve its function several studies have been carried out to assess *M. oleifera's* potency on renal function. This section explores various findings of *M. oleifera* on renal protection as documented by literature.

In their carefully designed study, El-behairy *et al.* (2019) investigated the effect of *M. oleifera* on renal toxicity induced by Titanium Dioxide Nanoparticles (TDN) in adult male albino rats. TDN-treated animals recorded a significant increase in urea and creatinine indices. A Significant improvement occurred in the urea and creatinine parameters of the group that received *M. oleifera.* The findings were also confirmed by histopathological findings. El-behairy *et al*. (2019) view is supported by Adeyemi & Elebiyo, (2014), who studied the protective effect *M. oleifera* diet against nickel-induced nephrotoxicity in rats. Male Wistar rats were assigned into six groups of five. During the experiment, the rats were orally exposed to a dosage of 20mg/kg of nickel sulphate (NiSO4) dissolved in normal saline solution. The rats were then divided into different groups and provided with either a normal diet or diets that were supplemented with varying concentrations of *M. oleifera*. This exposure and dietary regimen were maintained for a duration of 21 days.

Blood and kidney samples were collected for subsequent biochemical and histopathological analysis. The exposure to NiSO4 resulted in a reduction in the kidney-to-bodyweight ratio in rats, as well as a substantial increase in the levels of plasma creatinine, urea, and potassium. Furthermore, the exposure to NiSO4 resulted in a reduction in the plasma concentration of sodium. Nevertheless, the incorporation of *M. oleifera* into dietary treatments prevented the nickel-induced disruption of creatinine

and urea levels. The histopathological analysis demonstrated that exposure to NiSO4 resulted in structural damage to the renal tubules and glomerular walls. On the other hand, the inclusion of *M. oleifera* in the diets resulted in mitigation of the damages. The research findings indicated that the incorporation of *M. oleifera* into the dietary treatments provided substantial protection against nephrotoxicity caused by nickel. Similar to the findings of (Adeyemi & Elebiyo, 2014; El-behairy *et al.,* 2019), Abou-Zeid *et al*. (2021) have designed a study that explored the efficacy of *M. oleifera* ethanolic extract (MOE) in protecting against Tilmicosin (Til)-induced nephrotoxicity in Sprague Dawley rats. In the research, the experimental mice were administered a single dose of Til (75 mg/kg bw, subcutaneously), and/or MOE for a duration of 7 days (400 or 800 mg/kg bw, administered via oral gavage). The study findings indicated a strong association between Til-treatment and elevated serum levels of creatinine, urea, salt, potassium, and GGT activity. Additionally, there was a decrease observed in total protein and albumin concentrations. The levels of hydrogen peroxide and malondialdehyde in the renal tissue were found to be increased, whilst the activity of the superoxide dismutase and glutathione peroxidase enzymes were observed to be reduced.

The levels of renal tumor necrosis factor-alpha and interleukin-1 beta (IL-1β) and the mRNA expression of intermediate filament protein-encoding genes (desmin, nestin, and vimentin) in the kidney were up-regulated with histopathological alterations in renal glomeruli, tubules, and interstitial tissue. These toxic effects were markedly ameliorated by co-treatment of MOE with Til, in a dose-dependent manner. Collectively, the findings of this study suggest that the administration of MOE at a dosage of 800 mg/kg offers protection against Til-induced kidney impairment. This

protective effect is attributed to the high antioxidant and anti-inflammatory properties of MOE, rendering it suitable for usage as a protective supplement.

The findings from the above studies are indicative that administration of moringa as haematinic may not be detrimental to renal indices and it also support the claim that *M. oleifera* confer renal protection. The renal protection has been reported to be as result of the high antioxidant and anti-inflammatory properties.

2.14 Effect of *Moringa oleifera* on Lipid Profile

The lipid profile indices are useful in monitoring the health status of the cardiovascular system. Various research studies have investigated the effect of *M. oleifera* leaves on the lipid profile of experimental and clinical studies among such findings are discussed below. In their widely acclaimed work, Achuba FI *et al*. (2016) have discussed the effect of *M. oleifera* on rats fed with a crude oil-contaminated diet. They indicated that exposure of rats to a crude oil-contaminated diet significantly ($P < 0.05$) increased serum total cholesterol (TC), triacylglyceride (TAG), and low-density lipoprotein (LDL) but significantly decreased high-density lipoproteins (HDL) when compared to the control.

Moreover, the study indicated that rats fed *M. oleifera*-treated diets exhibited reduced TC, TAG, and LDL and a higher HDL compared to rats fed with crude oil-contaminated diet.

Interestingly, a similar finding has been reported by Aborhyem *et al.* (2016), who sought to assess the effect of *M. oleifera* consumption on lipid profile in hyperlipidemic

rats. According to the findings of the, after four weeks of feeding the rats with *M. oleifera*, total cholesterol and LDL levels were significantly decreased by 26.8% and 40.6 %, respectively, compared to baseline levels. Although there were slight increases in VLDL and TG of 5.6% and 5.1%, respectively, these remained within the normal range. Otherwise, there was a significant increase in LDL, VLDL, TC, and total TG levels in rats fed on an atherogenic diet only by 17.13%, 195.4%, 29.2%, and 193% respectively compared to baseline values after 4 weeks.

The findings from the above studies are indicative that administration of moringa as haematinic may not significantly affect lipid profile levels in the experimental model. Again, based on the findings reported by Aborhyem *et al*. (2016), *M. oleifera* could be used to maintain cardiovascular health as reduction in elevated levels of total cholesterol, LDL levels and elevation on VLDL is indicative of improved cardiovascular function.

2.15 Toxicity of *Moringa oleifera*

Numerous research studies have evaluated the toxicity and safety of *M. oleifera*, and the majority of those investigations have shown no evidence of any negative consequences of its ingestion in human trials. Similar to this, both in vitro and in vivo research have been used to evaluate the plant's potential toxicity. The majority of in vitro research used malignant and healthy human cell lines as indicators for the safety and toxicity of the *M. oleifera* extract therapy (Azlan *et al.,* 2022). In an animal experiment, rats used as test subjects received oral treatments of an aqueous leaf extract of *M. oleifera* at dosages of 400, 800, 1600, and 2000 mg/kg. The research found that the extreme daily dose of 2000 mg/kg was safe to administer, and no mortality was

recorded except for a dose-dependent drop in body weight for the rats treated throughout the course of a 21-day daily treatment cycle (Stohs & Hartman, 2015). In another study, the experimental rats were given treatment with 1000 and 3000 mg/kg of the leaf extract; genotoxicity was observed in rats treated with a 3000 mg/kg dose of the extract and not the lower (Asare *et al.,* 2012). However, according to Stohs & Hartman (2015), administration of 1000 mg/kg of the extracts is still too much for routine use. Furthermore, a cytotoxicity analysis of an aqueous seed extract *of M. oleifera* in mice indicated that even at a dosage of 2000 mg/kg, no systemic toxicity was seen and that the control group's erythrocytes, platelets, haemoglobin, and hematocrit levels did not alter significantly (Araújo *et al*. 2013). This finding disagreed with the findings reported by (Asare *et al.,* 2012).

The study of the acute toxicity and LD50 of 70% ethanolic *M. oleifera* leaf extracts in white albino rats and rabbits has also been carried out by Mohamed *et al*. (2015). In their studies, the experimental models were continually administered intraperitoneally with 150mg/mL of the extract every five (5) minutes, until mortality was recorded. According to the findings, the acute toxicity lethal dosage for rats was 6616.67 mg/kg, and for rabbits, it was 26,043.67 mg/kg. However, albino rats were also reported to have survived after receiving a 14 mL injection of a concentrated dosage over ten (10) minutes, indicating that excessive fluid buildup or water intoxication is more likely to be the cause of mortality than the extract's toxicity. This has also been supported by histopathological findings, which led to the conclusion that, when administered in moderation and over the course of a reasonable amount of time, ethanolic extracts of *M. oleifera* leaves have very low toxicity and are less harmful to animals (Mohamed *et al.,* 2015). According to the findings of a study that was conducted by Okumu *et al.*

(2016) on the acute toxicity of the aqueous-methanolic *M. oleifera* leaf extract on female Wistar albino rats, the rats were given an oral dose of 2000 mg/kg of the extracts, and blood samples were obtained to evaluate key signs of liver failure. The study revealed that administration of a 2000 mg/kg oral dose resulted in a significant increase ($p < 0.05$) in aspartate aminotransferase (AST) levels. However, there were non-significant increases ($p > 0.05$) in total bilirubin levels and a non-significant decrease ($p > 0.05$) in alanine aminotransferase (ALT) levels compared to the control group. In addition, the postmortem analysis, which showed a non-significant increase ($p > 0.05$) in the hepatic index (liver to body weight ratio) and only mild distortions in the structure of liver cells via transverse liver section analysis, hypothetically concluded that the LD50 for the *M. oleifera* aqueous-methanolic leaf extract in female Wistar albino rats is > 2000 mg/kg.

Olayemi *et al. (*2016) used biochemical, haematological, and histopathological investigations to assess the toxicity profiles of crude methanol extracts of *M. oleifera* seeds and leaves as well as the relative impact on vital organs in Wistar rats. Rats were given varying dosages of the extracts (100, 200, 400, and 1000 mg/kg body weight) daily for 28 days, and rats at all doses had their hearts, livers, lungs, spleens, and kidneys histopathologically evaluated. The study revealed that at a dose of 1000 mg/kg of the seed extract treatment, a physical evaluation of agitation, confusion, and disorientation was observed, however, these symptoms quickly subsided and there was no mortality. White blood cells and platelet levels, AST, ALT, and alkaline phosphatase levels (ALP) are some of the other indices that were assessed by the study. The study found that both seed and leaf extracts are safe to consume in moderation, it has the tendency to improve immunity and have the potential to provide hepatoprotection.

However, in comparison, the seed extracts demonstrated more potential in a long-term treatment as more notable changes were observed in the indices (Olayemi *et al.,* 2016). In addition, a study was done to assess the acute toxicity of *M. oleifera* leaf powder in Sprague Dawley rats, utilizing the toxicity categorization technique outlined in the Organization of Economic Co-operation and Development (OECD) Guideline 423. The rats involved in the experiment received doses of dry leaf powder ranging from 0 to 2000 mg/kg. Throughout the 14-day observation period, no negative effects were observed in any of the groups, as neither in the clinical signs nor gross pathology observation were made.

Additionally, using the toxicity classification approach outlined in OECD Guideline 423, an acute toxicity investigation on *M. oleifera* leaf powder in Sprague Dawley rats was carried out (Moodley, 2017). When the experimental rats were fed up to 2000 mg/kg of the dried leaf powder during the 14-day experimental period, no adverse effects were noticed in any of the groups, neither in the clinical symptoms nor in the gross pathological observation. The study concluded that the oral toxicity (LD50) of the dried leaf powder transcends beyond 2000 mg/kg dose. The findings from the documented literature give a general expression that *M. oleifera* is safe and possesses various pharmacological properties. The finding of this study is in agreement with Olayemi *et al.* (2016).

The findings indicated in the above studies show that doses above 1000mg/kg of Moringa could be detrimental to vital organs such as the kidney and liver. Therefore, caution should be taken when administering high doses of *M. oleifera* as a haematinic.

Also, the *M. oleifera* extract should be administered in moderation (< 1000mg/kg) over a reasonable period as prolong administration has the potential to induce toxicity.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location and Duration

The study was carried out at the Animal Science Department of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Asante Mampong Campus. This university is situated in Asante Mampong, which is located in the Asante Region of Ghana. Asante Mampong is geographically located within the transitional zone lying between the Guinea Savanna in the north and rainforest of the south of Ghana along the Kumasi-Ejura Road. It is about 65 km from Kumasi (Meteorological Service Department (MSD), 2013). Within the experimental period, the maximum temperature recorded was 31.6 ºC and the lowest was 22.3 ºC. The rainfall pattern was bimodal with the main rainy season occurring from April to July with an average of 1000 mm rainfall and minor season starting from August to November with an average of 350 mm rainfall (Meteorological Service Department (MSD), 2013). Asante-Mampong lies on the latitude 07^0 04 North and longitude 0^0 24 West and an altitude of about 457 m above sea level. The entire study lasted for thirty-two weeks starting on 25th January to 31st August, 2023. However, acclimatization and administration of treatments lasted for a period of six weeks, which started on the 12th of June to 10th of July 2023

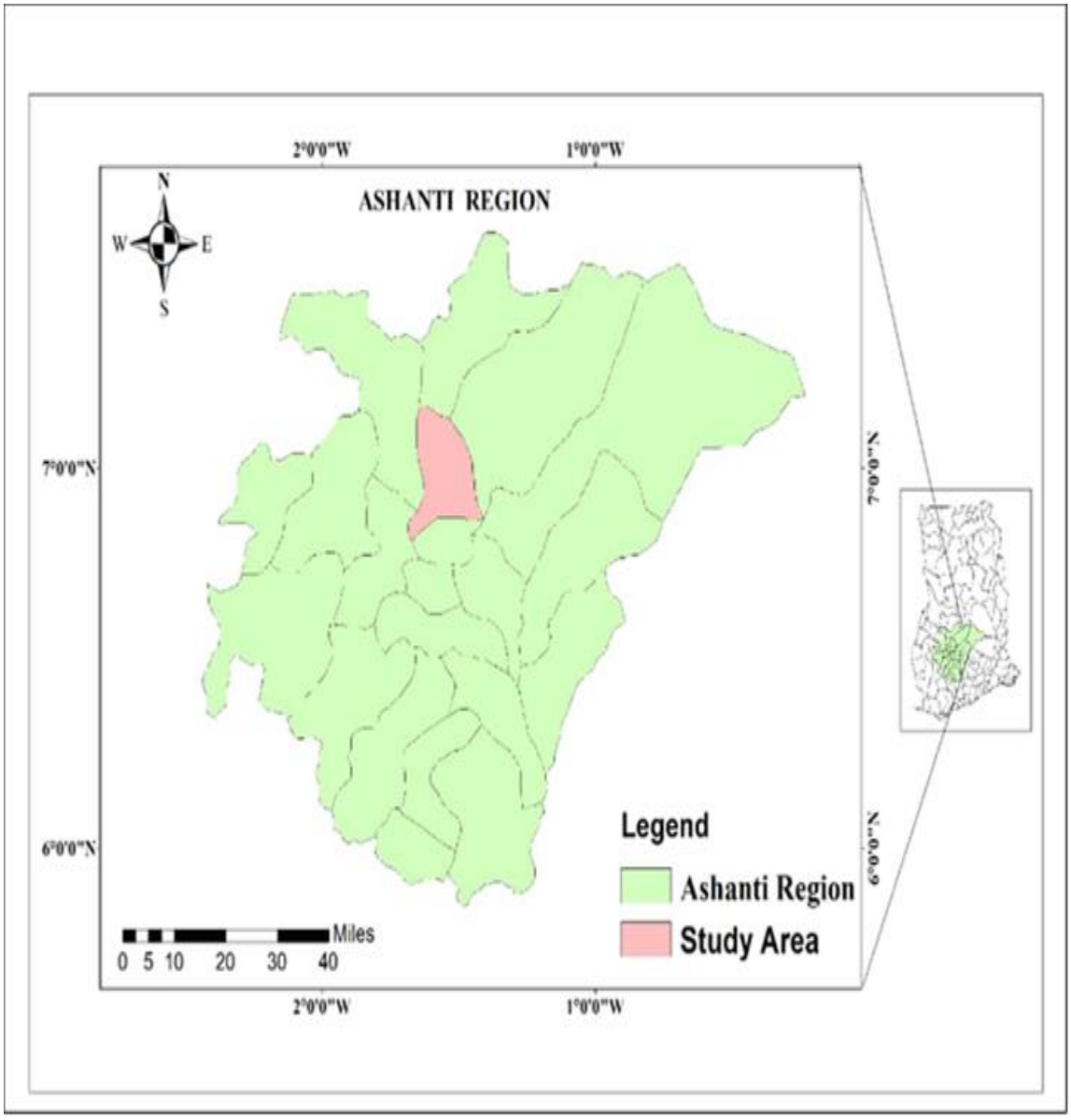

Figure 3.1: Map of the study area. Designed by using ArcMap version 10.7.

3.2 Experimental Design and Animals

The study utilized a completely randomized design (CRD). A total of twenty (20) male Wistar Rats (*Rattus novergicus*) aged eight weeks and weighing between 130 to 150 g were purchased from Noguchi Memorial Research Institute for Medical Research. The animals were transported to the animal farm located at the Animal Science Department of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (specifically, the Asante–Mampong Campus), where the experiment was

conducted. The animals were given a period of 14 days to acclimatize before randomly put into four groups, each consisting of five replicates. Treatments were administered via the oral route using sterile oral gavage (Instech Laboratories, Philadelphia, USA) for a period of twenty-eight days. The table below shows the treatment and dose administered.

Table 3.1: Treatment and dose administered

GROUP	TREATMENT	DOSE ADMINISTERED
1	9% Normal Saline	1mL/kg/bw
2	Iron (III) Hydroxide Polymaltose	100 mg/kg/bw
3	Ethanolic leaf extract of young M. *oleifera*	100mg/kg/bw
4	Ethanolic leaf extract of old *M. oleifera*	100mg/kg/bw

3.3 Maintenance of Experimental Animals

3.3.1 Housing

The experimental animals used in this research were housed in galvanized metal cages with a 2 mm wire mesh covering. The cages were divided into four compartments with dimensions 70 cm x 60 cm x 40 cm. Each compartment had an opening at the top to provide access to the interior. Softwood shreds were used as beddings for the experimental animals, the shreds were changed periodically to prevent the spread of infections. The animals were kept under a controlled environmental condition at a temperature of about 27 ± 3 °C under a 12-hour day-night cycle.

3.3.2 Feeding and Watering

Each treatment group was provided with an earthenware bowl and water bottle with nipple as feeders and waterers. Clean water was provided *ad libitum*. The experimental

animals were fed in the morning between 6:00 and 08.30 hours GMT each day. The experimental animals were fed with commercially formulated feed purchased from Galdus Ghana Limited. The feed was composed of the following: Maize, soya bean meal, wheat peas, corn gluten, rapeseed meal, nutricell, wheat, grits lecithin, feed-phosphate, lysine, sulfate, fishmeal, Limefine, sunflower oil, mould inhibitor, salt-NaCl, Vitamin E, premix, Choline chloride liquid, xylamase, phytase, sunflower hippo, and oat. The calculated analysis of the feed as provided by the manufacturer is shown in the table below.

Table 3.2: Ingredients of experimental feed

COMPOSITION	PERCENTAGE (%)
ME (Kcal/kg)	3150
Crude protein	22.00
Crude fat	7.50
Lysine	2.50
Methionine	1.30
Meth + Cyst	0.60
Calcium	0.95
Sodium	0.95
Phosphorus	0.60
Anti-oxidant	E321
Enzymes	4ª11/ 4ª24
Mould inhibitor	Added
Vitamins	Added

***Source**: Label, Koudjis Animal Nutrition*

3.4 Collection of leaves of *Moringa oleifera*

Leaves of *M. oleifera* were identified, and collected at Nyamebekyere a suburb in Mampong municipality, on the 21st day of March 2023. Plant authentication was done

by a botanist at the crop science department of AAMUSTED. In this study, leaves of *M. oleifera* were considered young when their appearance was whitish-green and measured < 0.9 to 1.8 cm in length by 0.5 to 0.96 cm in width. Also, *M. oleifera* leaves were considered old when their appearance showed a dark green coluor and measured ≥ 0.9 to 2.54 cm in length by ≥ 0.5 to 1.5 cm in width (Macário *et al.,* 2020; Odukuya *et al.,* 2022).

3.5 Plant Extraction

The leaflets were separated from the stem and cleaned with distilled water to remove any debris. They were allowed to air dry for two weeks at room temperature (26 ± 1°C) at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development's Science laboratory. The leaves were then pulverized to a fine powder using the Retsch Milling solution at 2000 rpm for five minutes. Fifty (50) g of the coarse form of the sample (leaves) was macerated for 3 days in 500 mL of 90% ethanol in sterile plastic containers with intermittent shaking at 26 °C to ensure adequate dilution and extraction. The extract was then filtered with Whatman filter paper (1.5 Sigma Aldrich, USA) and then concentrated to semi-solid form at 42 °C (to avoid denaturation of the active ingredients) using a Clifton water bath. 16g and 45g of YMoE and OMoE crude extracts, respectively, were dissolved in 100ml of sterile distilled water to make a stock solution with a concentration of 0.16g/mL and 0.4568g/mL, respectively. The preparation of sub-stocks in microliters was done by diluting the stock solution with sterile distilled water to the concentration of interest (100mg/bw/day). The stock solution was kept refrigerated (-4^0 C) until the time of treatment administration, which started on June 12, 2023.

3.6 Preparation of Stock Solution of Standard Hematinic Drug

The standard hematinic drug used in this study was iron (III) hydroxide polymaltose, which is marketed under the brand name Polyfer. Polyfer was purchased from Laugh Pharmacy in Mampong-Ashanti. 2,626mg of the drug containing 50mg of elemental iron was weighed and dissolved in 50 mL of sterile distilled water, giving a concentration of 52.52mg/mL. The stock solution was kept in a functional laboratory refrigerator at a constant temperature of -40°C until the period of administration.

3.7 Dose Calculation and Administration of Treatments

The mean body weight (BW) of the experimental animals in grams for each treatment group was converted to kilograms. The resulting mean body weight in kilograms was multiplied by the dosage to be given to each treatment per kilogram of their body weight, the resulting value was divided by the concentration of the treatments. The same procedure was followed to determine the dose calculation for the groups that were administered 100 mg/kg/bw of young *M. oleifera* (YMoE) and 100mg/kg/bw Iron (III) Hydroxide Polymaltose. The dose used in this study was adapted from the studies carried out by Allahmoradi *et al.* (2019); Hassan *et al.* (2020). Before the extract was administered to the experimental rats, the stock solutions were allowed to cool to ambient temperature a few hours before the preparation of the dose.

3.8 Procedure for Phytochemical Screening

The leaves of young and old *M. oleifera* were screened separately for the phytochemical constituents using standard phytochemical reagents and procedure as described previously by De *et al. (*2010) and Effah-Yeboah *et al.* (2021).

3.8.1 Test for Anthraquinones

Born Tranger's test was used to test for the presence of anthraquinones. Ethanolic extract of young and old leaves of the *M. oleifera* Plant was heated at 27 ^{0}C for 10 minutes in weak sulphuric acid. After that, it was filtered and cooled. The filtrate was then extracted with chloroform and treated with dilute ammonia. The presence of anthraquinone derivatives was shown when the layer changed from pink to red.

3.8.2 Test for Steroids and Triterpenoids

The Salkowski test was used to determine the presence of steroids and triterpenoids. Extracts were treated with chloroform with a few drops of strong sulfuric acid poured gently down the side of the test tube, agitated briskly, and left to stand. After a few minutes, the emergence of red colour at the lower layer indicated the presence of steroids, whilst triterpenoids were detected by the production of yellow colour at the interface of the layer.

3.8.3 Test for Alkalnoids

A few drops of 35 % dilute HCL and 0.5 ml Wagner’s reagent (a solution of iodine and potassium iodide) were added to a portion of the extract. A brown flocculent precipitate shown indicated the presence of an alkaloid.

3.8.4 Test for Glycoside

Molisch’s test was used to detect the presence of glycoside. A few drops of Molisch’s reagent (a solution of α-naphthol in ethanol) to the neutralized extract. A red or pink color indicates the presence of glycosides.

3.8.5 Test for Flavonoids

A portion of the extract was dissolved in 98% concentrated H_2SO_4, formation of an intense color was observed; this indicated the presence of flavonoids.

3.8.6 Test for Tannins

A portion of the extract was mixed with a few drops of 0.1 % Ferric chloride. The presence of a brownish-green coloration indicated the presence of hydrolysable tannins.

3.8.7 Test for Saponins

About 0.5 ml of the extract was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth (5 to 10 minutes) with a honeycomb structure indicating the presence of saponins.

Plate 3.1: Researcher performing phytochemical screening

3.9 Procedure for Dissection

Dissection of experimental rats was done at the university's biology laboratory (Akenten Appiah-Menka University of Skills Training and Entrepreneurial

Development, Mampong Campus). The rats were placed supinely on a dissection board and pinned down to prevent movement during the dissection procedure. The experimental animals were examined macroscopically, weighed, and sacrificed under mild chloroform anesthesia. An abdominal incision was made through the middle line on the ventral surface of the rats using a sterilized surgical blade, scissors, pins, and forceps to expose the peritoneal organs.

Plate 3.2: Researcher dissecting, drawing cardiac blood samples, and dispensing blood samples into an EDTA tube

3.10 Parameters Measured

3.10.1 Body Weight

The body weight (g) of the experimental rats for each treatment was determined using a Camry top-loading sensitive scale with a reading or sensitivity of 0.1 g, produced in China by the Jadever Company Limited. The scale was set to zero after the weight was initially measured on an empty container. After being individually placed in the container, the rats' body weights were then measured and recorded once they had

stabilized within the container. Rats were kept from escaping the container while being weighed.

3.10.2 Daily Feed Intake

Feed intake was all measured in grams (g/day) and recorded for each treatment group. Feed intake was taken daily and calculated as the difference between the feed given and the feed left over.

3.10.3 Determination of Haematological Indices

3mls of blood sample was taken via cardiac puncture on day 28, using a sterile syringe and needle manufactured by Jiangsu Shenli Medical Production Company Limited, China, and distributed by Letap Pharmaceuticals Limited, Ghana, and transferred into ethylenediaminetetraacetic acid (EDTA) tubes for haematological assays. The white blood cell (WBC), red blood cell (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haemoglobin (HGB), and platelet (PLT) were determined using a haematology auto-analyzer (Automatic Haematology Analyzer, with a model number Rayto RT-7600s made in Guangzhou, China) at Ashanti Mampong Maternity Hospital.

3.10.4 Determination of Renal and Liver Biochemical Indices

5mls of blood was drawn into clean vacutainer tubes and centrifuged for five minutes at 13000 rpm. Sera was aspirated, and biochemical parameters including Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphate, Gamma-glutamyl transferase, Total protein, Total bilirubin, creatinine, Blood urea nitrogen, Total cholesterol, High-density lipoprotein-cholesterol, Low-density lipoprotein-cholesterol,

Triglycerides, very low-Density lipoprotein-cholesterol concentrations in serum were examined using BS-120 Mindray Chemistry Analyzer (Mexban & Hussein, 2015).

3.10.5 Histological Analysis of Liver and Kidney

The liver and kidney were harvested and fixed in 10% formalin for routine histological techniques. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene, and embedded in paraffin wax. Serial sections of 7-micron thickness were obtained using a rotatory microtome. The deparaffinized sections were stained with the hematoxylin and eosin (H&E) method (Mahmood *et al.,* 2021). The tissues were then examined using a light microscope, and photomicrographs of the sections of interest were taken for further studies.

3.11 Data Analysis

Data on haematological indices, renal and liver function tests, feed intake, body weight and lipid profile were expressed in terms of mean ± standard error of the mean (SEM). Data was analyzed using Minitab statistical software (Version 20.0). Parameters in groups were compared by one-way ANOVA, means were separated using Tukey HSD. All data were analyzed at a 95% confidence interval, and values were considered statistically significant at $p < 0.05$.

3.12 Ethical Considerations

Ethical approval was obtained from the Committee for Human Research and Ethics of the University of Energy and Natural Resource, Sunyani with reference number CHRE/AP/217/024. This research also followed The Guide for the Care and Use of

Laboratory Animals by the National Institutes of Health in the United States (NIH Publication No. 85-23).

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening of Old and Young *Moringa Oleifera* Leaves

The results of the phytochemical screening of young and old *M. oleifera* Leaves (YMoL and OMoL) revealed that there was diversity in the phytochemical constituents of the two leaves (Table 4.1). Triterpenoids, glycosides, flavonoids, and saponins were found in both leaves, however, alkaloids, and tannins were only found in the young leaves of *M. oleifera.* Anthraquinones, steroids, and terpenoids were not detected in either of the leaf types.

Table 4.1: Phytochemical constituents of young and old *Moringa oleifera* leaves

Detected = ++, Not detected = ---, YMoL = Young M. oleifera leaves, OMoL = M.

Phytochemical	YMOL	OMoL
Anthraquinones	---	---
Steroids	---	---
Triterpenoids	++	++
Alkaloids	++	---
Glycosides	++	++
Terpenoids	---	---
Flavonoids	++	++
Tannins	++	---
Saponins	++	++

oleifera leaves.

4.2 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, Ymoe, and Omoe Treatments on Feed-intake

Table 4.2 gives the summary of the weekly feed consumption rate by the experimental animals during the 28 days of treatment administration.

Table 4.2: Effect of Normal saline, Iron (III) Hydroxide Polymaltose, YMoE, OMoE on weekly feed intake

Week	Normal saline	Iron (III) Hydroxide Polymaltose	YMoE	OMoE	p-value
1	59.70±1.29^{c}	58.57±4.09^{c}	6.71±2.18^{b}	96.29±4.81^{a}	0.001
2	61.71±6.68^{b}	66.29±2.09^{b}	3.00±1.72^{a}	83.14±2.67^{a}	0.001
3	64.57±1.85^{b}	69.71±1.41^{b}	80.14±1.92^{a}	78.14±1.65^{a}	0.001
4	69.57±4.59	77.57±4.08	79.57±2.92	80.29±2.47	0.165

^{abc} *Means with different superscripts in the same row are significantly different ($p < 0.05$). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval.*

There was a significant ($P < 0.05$) increase in the weekly feed-intake of the experimental models administered with YMoE and OMoE treatments. In week one, the OMoE had a better treatment effect compared to YMOE, iron (III) hydroxide polymaltose and normal saline treatments. A comparison between YMoE, iron (III) hydroxide polymaltose and normal saline treatments also revealed that YMoE treatment had better treatment effect. In week two, the OMoE and YMoE treatments showed a statistically significant ($P < 0.05$) treatment effect on feed-intake when compared to iron (III) hydroxide polymaltose and the normal saline treatments.

Even though the iron (III) hydroxide polymaltose and the normal saline treatments were statistically similar, a comparison of the means of the two treatments showed that iron (III) hydroxide polymaltose had a better effect on feed intake than the normal saline treatment. Moreover, in week three, the YMoE and OMoE treatments was significant ($P < 0.05$) when compared to the normal saline and iron (III) hydroxide poly-maltose treatments. YMoE treatment recorded the highest average feed-intake for week three. The normal saline and iron (III) hydroxide poly-maltose treatments were similar statistically, however, a comparison between the means of normal saline and iron (III) hydroxide poly-maltose treatments showed that the iron (III) hydroxide poly-maltose treatment had a better effect on feed-intake than the normal saline treatment. In week four, an insignificant ($P > 0.05$) feed intake was observed in all the treatments, conversely, in terms of their mean, OMoE and YMoE treatment had a better effect compared to the normal saline and iron (III) hydroxide poly-maltose treatments. Iron (III) hydroxide poly-maltose treatment also had a higher feed-intake level than the normal saline treatment.

4.3 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, Ymoe, and Omoe Treatments on Body Weight

The administration of treatments did not cause any statistical significance ($P > 0.05$) on days 1, 7 and 14 (Table 4.3).

Table 4.3: Effect of Normal saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE treatments on body weight of the experimental *Rattus novergicus*

Day	Normal Saline	Iron (III) Hydroxide polymaltose	YMoE	OMoE	p-value
1	181. 00±6.62	190.2±14.2	194.0±18.1	203.60±9.69	0.854
7	189.20±6.89	191.2±11.9	215.4±13.7	221.0±10.7	0.062
14	194.00±8.39	198.4±12.2	228.0±13.5	232.2±11.8	0.068
21	203.00 ± 9.96^{b}	202.6 ± 10.1^{b}	242.6 ± 14.0^{a}	248.0 ± 10.9^{a}	0.015
28	207.0 ± 13.7^{c}	215.2 ± 13.6^{bc}	250.4 ± 15.6^{ab}	257.0 ± 11.7^{a}	0.047

[abc:] Means with different superscripts in the same row are significantly different ($p < 0.05$). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval.

On day 21, the YMoE and OMoE treatments showed a statistically significant difference ($P < 0.05$) when compared with the normal saline and iron (III) hydroxide polymaltose treatments. The normal saline and iron (III) hydroxide polymaltose treatments showed a similar treatment effect. During day 28, the OMoE treatment was statistically similar to the YMoE treatment. Iron (III) hydroxide polymaltose treatment had a statistically insignificant ($P > 0.05$) mean body weight when compared with YMoE and normal saline treatments. However, a statistically significant ($P < 0.05$) level was observed on day 28 when OMoE treatment was compared with the iron (III) hydroxide polymaltose and normal saline treatments. Also, YMoE treatment was statistically different from the normal saline treatment but similar to OMoE.

4.4 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, Ymoe, and Omoe Treatments on Haematological Indices

The study investigated the effects of normal saline, Iron (III) Hydroxide Polymaltose, Young *M. oleifera* Extract (YMoE), and Old *M. oleifera* Extract (OMoE) on various hematological indices. The results indicated no significant changes in Red Blood Cells (RBCs), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Platelet Volume (MPV), White Blood Cells (WBCs), and Platelets across the treatments ($p > 0.05$). This suggests that the administration of these substances did not markedly alter these specific haematological parameters.

However, a significant effect was observed in haemoglobin (Hbg) levels ($p < 0.05$), where both OMoE and Iron (III) Hydroxide Polymaltose treatments significantly ($p < 0.05$), increased haemoglobin levels compared to the normal saline control. Specifically, OMoE and Iron (III) Hydroxide Polymaltose showed significantly higher levels of haemoglobin, indicating a potential benefit of these treatments in increasing haemoglobin concentration. In contrast, the YMoE treatment increased haemoglobin levels, but the increase was not statistically significant when compared to normal saline.

Indices such as HCT, MCV, MCH, MCHC, MPV, and Platelets, no significant ($P > 0.05$) differences were observed between the treatment groups and the control. The mean WBC count was slightly higher in the YMoE and OMoE groups compared to the normal saline and Iron (III) Hydroxide Polymaltose groups, but these differences were not statistically significant ($p = 0.992$). The RBC count showed a trend of being higher

in the Iron (III) Hydroxide Polymaltose and OMoE groups compared to the normal saline group, but this was not statistically significant ($p = 0.215$), (Table 4.4).

Table 4.4: Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE treatments on haematological indices

Blood Indices	Normal Saline	Iron (III) Hydroxide polymaltose	YMoE	OMoE	P-value	Reference range
WBC (10^9/L)	4.61±1.71	4.54±0.894	4.96±0.936	4.85±0.618	0.992	2.45 - 9.55
RBCs(10^12/L)	6.050±0.686	6.970±0.214	6.810±0.344	7.353±0.160	0.215	5.89 - 7.35
Hbg (g/dL)	11.77±1.60[b]	15.300±0.721[a]	14.36±0.219[ab]	16.733±0.273[a]	0.025	11.57 - 14.63
HCT (%)	32.27±6.34	39.267±0.817	37.83±1.39	39.33±2.10	0.462	35.85 - 47.75
MCV (fL)	56.23±2.96	53.43±1.24	55.63±1.10	58.13±1.03	0.312	55.41 - 68.58
MCH (pg)	20.33±0.296	21.67±0.867	21.667±0.751	21.600±0.709	0.405	17.96 - 22.03
MCHC (g/dL)	37.200±0.666	38.933±0.869	38.17±1.34	39.27±1.96	0.697	29.38 - 33.83
MPV (fL)	6.56±0.176	6.733±0.120	6.700±0.100	6.700±0.208	0.819	6.2 - 11.7
PLATELETS (10^9/L)	396.3±28.3	419.7±28.6	401.7±22.3	423.7±28.4	0.863	467.5-1004.5

[abc] *Means with different superscripts in the same row are significantly different ($p < 0.05$). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval. White Blood cells (WBC), Red Blood Cells (RBC), Haemoglobin (Hbg), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin Concentration (MCH), Mean Platelets volume (MPV).Reference range was adapted from* Patel *et al.,* (2024).

4.5 Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE Treatments On Liver Function

The results from the liver function tests indicate that there were no significant differences ($p > 0.05$) among the groups treated with Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE in all measured liver indices. Specifically, ALT levels ranged from 23.67±1.76 U/L in the Normal Saline group to 30.67±4.81 U/L in the OMoE group, AST levels ranged from 158.7±50.3 U/L in the OMoE group to 259.0±37.2 U/L in the Normal Saline group, ALP levels were between 258.3±68.8 U/L in the Normal Saline group and 298.7±21.1 U/L in the Iron (III) Hydroxide Polymaltose group, GGT levels varied from 5.33±1.86 U/L in the OMoE group to 15.7±14.2 U/L in the Normal Saline group, total protein levels ranged from 68.87±4.87 g/L in the OMoE group to 74.00±2.60 g/L in the Iron (III) Hydroxide Polymaltose group, and total bilirubin levels ranged from 12.27±1.36 μmol/L in the YMoE group to 24.33±8.55 μmol/L in the Normal Saline group. All these values fell within their respective reference ranges, indicating no significant impact of the treatments on liver function.

Table 4.5: Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE treatments on liver function test

	Normal Saline	Iron (III) Hydroxide polymaltose	YMoE	OMoE	p-value	Reference Range
ALT (U/L)	23.67±1.76	28.00±1.55	26.33±5.04	30.67±4.81	0.813	19.78-50.55
AST (U/L)	259.0±37.2	200.7±21.3	201.67±9.21	158.7±50.3	0.281	94.34-228.28
ALP (U/L)	258.3±68.8	298.7±21.1	277.0±99.6	288.3±61.2	0.971	137.35-437.41
GGT (U/L)	15.7±14.2	9.00±1.15	7.33±1.76	5.33±1.86	0. 767	5.0-55.0
T. Protein (g/L)	70.80±2.32	74.00±2.60	69.63±3.13	68.87±4.87	0.726	50.27-60.53
T. Bilirubin(µmol/L)	24.33±8.55	18.47±3.41	12.27±1.36	19.73±4.96	0.493	5.27-6.53

Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (alkaline phosphate), GGT (Gamma-glutamyl transferase), T. Protein (Total protein), T. Bilirubin (Total Bilirubin).Reference range was adapted from Patel *et al,* (2024).

4.6 Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Lipid profile

The results from the lipid profile analysis of normal saline, Iron (III) Hydroxide Polymaltose, Young *M. oleifera* Extract (YMoE), and Old *M. oleifera* Extract (OMoE) revealed no significant differences across all measured lipid profile indices ($p > 0.05$). Total cholesterol levels exhibited slight variations among the treatment groups but remained within the reference range (1.50 - 3.00 mmol/L), with no significant trend observed ($p = 0.495$). HDL cholesterol levels were stable across all treatments, fitting within the reference range (0.50 - 1.50 mmol/L), and showed no significant differences ($p = 0.998$). LDL cholesterol levels also remained within the reference range (0.50 - 1.50 mmol/L) without significant differences ($p = 0.962$). Triglyceride levels varied slightly but stayed within the reference range (0.50 - 2.00 mmol/L), with no significant differences ($p = 0.816$). VLDL cholesterol levels showed minor variations but were within the reference range (0.20 - 0.90 mmol/L), and no significant trend was observed ($p = 0.359$). Finally, coronary risk values varied slightly across treatments, all within the reference range (2.5 - 5.0), with no significant differences ($p = 0.653$). Overall, the treatments did not significantly impact the lipid profile levels within the observed period as presented in table 4.6.

Table 4.6: Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, OMoE and treatments on Lipid profile

Parameter	Normal Saline	Iron (III)Hydroxide Polymaltose	YMoE	OMoE	p-value	Reference Range
T. CHOL(mmol/L)	3.073±0.308	3.206±0.119	3.315±0.477	3.143±0.158	0.495	1.50 - 3.00
HDL (mmol/L)	1.017±0.316	1.0356±0.093	1.0244±0.042	1.0241±0.042	0.998	0.50 - 1.50
LDL. (mmol/L)	1.5273±0.0558	1.5508±0.0916	1.441±0.263	1.8669±0.416	0.962	0.50 - 1.50
TRIG.(mmol/L)	1.228±0.156	1.364±0.131	1.306±0.238	1.468±0.179	0.816	0.50 - 2.00
VLDL(mmol/L)	0.5582±0.0708	0.6200±0.0594	0.849±0.1889	0.6671±0.0812	0.359	0.20 - 0.90
C. RISK (mmol/L)	4.7568±0.0738	4.212±0.299	4.274±0.417	4.455±0.393	0.653	2.5 - 5.0

Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. T. CHOL. (Total cholesterol), HDL.CHOL. (High-density lipoprotein-cholesterol), LDL.CHOL. (Low-density lipoprotein-cholesterol), TRIG. (Triglycerides), VLDL.CHOL. (Very low density -lipoprotein -cholesterol). reference range was adapted from (National Research Council, 2011).

4.7 Effect of Normal saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Renal Function Test

Table 4.7 shows the effect of normal saline, iron (III) hydroxide polymaltose, YMoE, OMoE treatments on renal function over four weeks, focusing on urea and creatinine levels. Urea levels across all treatments, including Normal Saline, Iron (III) Hydroxide Polymaltose, Young *M. oleifera* Extract (YMoE), and Old *M. oleifera* Extract (OMoE), were not significantly different ($p > 0.05$), with values remaining within the reference range (21.74-48.2 mmol/L). However, creatinine levels showed a significant difference ($p = 0.006$), with Normal Saline and Iron (III) Hydroxide Polymaltose-treated groups showing higher creatinine levels (0.644 ± 0.02 µmol/L and 0.681 ± 0.04 µmol/L, respectively) compared to the YMoE and OMoE groups (0.555 ± 0.03 µmol/L and 0.485 ± 0.02 µmol/L, respectively), which were within the reference range of 0.3-0.78 µmol/L. This indicates that while urea levels were unaffected by the treatments, *M. oleifera* extracts were associated with lower creatinine levels, suggesting a potential renal protective effect.

Table 4.7: Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE treatments on renal function test

Parameter	Normal Saline	Iron (III) hydroxide polymaltose	YMoE	OMoE	p-value	Reference range
Urea(mmol/L)	26.49±5.23	23.30 ± 6.71	24.70± 5.76	25.27 ± 5.73	0.984	21.74-48.2
Creatinine (µmol/L)	0.644 ± 0.02	0.681 ± 0.04	0.555 ± 0.03	0.485 ±0.02	0.006	0.3-0.78

Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. *Reference range was adapted from* Patel *et al,* (2024).

4.8 Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Renal Histology

Plate 4.8 above shows the histopathological examination of the kidney following four weeks after treatment administration. Showing normal histological structure for the normal saline treatment (A), young and old *M. oleifera* treatments (C and D respectively). Diffuse degeneration of renal epithelium was observed in the iron (III) hydroxide polymaltose treatment (B). No tubular congestion was observed in any of the groups. H&E. 100X. Arrows point to (G) glomeruli, (BC) Bowman's Capsules, (BS) Bowman's Space, (PCT) Proximal Convoluted Tubules, (DCT) Distal Convoluted Tubules, (RT) Renal Tubules**.**

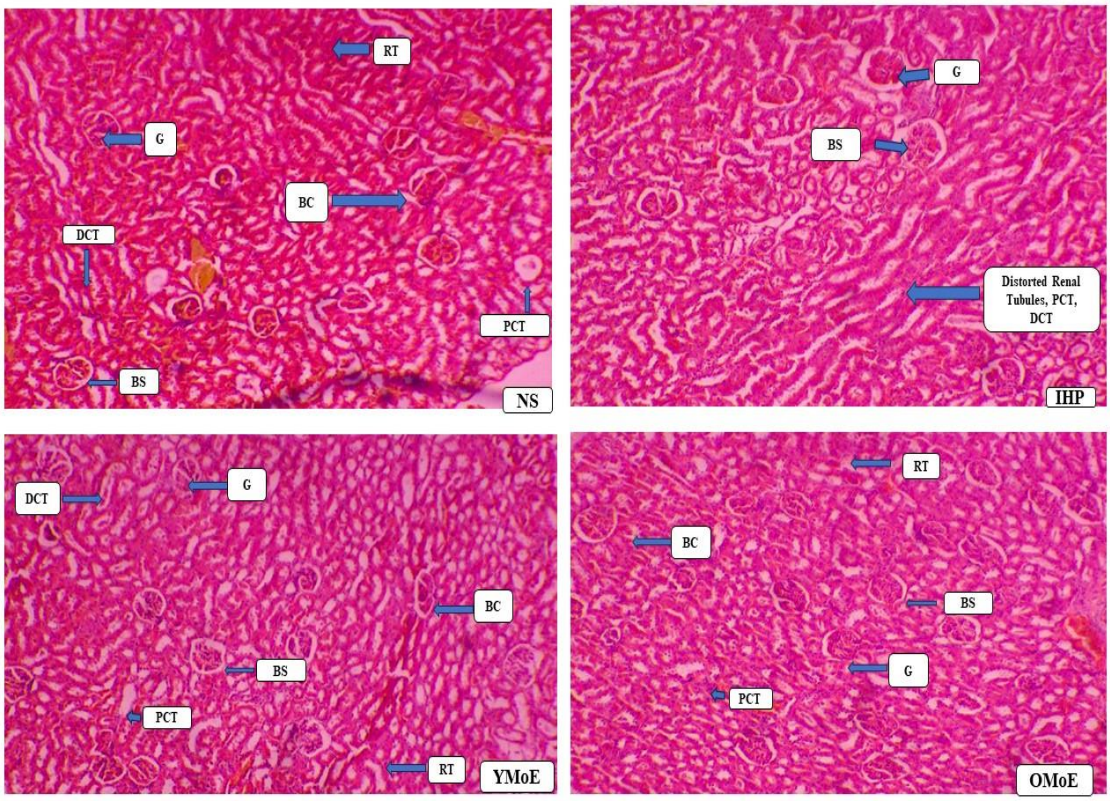

Plate 4.1: Effect of Normal saline, Iron (III) Hydroxide Polymaltose, YMoE, and OMoE Treatments On Renal Function Test

4.9 Effect of Normal Saline, Iron (III) Hydroxide Polymaltose, YMoE, OMoE on Liver Histology

Plate 4.9 shows the histopathological examination of the liver following four weeks of treatment administration. Micrographs showing normal histological structures of the treatment groups (Normal saline (A), Iron (III) hydroxide polymaltose (B), young *M oleifera* (C), *M. oleifera* (D). The central vein indicated by an arrow, hepatic cords, and sinusoids were undeformed. No fatty infiltration of hepatic cells and vacuolar degeneration were seen.

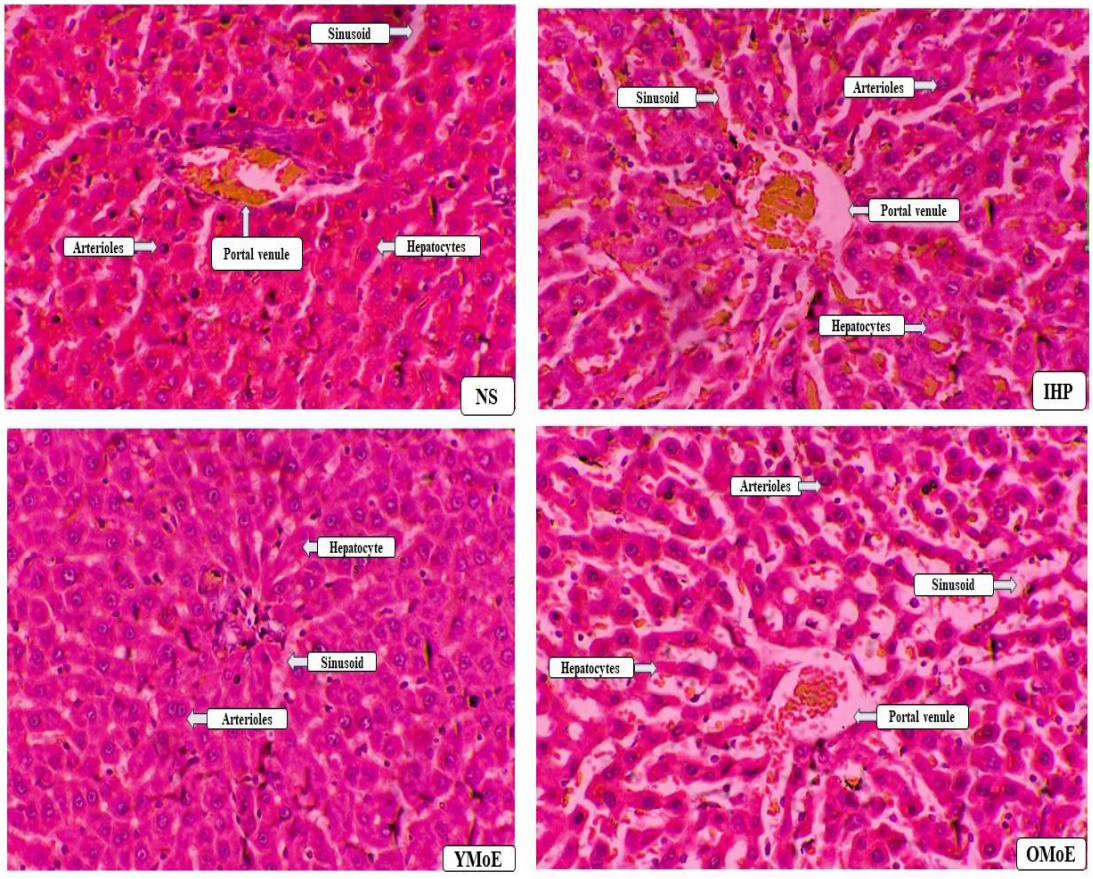

Plate 4.2: Effect of Normal saline, Iron (III) Hydroxide Polymaltose, YMoE, OMoE on Liver Histology

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical Screening

Phytochemicals, also known as bioactive compounds, are non-nutritive plant chemicals with the capacity to exert physiological effects on farm animals. Phytochemical constituents identified in the ethanolic extracts of both young and old *M. oleifera* leaves in the current study is in agreement with a study conducted by Fowoyo & Oladoja (2015); Akinyeye *et al.*(2014). The presence of alkaloids and tannins found in the young leaves of *M. oleifera* which was absent in the old *M. oleifera* extract this is in concordance with studies by Mutwedu (2022). Alkaloids have been associated with various pharmacological properties, such as anti-inflammatory and analgesic effects, as well as potential anticancer activity (Nugraha *et al.,* 2019; Adamski *et al.,* 2020). Tannins, on the other hand, have been shown to possess antioxidant and antimicrobial properties (Sung *et al.,* 2012). Therefore, the presence of these compounds in the young leaves suggests that the young *M. oleifera* leaves may have greater potential for medicinal use compared to the older leaves.

The findings of the qualitative phytochemical screening support Nobossé *et al*. (2018) hypothesis that the phytochemical content and antioxidant activity of fresh *M. oleifera* leaves are influenced by the age of the leaves and the extraction solvent used, as well as their interaction. In their study, they reported that old (aged 45 days) *M. oleifera* leaves are best suited for the production of extracts with the most potent antioxidant activity, this assertion contradicts the findings of this study as the individual secondary metabolites in young *M. oleifera* leaves were higher as compared to the older

moringa oleifera leaves. The disparity between the finding of this present study and that of Nobossé *et al*. (2018) could be attributed to the type of menstruum used in the extraction process. Sigh *et al.* (2022) have reported on a range of phytochemicals in the stem, leaves and pod of *M. oleifera*. This assertion is in line with the findings of the present study. However, Vongsak *et al.* (2013) have also indicated that the presence and amount of these metabolites vary with geographical location, soil type, age of the leave, and climate condition, aside from these factors the presence and quantity of these metabolites are highly influenced by the menstruum used in the extraction process. These are factors that contribute to the degradation of phytochemicals in plants. This could be the reason why alkalnoids and tannins were not detected in the old *M. oleifera* leaves.

Studies have shown that *M. oleifera* shows an abundance of bioactive compounds that provide several health benefits (Kashyap *et al.,* 2022; Rodríguez-Pérez *et al.,* 2015; Chhikara *et al.,* 2021; Milla *et al.,* 2021). Every part of the *M. oleifera* is reported to possess the presence of a group of compounds known as glucosinolates. These compounds are known to have diverse biological activity such as anti-hypotensive, chemoprevention, and antibacterial activities (Sigh *et al.,* 2022). The presence of triterpenoids, alkaloids, glycosides, flavonoids, tannins, and saponin detected in the qualitative phytochemical screening affirms the diverse pharmacological activities that have been attributed to *M. oleifera* (Esmeeta *et al.,* 2022). *M. oleifera* is highly regarded because of its rich content of advantageous phytochemicals which are predominantly present in its leaves (Padayachee & Baijnath, 2019; Namadina *et al.,* 2019). Phytochemicals possess numerous possible biological impacts, hence enhancing the plant's status as a valuable source of food and medicine (Lee *et al.,* 2017). Triterpenoids

identified in the young and old *M. oleifera* leaves in his study could exert positive health effect on humans this assertion is in concordance with a report by Mahmood *et al.* (2018) who have indicated that triterpenoid is known to demonstrate anti-inflammatory characteristics that have the potential to alleviate disorders associated with inflammation.

Moreover, it has been observed that triterpenoids exhibit antioxidant properties, hence mitigating the detrimental effects of oxidative stress and cellular impairment (Mahmood *et al.,* 2018). Triterpenoids have been found to possess anti-inflammatory activities. Chronic inflammation has the potential to impede the synthesis and functionality of red blood cells, hence playing a role in the development of anaemia (McCranor *et al.,* 2014). Triterpenoids have the potential to indirectly aid in the management of anaemia and improve blood parameters through their anti-inflammatory properties (Swallah *et al,.* 2023). The significance of alkaloids detected in the phytochemical screening of this study suggests that it may play a role in the improvement in the body's capacity to absorb vital minerals, such as iron, which is required for the prevention and treatment of iron-deficiency anaemia, the most frequent type of anaemia. Iron-deficiency anaemia is the most prevalent type of anaemia. Both the production of red blood cells and the amount of haemoglobin in the blood can be impacted by an increase in the body's ability to absorb iron (Leone *et al.,* 2015). The presence of glycosides in the leaves of both young and old *M. oleifera* has been associated with potential benefits for cardiovascular well-being, including the regulation of blood pressure and the mitigation of heart disease risk this is in agreement with a study by Chumark *et al.* (2008). Tannins, a type of polyphenolic chemical, have antioxidant properties that help reduce oxidative stress and also exhibit antibacterial

properties (Rana, 2022). The presence of flavonoids in the leaves of *M. oleifera* in this study is in line with an earlier study by Pandey and Rizi (2009). The biological activity of Flavonoids is to safeguard cells against oxidative impairment, provide antioxidant and anti-inflammatory properties that shield red blood cells from oxidative stress and inflammation. By preserving red blood cell integrity, flavonoids may indirectly contribute to improved blood parameters, especially when oxidative stress is a contributing factor to anaemia as indicated by Pandey and Rizi (2009).

Saponins detected in the qualitative phytochemical screening in this study agrees with a study by Borges *et al*. (2015). Saponins have the potential to demonstrate immunomodulatory properties, which could potentially enhance the functioning of the blood and immune system Borges *et al*. (2015). Furthermore, the phytochemicals identified in this current study is in agreement with a study by Odukoya *et al.* (2022) who has reported on phytochemical such as saponins, tannins, and alkaloid in their study on the chemical constituents of crude extract of *M. oleifera* leaf and biochemical response of Wistar rat administered with the crude extract. The study indicated Saponins in *M. oleifera* leaves possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic; permeabilization of the intestine) properties. Even though it has been shown that some saponins are particularly poisonous under research conditions, acute poisoning from saponins is comparatively uncommon in animals and humans (Fowomola, 2010; Fila *et al.,* 2012). The beneficial effect of saponins includes lowering blood cholesterol levels, cancer prevention, bone health, and stimulation of the immune system. Both tannins and alkaloids have also been reported to have the ability to prevent some basic animal and human diseases (Kasolo *et al.,* 2010). In general, the

presence of these Phytochemicals could account for the much-touted medicinal properties of these leaves in various disease conditions.

5.2 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Feed Intake

The highest feed intake recorded under OMoE and YMoE treatments in the present study is in accordance with a study done by Ghebreselassie *et al.* (2011). Asogwa *et al*. (2017) have also found that the administration of *M. oleifera* caused an increase in total feed intake in rats. The finding by Asogwa *et al*. (2017) is in concordance with the findings of this study. The observed significant feed intake in the rats could be attributed to the fact the taste of *M. oleifera* leaves was not objectionable to the experimental rats (Asogwa *et al.,* 2017). This could be the causal factor of the observed high feed intake levels. Crude fiber consists primarily of indigestible cellulose. When compared to other forage plants, *M. oleifera* leaves have a relatively low fiber content (Su & Chen, 2020). Fiber content is what determines the extent and rate of feed digestibility.

It is worth highlighting that the crude fiber content of *M. oleifera* leaves has been reported to be 6.00 - 9.60 % by Sultana (2020) which was considered to be at the acceptable level. This makes moringa leaves a promising ingredient for human and animal diets. The observed low fiber content could also be the reason for the observed relative increase in feed intake among the treatment that received old and young *M. oleifera* extract when compared with those administered with normal saline and Iron (III) hydroxide polymaltose. The finding on the feed intake also suggests that both old and young leaves of *M. oleifera* have similar effects on daily feed intake. The observed reduction in feed intake in week four the treatments could be attributed to reduced

lusciousness that may have resulted from prolonged administration of the extract. Similar results were obtained in an experiment by Zaher *et al.* (2020) who reported that high and prolonged administration of Moringa in diet may result in reduced feed intake and weight as Moringa is thought to comprise various anti-nutritional compounds such as phytase, tannins, cyanide, and oxalates, which may alter the digestibility, metabolism, and absorption of nutrients (Zaher *et al.,* 2020).

It is interesting to note that the observed insignificant feed intake in week four could imply that prolonged intake of *M. oleifera* extract might be effective in weight management, as reduced feed intake could invariably result in reduced weight gain. Furthermore, the observed drop in feed consumption during the fourth week may be attributed to a slow metabolic process of ingested nutrients in the gastrointestinal system. This phenomenon could potentially be influenced by the documented concentration of saponins detected in the extract or a decline in appetite (Odukoya *et al.,* 2022). Previous studies have indicated that elevated levels of saponins can lead to significant decrease in the assimilation of essential nutrients within the gastrointestinal system, mostly attributed to the phenomena commonly referred to as "autointoxication" or "leaky gut" (Jagadeesan, *et al.,* 2022). Similar conclusions were drawn by Nwamarah *et al.* (2015) who observed that aqueous *M. oleifera* extract altered feed intake in Wistar rats and subsequently weight of the rats. This finding supports the claim proposed by Chandra (2012) and Oyewo (2013) that one of the causes of hunger is a deficiency in the levels of vitamins, minerals, and other essential nutritional components. Although *M. oleifera* is so rich in nutrients, it can offer many of these without requiring a significant quantity. This can keep the hunger urges from striking when they are unwanted. The leaves of *M. oleifera* have been found to provide 42 % of

the recommended daily protein together with vitamin and mineral needed. *M. oleifera* has a unique combination of antioxidants and complex proteins that provide an array of health advantages. As a result of enhanced wellbeing induced by the antioxidants and complex proteins in *M. oleifera,* responses to cravings are significantly reduced in individuals with weight problems (Chandra, 2012).

This study is in concordance with earlier studies by Oyewo (2013) and Nwamarah *et al.* (2015) who have reported that prolonged administration of *M. oleifera* could result in decreased feed intake levels. The findings of this study followed a similar trend as the administration of young and old moringa leaf as extract did not produce any level of significance when compared with the other treatments. The findings further affirm the claim that moringa could be effective in the management of obesity (Simeon *et al.,* 2021). Young and old *M. oleifera* leaves showed similar effects suggesting that either of the leaves or both could be used in the management of weight-related problems.

5.3 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Body Weight

The present study revealed that the effect of the administration 100 mg/kg/bw of ethanolic leaf extract of young and old *M. oleifera*, Iron (III) hydroxide polymaltose on body weight had a significant effect on days 21 and 28 when compared with the normal saline treatment. The observed increase in body weight could be ascribed to increased feed intake that was observed among the moringa-treated groups. This finding also follows a similar report by Abijo *et al.* (2019) who found that administration of *M. oleifera* at a dose of 100 mg/kg and 200mg/kg for a period of six weeks significantly increased body weight. Abijo *et al.* (2019) further indicated that *M. oleifera* has the

potency to increase feed intake which resulted in a significant increase in weight of animals in the treatment group when compared with the control group. Even though an insignificant feed intake level was observed among the treatments on the fourth week of treatment administration, however, this did not have any significant effect on the body weight of the rats. The observed improvement in body weight gain following administration of young and old *M. oleifera* may be attributed to the rich content of nutrients in the old leaves of moringa which was efficiently metabolized for growth (Ayo-Ajasa *et al.* 2016).

The finding of this study aligns with research conducted by Nambiar & Seshadri (2001), who reported a notable difference in feed intake and weight gain among vitamin A deficient rats that were fed dehydrated moringa leaves as opposed to synthetic vitamin A. This conclusion aligns with the results reported by previous studies by Osman *et al.,* 2012; Ekundina *et al.,* 2015 which also documented an increase in body weight in rats that were fed diets and extracts containing moringa leaves. Similar results were obtained in the experiment by Madukwe *et al.* (2013) who reported that rats whose diets were supplemented with dry *M. oleifera* leaf powder had higher mean weight gain than the control. Their findings suggest that supplementation of food with *M. oleifera* leaf results both in increased food intake and weight gain. However, the study did not comment on whether young or old leaves of *M. oleifera* possess the weight gain effect. Awodele *et al.* (2012) and Fernand *et al.* (2017) have reported a contrast result to that of the findings of this study. Their study found no significant increase in body weight following the administration *M. oleifera* extracts regardless of the dosage, when compared to the control group. This observation suggests that the administered extract did not induce any significant changes in the metabolic processes of the animals under

study, potentially influencing their hormonal regulation and body weight. Again, in contrast to the results of this research, Suprihartini *et al.* (2023) found that the average increase in body weight of Wistar rats fed with moringa fermented flour was highest in the group of non-anemic rats fed with standard feed. However, the statistical test result showed no significant difference in the rats' weight between the treatment of standard feed and those fed with moringa fermented flour.

The reported insignificant difference in body weight was attributed to the biochemical changes that occur to the vital nutrient composition in moringa during fermentation. The findings of this investigation exhibit notable disparities when compared to the outcomes reported by Atsukwei *et al.* (2014). The results of their study showed a notable decrease in body weight among female rats who were administered *M. oleifera* extract while being fed a high-fat diet. The notable reduction in body weight seen in their study was attributed to the suppression of cholesterol deposition in body tissues or the inhibition of 3-Hydroxy-3-Methyl-Glutaryl-CoenzymeA (HMG CoA) reductase activity. HMG CoA reductase is a crucial regulatory enzyme in the biosynthetic pathway of cholesterol (Rostam *et al.,* 2018). The relevance of this finding suggests that the extract may be a viable recommendation for individuals seeking to achieve weight loss goals. Nevertheless, the researchers also noted that the administration of *M. oleifera* extract did not yield a statistically significant impact on the body weight of male rats. This observation may suggest that the extract possesses potential utility in the context of weight management.

5.4 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Haematological Indices

Both the young and old *M.oleifera* leaf extracts had no significant impact on various b lood parameters, including red blood cells, hematocrit, mean corpuscular volume, mea n corpuscular haemoglobin concentration, mean platelets volume, platelets, and white blood cells, when compared to the normal saline and Iron (III) hydroxide polymaltose treatments

Although the absence of statistical significance was observed, the levels of these indices remained in the normal range as indicated by Patel *et al.,(*2024), suggesting that there was no detrimental effect on the blood indices resulting from the administration of young and old *M. oleifera* extracts administration. The results are consistent with the findings made by Awodele et al., (2012), who found that *M. oleifera* extracts did not cause any notable changes in blood composition.

The normal range of white blood cell counts suggests a potential benefit for individuals with weakened immune systems or those seeking to enhance immunity. The observed increase in white blood cell counts with the young *M. oleifera* extract may be attributed to its nutritional components, consistent with findings by Gupta et al. (2010).

This suggests that Moringa does not only have the potency to improve blood indices it also has an immunomodulatory effect by stimulating the immune system through cellular and humoral immunity (Jayanthi *et al.,* 2015). Similarly, the observed increase effect on platelet levels signifies that both young and *M. oleifera* could improve the rate of clothing as platelets function by maintaining the integrity of the vascular tree,

producing the platelet plug in the first phase of blood clothing, and by producing platelet factor 3, an essential component of the coagulating cascade this is in agreement with the study by Ifeanyi (2018). Platelets might potentially control inflammatory processes by interacting with leukocytes and secreting cytokines and their associated inflammatory mediators. Platelets are rapidly deployed to areas of injury or infection and are able to do so very quickly (Hally *et al.,* 2020).

The observed similarity in the effect of old and young *M. oleifera* leaf extract and Iron (III) hydroxide polymaltose on haematological parameters, particularly with regard to retaining normal ranges, implies that *M. oleifera* could serve as a safe and potentially beneficial dietary source of iron and other essential nutrients for individuals who prefer natural alternatives to iron supplements. This finding affirms the potential nutritional advantages of incorporating *M. oleifera* into one's diet as a supplementary measure to promote general well-being, particularly for individuals who suffer from iron deficiency anaemia (Olusanya, 2018). The observed significant increase in haemoglobin concentration by the old and young *M. oleifera* treatments suggests that *M. oleifera* leaves may contain an appreciable amount of iron and also facilitate its absorption as an adequate amount of this element is necessary for Haemoglobin synthesis and for animal tissues such as the kidneys and bones to take part in the production of RBCs this is similar to an earlier report by Aboubacar Coulibaly *et al*. (2020).

Improved haemoglobin levels can enhance oxygen-carrying capacity, which can possibly benefit individuals with conditions such as iron-deficiency anaemia. This finding agrees with earlier studies by Srole and Ganz (2021). Again, the reported

presence of phytochemical constituents in the extract and also the presence of minerals and vitamins may have contributed to the significant increase in haemoglobin concentration in the rats that were administered with o young and old *M. oleifera* extracts. The bone marrow's ability to produce blood is directly influenced by these constituents, which are known as haemopoietic factors (Nfambi *et al.,* 2015). Previous studies have documented that *M. oleifera* contains a total of 19 amino acids and 14 fatty acids. These amino acids have been identified as crucial components in the body's protein synthesis processes, including the synthesis of plasma proteins that are important in the defense mechanisms of the body Urso and Maffia (2016). Studies have also indicated that *M. oleifera* also contains various micronutrients such as iron, zinc, copper, calcium, manganese, magnesium, potassium, sodium (Mulyaningsih and Yusuf, 2018), sulfur, vitamin E, beta carotene, thiamine, riboflavin, niacin, pyroxide, biotin, ascorbic acid, cholecalciferol, selenium, tocopherol and vitamin K (Shunmugam, 2016; Kumar and Sharma, 2023; Chen *et al.,* 2023). There is a potential for ethanol used as an extraction solvent in the study to have extracted most of these chemicals, hence potentially influencing the observed effects on the haematological parameters. These compounds have played an essential part in the formation and maturity of the body's immune systems, particularly in relation to the cellular components involved in hemopoiesis. The essentiality of micronutrients found in *M. oleifera* leaves lies in their role in facilitating the growth, differentiation, and proliferation of cells within the immune system. The amino acids in *M. oleifera* are significant in the process of globin production, which plays a crucial role in the synthesis of haemoglobin (Dzuvor *et al.,* 2022). Iron, a trace metal found in *M. oleifera*, plays a crucial role in the synthesis of haemoglobin, a protein found in red blood cells as reported by Ameh and Alafi, (2018). The findings from this study are suggestive that both young and old *M. oleifera* extract

had a similar effect of haematological indices which is comparable Iron (III) hydroxide polymaltose.

5.5 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Liver Function Test

The observed insignificant levels of the liver biomarkers suggest that both young and old *M. oleifera* extract did not cause any hepatocellular damage this is in agreement with a study reported by Akpanyung *et al*. (2018); Karthivashan *et al. (*2015); Hamza (2010) who found that *M. oleifera* extract has demonstrated a potential protective effect against liver damage caused by hepatotoxic substances such as carbon tetrachloride and acetaminophen. Its administration has been associated with a reduction in the concentrations of liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase, which serve as indicators of hepatic injury (Patel *et al.,* 2024).

Furthermore, Moringa leaf extract has been observed to enhance the activity of antioxidant enzymes within the liver and elevate the levels of glutathione, a potent antioxidant. These findings collectively imply that the utilization of both young and old *M. oleifera* leaf extract may confer a safeguarding effect against liver damage. More so, the findings from this study also suggest that administration of *M. oleifera* at low dose 100 mg/bw/kg is safe and it did not cause any significant change in the liver enzymes. A similar trend has been reported by Adedapo *et al.* (2020) in their work on safety evaluations of the aqueous extract of the leaves of *M. oleifera* in rats. In their study, different doses of moringa extract were administered to evaluate their effects on liver enzymes. The 400 and 1600 mg/kg doses caused a significant increase in ALT and

AST levels, indicating potential liver cell damage. However, the 800 mg/kg dose resulted in a significant decrease in these enzymes, suggesting a potential protective effect on the liver. Additionally, the 1600 mg/kg dose caused a significant increase in ALP levels, indicating potential cholestasis. Overall, the findings suggest that the 800 mg/kg dose may be the safest for the medicinal use of this extract. The observed effect of both young and old *M. oleifera* extract on liver enzymes could be as result of phytochemicals such as triterpenoids, alkanoids, glycoside, flavonoids, and saponins detected in the leaves through the qualitative analysis. The collected data are consistent with Upadhyay *et al.* (2015), who has asserted that there are many flavonoids in Moringa, such as quercetin, kaempferol, isoquercetin, rhamnetin, etc., but quercetin is thought to be the one that possesses the hepatoprotective effect. Arora and Arora (2021) found that the administration of *M. oleifera* extract over a period of three weeks as a consistent dietary regimen resulted in a notable decrease in liver damage. This observed impact can be attributed to the presence of many phytochemicals within the plant.

5.6 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Lipid Profile

Lipid profile plays a pivotal role in assessing cardiovascular health, and their effective control is essential for mitigating the possibility of developing heart-related ailments (Masenga *et al.,* 2023). The primary finding from this study indicated that no significant effect was observed on the lipid profile parameters following the administration of young and old moringa extracts. This finding is in concordance with earlier studies reported by Phimarn *et al.* (2021) and Omodanisi *et al.* (2017).

Having lipid profile values within normal ranges is indicative that administration of either young or old M. *oleifera* extracts has the potency to improve cardiovascular health and overall well-being. It also suggests a reduced risk of heart disease, atherosclerosis, and complications related to cardiovascular disease (Powell-Wiley *et al.,* 2021). The finding of this study also affirms the claim that *M. oleifera* possesses an anti-lipidemic effect owing to its diverse phytochemical constituents (Sharma *et al.,* 2022; Kesharwani *et al.,* 2014; Prabu *et al.,* 2019). This finding is in agreement with a study by Sandoval and Jimeno (2013), who reported that the administration of *M. oleifera* capsules had a similar effect on lipid profile indices (HDL, LDL, total cholesterol, and triglycerides) when compared with the placebo group. The study further indicated that the administration of *M. oleifera* did not have any adverse effects, making it safe for consumption. Similar conclusions were drawn by Sari and Suwondo (2022), who indicated that administering a treatment consisting of Moringa leaf extract for a period of at least 14 days and up to 30 days to hyperlipidemic individuals successfully lowers total cholesterol, LDL, and triglyceride levels while simultaneously raising HDL.

Sari and Suwondo concluded that complementary therapy consisting of *M. oleifera* extract holds a promising future for the treatment and management of hyperlipidemia. Similar to the findings of this current study, other studies have also reported on the hypolipidemic effect of *M. oleifera*. According to research results published by Kim & Kim (2019), male C57BL/6J mice that were fed a high-fat diet (HFD) supplemented with 0.1% leaf powder of *M. oleifera* for seven weeks showed several beneficial effects. Notably, the supplements prevented their bodies from accumulating fat and from developing hypercholesterolemia, or high cholesterol. In addition, there was a decrease

in the elevated levels of triglycerides (TG), cholesterol (CHO), and low-density lipoprotein cholesterol (LDL-C) that the HFD induced. These results imply that the addition of *M. oleifera* leaf powder to the diet protected against lipid metabolism and fat deposition, which may have enhanced the mice's cardiovascular health. In the same way, a study by Madkhali *et al.* (2019) presents further findings about the possible advantages of *M. oleifera* extract in relation to lipid metabolism. In this study, Albino Wistar rats were subjected to a high-fat diet (HFD) and afterward received oral administration of methanolic *M. oleifera* extract at doses of either 200 or 400 mg/kg/day for a duration of three weeks. Notably, these rats exhibited prominent improvements in their lipid profiles. The administration of the *M. oleifera* extract resulted in significant decreases in levels of cholesterol (CHO), triglycerides (TG), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL). Concurrently, it resulted in an elevation of high-density lipoprotein (HDL) concentrations.

The results on lipid profile of this current study suggests that incorporation of M. *oleifera* leaves in a diet may have a positive impact on lipid profiles and cardiovascular well-being. This may be achieved by through a reduction in levels of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), as well as an increase in levels of high-density lipoprotein (HDL). Even though most of the documented studies on the effect of *M. oleifera* on lipid profile did not indicate which of the leaf type whether young or old has the lipidemic effect, however, the findings from this study shows that both the young and old leaves of *M. oleifera* have a similar effect on maintaining the lipid profile value in the normal range as indicated by Patel *et al.* (2024).

5.7 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Renal Function Test

The indicators utilized in renal function tests are employed to evaluate the proper functioning of the kidneys. These markers have the potential to exhibit both radioactive and non-radioactive properties (Gounden, 2018). These measurements provide information about the glomerular filtration rate as well as the kidneys' ability to concentrate and dilute substances, which is indicative of their tubular function (Lopez-Giacoman *et al.,* 2015). The presence of changes in the values of these indicators is indicative of renal failure (Gowda *et al.,* 2010). In this study, results show that administration of 100 mg/bw/kg of old and young *M. oleifera* extract did not have any significant effect on serum urea levels when compared with rats that received a similar dose of iron (III) hydroxide polymaltose and normal saline this finding conforms to the findings of Saleh & Sarah (2019). As indicated by Gowda *et al.* (*2010*), urea is a significant nitrogenous compound that serves as the primary result of the degradation of proteins and amino acids.

Elevated blood urea nitrogen (BUN) levels have been observed in conjunction with renal diseases, obstruction of the urinary system caused by a renal calculus, congestive heart failure, desiccation, pyrexia, shock, and gastrointestinal hemorrhage Gowda *et al*. (2010). In comparison with the findings indicated by Gowda *et al.* (2010), a similar finding was observed as the administration of old and young *M. oleifera* extract did not have any detrimental effect on the renal function of the experimental rats. This finding is in line with a study by Khalid *et al.* (2023) who have reported that *M. oleifera* has been shown to be non-toxic and has been shown to promote nephron-hepatic function. Saleh & Sarhat (2019) have also reported that due to the numerous ethnomedical

properties of *M. oleifera* in reducing Blood Urea Nitrogen (BUN), it is considered the preferred remedy for the treatment of kidney disorders. The observed variations in Creatinine levels across the treatments (Normal saline, Iron (III) hydroxide polymaltose, Young *M. oleifera* extract (YMoE), and Old *M. oleifera* extract (OMoE) indicate that the different treatments exerted distinct impacts on renal function. The observed decrease in Creatinine levels in the YMoE and OMoE groups, as compared to the Iron (III) hydroxide polymaltose group, suggests the possibility of moringa having protective effects on renal function. Reduced levels of creatinine are frequently correlated with enhanced renal function, hence indicating possible therapeutic significance. This observation may imply a beneficial influence of *M. oleifera* extracts on renal health. This agrees with a study by Khalid *et al.* (2023) who reported that creatinine is produced from muscle creatine and is excreted through the kidneys at a constant rate.

An increase beyond the reference values is an indicator of impaired kidney disease. Their study further reported that the administration of *M. oleifera* extract 150mg/kg reduced creatine levels, while the dose of 500mg/kg increased creatine levels. The rise in creatine levels was attributed to other factors such as stress, dehydration, and season and not necessarily the effect of the extract. In support of the findings of this current study, research conducted by Karthivashan *et al.* (2016), Ahmed *et al.* (2020), Tang *et al.* (2017), and Ling & Kuo (2018) offer significant contributions to our understanding of the possible renal protective properties of *M. oleifera* extracts. Collectively, the results of this research give evidence that suggests that extracts from *M. oleifera* may have renal protective properties. They point to the potential benefits of moringa extracts in enhancing kidney function, by lowering markers of kidney damage (such as

creatinine and Blood urea nitrogen), and maybe moderating oxidative stress that might have an impact on renal health.

5.8 Effect of Normal Saline, Iron (Iii) Hydroxide Polymaltose, YMoE, and OMoE Treatments on Renal and Liver Histology

The histological investigation provides understanding into the structural condition of renal tissues following a four-week period of treatment administration. Significantly, the treatment that received normal saline (A) showed a kidney section characterized by an intact histological architecture. Likewise, it was observed that the young and old *M. oleifera* treatments (C and D) displayed a kidney section that showed a histological structure indicative of normalcy. This finding implies that the administration of these extracts did not result in any structural abnormalities or detrimental effects on the kidney tissues, suggesting the possible protective effects of young and old *M. oleifera* extracts on kidney function. This conclusion is very similar to the findings reported by El-Gharabawy *et al*. (2019), who suggested that *M. oleifera* has the potential to have beneficial effects on the liver and kidneys by repairing the damage that Monosodium glutamate causes to these organs, as demonstrated by observations made in rats.

Similarly, a study by Wijayanti *et al.* (2023), reported that malondialdehyde (MDA) levels in the liver and kidneys of hepatotoxic and nephrotoxic rats were reduced in response to the administration of *M. oleifera* leaf extract, which was found to have therapeutic effects. Histological examinations of the liver and kidneys also revealed that the extract restored damages caused by gentamicin. *M. oleifera* leaf extract could prevent hepato-nephrotoxicity and the accompanying oxidative stress. In contrast, Iron (III) hydroxide polymaltose treatment exhibits widespread deterioration

of the renal epithelium. This finding elicits concerns over the possible detrimental impacts of prolonged usage of synthetic Iron (III) hydroxide polymaltose on the integrity of renal tissue. The kidneys were responsible for the elimination of iron (III) hydroxide polymaltose, the results from this study suggest that high dose or prolonged usage of iron (III) hydroxide polymaltose as an iron supplement for boosting blood or treating iron deficiency anaemia may cause degenerative changes or abnormal function of the urinary system (Abdulazeez *et al.,* 2020). Histopathological studies also provided crucial evidence to support the biochemical analysis and antioxidant status of the liver (Jiang *et al.,* 2012). Results from the liver histology revealed that the administration of normal saline, iron (III) hydroxide polymaltose, and 100 mg/kg/bw of young and old *M. oleifera* did not have any detrimental effect on the liver architecture. The liver structures were undeformed, this is indicative that both old and young *M. oleifera* exhibited a similar effect when compared with the normal saline and, iron (III) hydroxide polymaltose treatments.

This affirms the already established claim that *M. oleifera* is nontoxic and has hepatoprotective effect. This finding correlates with a report by Aborhyem *et al*. (2016), who stated that consumption of *M. oleifera* daily as an herb and spice should be encouraged as it has no detrimental effect on liver function and histology. Contrary to what Aborhyem *et al.* (2016) have reported, Abdulazeez *et al.* (2020) have reported that in as much as *M. oleifera* may have a beneficial effect on liver function and histology, they have cautioned that high doses (1000 and 2000 mg/kg) of moringa extract caused a deleterious effect on the liver, kidney, and the brain on Wistar rats. Therefore, moderate to low doses of *M. oleifera* given via oral administration may be safe and may not have cytotoxic effects on the brain, liver, or kidneys**.**

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The findings of this research shed light on the following key observations:

1. Qualitative phytochemical screening revealed the presence of triterpenoids, glycosides, flavonoids, and saponins in both young and old *M. oleifera* leaves. Notably, alkaloids and tannins were exclusive to young leaves, while anthraquinones, steroids, and terpenoids were absent in both. The administration of old and young *M. oleifera* extracts exhibited significant effect on feed intake in rats.
2. Haematological parameters, such as red blood cells, mean corpuscular volume, mean corpuscular haemoglobin concentration, and mean platelet volume, remained largely unaffected by both extracts. However, there was a significant effect on haemoglobin which mirrored the effects of iron (III) hydroxide polymaltose.
3. Liver enzymes, renal indices and lipid profile stayed within normal physiological ranges and were not significantly altered by the administration 100 mg/kg/bw of young and old *M. oleifera* leaf extracts. Blood urea nitrogen remained largely unaffected following the administration of 100 mg/kg/bw of young and old *Moringa oleifera* extracts.
4. Histopathological examinations of the kidney and liver revealed normal architecture for the normal saline and *M. oleifera* treatments.

6.2 Recommendations

Based on the findings of this current study, the following recommendations are suggested;

i. Further research should aim to isolate, identify, and quantify the exact phytochemical constituents present in both young and old leaves of *M. oleifera*, to elucidate their respective impacts on body weight, blood parameters, renal and liver histology.

ii. Dose-response research is required to ascertain the effects that are dependent on concentration and to develop the most effective dosages for prospective therapeutic uses.

iii. Further studies should focus on the evaluation of iron concentration in the leaves of young and old *M. oleifera.*

iv. Additional investigation is imperative to clarify the fundamental mechanism and clinical ramification of the observed histological findings, particularly concerning the influence of iron (III) hydroxide polymaltose on renal function and integrity.

REFERENCES

Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi, V., Adeoye, A. M., Adetokunboh, O. O., … Amini, S. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 396(10258), 1204–1222

Abdulazeez, N., Yusuf, H. I., Karofi, D. H., Pilau, N. N., Abubakar, A. A., Ibrahim, B., & Abdullahi, A. M. (2020). Effects of Moringa oleifera biochemical constituents on kidney, liver and brain of Wister rats. Science, 8(4), 128-132.

Abijo, A. Z., Adeeyo, O. O., Komolafe, O. A., Saka, O. S., & Abodunrin, V. K. (2019). Effects of moringa oleifera on the developing cerebrum of young wistar rats. *Anatomy Journal of Africa*, *8*(1), 1336-1341.

Aborhyem, S., Ismail, H., Agamy, N., & Tayel, D. (2016). Effect of Moringa oleifera on lipid profile in rats. *Journal of High Institute of Public Health*, 46(1), 8-14.

Abou-Zeid, S. M., Ahmed, A. I., Awad, A., Mohammed, W. A., Metwally, M. M. M., Almeer, R., Abdel-Daim, M. M., & Khalil, S. R. (2021). Moringa oleifera ethanolic extract attenuates tilmicosin-induced renal damage in male rats via suppression of oxidative stress, inflammatory injury, and intermediate filament proteins mRNA expression. Biomedicine and Pharmacotherapy, 133(September 2020).

Abu, B. A., Buttner, N., Garror, O. D., Stefanic, R., Sandow, A., & Pereko, K. A. (2021). Qualitative assessments of anaemia□related programs in Ghana reveal gaps and implementation challenges. Annals of the New York Academy of Sciences, 1492(1), 27-41.

Achuba FI, Ubogu LA, & Ekute BO. (2016). Moringa oleifera Attenuates Crude Oil Contaminated Diet Induced Biochemical Effects in Wistar Albino Rats. *Pharmaceutical and Biosciences Journal*, October, 70–77.

Adamski, Z., Blythe, L. L., Milella, L., & Bufo, S. A. (2020). Biological activities of alkaloids: from toxicology to pharmacology. *Toxins*, *12*(4), 210.

Adedapo, A. A., Ogunmiluyi, I. O., Falayi, O. O., Ogunpolu, B. S., Oyagbemi, A. A., Orishadipe, A., ... & Oguntibeju, O. O. (2020). The lyophilized aqueous leaf extract of Moringa oleifera blunts streptozocin-induced diabetes in rats through upregulation of GLUT 4 signaling pathway and anti-oxidant effect. *Scientific African*, *10*, e00619.

Adeyemi, O. S., & Elebiyo, T. C. (2014a). Moringa oleifera Supplemented Diets Prevented Nickel-Induced Nephrotoxicity in Wistar Rats. *Journal of Nutrition and Metabolism*, 2014.

Adias, T. C., Ajugwo, A. O., Erhabor, T., & Nyenke, C. U. (2013). Effect of Pumpkin Extract (Telfairia occidentalis) on Routine Haematological Parameters in Acetone-Induced Oxidative Stress Albino Rats. November 1–4.

Adusei, S., Azupio, S., & Tei, E. (2022). Phytochemistry, nutritional composition and pharmacological potential of Moringa oleifera: A comprehensive review. International *Journal of Plant Based Pharmaceuticals*, 2(2), 228–238.

Ahmed, N. F., Sadek, K. M., Soliman, M. K., Khalil, R. H., Khafaga, A. F., Ajarem, J. S., ... & Allam, A. A. (2020). Moringa oleifera leaf extract repairs the oxidative misbalance following sub-chronic exposure to sodium fluoride in nile tilapia Oreochromis niloticus. Animals, 10(4), 626.

Airaodion, A. I., Ogbuagu, U., Ogbuagu, E. O., Ekenjoku, J. A., & Airaodion, E. O. (2019). Protective Effect of ethanolic leaf extract of Moringa oleifera on haematological indices of rats fed with crude oil-treated diet. *International Journal of Bio-Science and Bio-Technology*, *11*(8), 84-92.

Akinyeye, A. J., Solanke, E. O., & Adebiyi, I. O. (2014). Phytochemical and antimicrobial evaluation of leaf and seed of Moringa oleifera extracts. *International Journal of Research in Medicine and Health Sciences*, *4*(6), 2307-2083.

Akpanyung, E. O., Nwaokonko, D. U., Ekong, M. B., & Ekpo, M. M. (2018). Evaluation of the protective effect of Moringa oleifera leaf extract against aluminium induced liver damage in male albino Wistar rats. *International Journal of Sciences*, 7(2), 20-30.

Akter, S., Netzel, M., Tinggi, U., Fletcher, M., Osborne, S., & Sultanbawa, Y. (2020). Interactions between phytochemicals and minerals in Terminalia ferdinandiana and implications for mineral bioavailability. Frontiers in Nutrition, 7, 598219.

Alain Mune Mune, M., Nyobe, E. C., Bakwo Bassogog, C., & Minka, S. R. (2016). A comparison on the nutritional quality of proteins from Moringa oleifera leaves and seeds. Cogent Food and Agriculture, 2(1).

Ali Redha, A., Perna, S., Riva, A., Petrangolini, G., Peroni, G., Nichetti, M., Iannello, G., Naso, M., Faliva, M. A., & Rondanelli, M. (2021). Novel insights on anti-obesity potential of the miracle tree, Moringa oleifera: A systematic review. *In Journal of Functional Foods* (Vol. 84, p. 104600). Elsevier Ltd.

Allahmoradi, M., Alimohammadi, S., & Cheraghi, H. (2019). Protective effect of Cynara scolymus l. on blood biochemical parameters and liver histopathological changes in phenylhydrazine-induced hemolytic anaemia in rats. *Pharmaceutical and Biomedical Research*, *5*(4), 53-62.

Allen, J. C., Issa, J. Y., & Cai, W. (2014). Calcium content, in vitro digestibility, and bioaccessibility in leaves of spinach (Spinacia oleracea), sweet potato (Ipomea batatas), and drumstick tree (*Moringa oleifera*). F1000Research, 3, 65.

Almatrafi, M. M., Vergara-Jimenez, M., Murillo, A. G., Norris, G. H., Blesso, C. N., & Fernandez, M. L. (2017). Moringa leaves prevent hepatic lipid accumulation and inflammation in guinea pigs by reducing the expression of genes involved in lipid metabolism. *International Journal of Molecular Sciences*, 18(7).

Al-Naseem, A., Sallam, A., Choudhury, S., & Thachil, J. (2021). Iron deficiency without anaemia: A diagnosis that matters. Clinical Medicine, *Journal of the Royal College of Physicians of London*, 21(2), 107–113.

Aly, O., Abouelfadl, D. M., Shaker, O. G., Hegazy, G. A., Fayez, A. M., & Zaki, H. H. (2020). Hepatoprotective effect of Moringa oleifera extract on TNF-α and TGF-β expression in acetaminophen-induced liver fibrosis in rats. *Egyptian Journal of Medical Human Genetics*, 21(1).

Amaglo, N. K., Bennett, R. N., Curto, R. B. L., Rosa, E. A., Turco, V. L., Giuffrida, A., ... & Timpo, G. M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa oleifera L., grown in Ghana. *Food Chemistry*, *122*(4), 1047-1054.

Ameh, S. S., & Alafi, O. F. (2018). Effect of ethanol extract of Moringa oleifera leaves in protecting anaemia induced in rats by aluminium chloride. IOSR J Biotechnol Biochem, 4(6), 34-52.

Andlid, T. A., D'Aimmo, M. R., & Jastrebova, J. (2018). Folate and Bifidobacteria. In The Bifidobacteria and Related Organisms: Biology, Taxonomy, Applications. Elsevier Inc.

Araújo, L. C. C., Aguiar, J. S., Napoleão, T. H., Mota, F. V. B., Barros, A. L. S., Moura, M. C., Coriolano, M. C., Coelho, L. C. B. B., Silva, T. G., & Paiva, P. M. G. (2013). Evaluation of cytotoxic and anti-inflammatory activities of extracts and lectins from *Moringa oleifera* seeds. PLoS ONE, 8(12), 1–15.

Arora, S., & Arora, S. (2021). Nutritional significance and therapeutic potential of *Moringa oleifera*: The wonder plant. Journal of Food Biochemistry, 45(10), e13933.

Aryeetey, R., Atuobi-Yeboah, A., Billings, L., Nisbett, N., van den Bold, M., & Toure, M. (2022). Stories of Change in Nutrition in Ghana: a focus on stunting and anaemia among children under-five years (2009–2018). Food Security, 14(2), 355-379.

Asare, E. V., Wilson, I., Benneh-Akwasi Kuma, A. A., Dei-Adomakoh, Y., Sey, F., & Olayemi, E. (2018). Burden of Sickle Cell Disease in Ghana: The Korle-Bu Experience. Advances in Haematology, 2018.

Asare, G. A., Gyan, B., Bugyei, K., Adjei, S., Mahama, R., Addo, P., Otu-Nyarko, L., Wiredu, E. K., & Nyarko, A. (2012). Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. Journal of Ethnopharmacology, 139(1), 265–272.

Asogwa, I. S., Ani, J. C., & Okoye, E. C. (2017). The Hypolipidemic Effect of Moringa Leaf Powder Supplementation in High Fat Diet Fed Rats. *Journal of Nutritional Ecology and Food Research*, *4*(2), 161-166.

Asomugha, A. L., Ezejindu, D. N., Asomugha, R. N., Anyabolu, A. E., & Ojukwu, P. C. (2015). Evaluation of toxicity effect of graded doses of Moringa oleifera leaf extract on blood indices using 20 adult Wistar rats. *Int J Biom Adv Res,* 6(2), 98-102.

Atsukwei, D., Eze, E. D., Adams, M. D., Adinoyi, S. S., & Ukpabi, C. N. (2014). Hypolipidaemic effect of ethanol leaf extract of Moringa oleifera Lam. in experimentally induced hypercholesterolemic wistar rats. *Int J Nutr Food Sci*, *3*(4), 355-360.

Awodele, O., Oreagba, I. A., Odoma, S., da Silva, J. A. T., & Osunkalu, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). Journal of ethnopharmacology, 139(2), 330-336.Moringa oleifera leaves extract in adult Wistar rats. *Journal of natural sciences Research*, 5(3), 1-9.

Aya, V. E., Alaku, A. I., Barde, R. E., & Adua, M. M. (2015). Effects of Moringa oleifera leaves on Body weight and Haematological parameters of Rabbits infected with Trypanasoma congolence Effects of Moringa oleifera leaves on Body weight and Haematological parameters of Rabbits infected with Trypanasoma congolence. December.

Ayo-Ajasa, O. Y., Abiona, J. A., Fafiolu, A. O., Egbeyale, L. T., Njoku, C. P., Omotayo, I. G., ... & Abel, F. A. S. (2016). Performance characteristics of broilers fed graded levels of Moringa oleifera leaf meal. *Malaysian Journal of Animal Science*, 19(1), 23-31.

Azlan, U. K., Mediani, A., Rohani, E. R., Tong, X., Han, R., Misnan, N. M., Jam, F. A., Bunawan, H., Sarian, M. N., & Hamezah, H. S. (2022). A Comprehensive Review with Updated Future Perspectives on the Ethnomedicinal and Pharmacological Aspects of Moringa oleifera. Molecules, 27(18).

Baird, D. C., Batten, S. H., & Sparks, S. K. (2022). Alpha-and Beta-thalassemia: rapid evidence review. American family physician, 105(3), 272-280.

Baldi, A., & Pasricha, S. R. (2022). Anaemia: Worldwide Prevalence and Progress in Reduction. In Nutritional Anaemia (pp. 3-17). Cham: Springer International Publishing.

Balk, J., Connorton, J. M., Wan, Y., Lovegrove, A., Moore, K. L., Uauy, C., Sharp, P. A., & Shewry, P. R. (2019). Improving wheat as a source of iron and zinc for global nutrition. Nutrition Bulletin, 44(1), 53–59.

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2015). Antibacterial activity and mode of action of selected glucosinolate hydrolysis products against bacterial pathogens. *Journal of Food Science and Technology*, 52(8), 4737-4748.

Briglia, M., Rossi, M. A., & Faggio, C. (2017). Eryptosis: ally or enemy. *Current Medicinal Chemistry*, *24*(9), 937-942.

Buttner, N., Garror, O. D., Stefanic, R., Sandow, A., & Abu, B. A. Z. (2020). Reveal gaps and implementation challenges Original Article Qualitative assessments of anaemia-related programs in Ghana reveal gaps and implementation challenges. January 2021.

Camaschella, C. (2015). Iron deficiency: new insights into diagnosis and treatment. *Hematology 2014, the American Society of Hematology Education Program Book*, *2015*(1), 8-13.

Center for disease control and prevention (2022). Center for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)

Chaparro, C. M., & Suchdev, P. S. (2019). Anaemia epidemiology, pathophysiology, and etiology in low□and middle□income countries. *Annals of the New York Academy of Sciences,* 1450(1), 15-31.

Chaparro, C. M., & Suchdev, P. S. (2019). Anemia epidemiology, pathophysiology, and etiology in low□and middle□income countries. *Annals of the New York Academy of Sciences*, *1450*(1), 15-31.

Chen, B., Miao, J., Ye, H., Xia, Z., Huang, W., Guo, J., ... & Cao, Y. (2023). Purification, Identification, and Mechanistic Investigation of Novel Selenium-Enriched Antioxidant Peptides from Moringa oleifera Seeds. *Journal of Agricultural and Food Chemistry*, 71(11), 4625-4637.

Chhikara, N., Kaur, A., Mann, S., Garg, M. K., Sofi, S. A., & Panghal, A. (2021). Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: An updated review. *Nutrition & Food Science*, 51(2), 255-277.

Chivapat, S., Sincharoenpokai, P., Saktiyasuthorn, N., Shuaprom, A., Sakpetch, A., & Rungsipipat, A. (2011). Acute and chronic toxicity of Moringa oleifera Linn leaves extracts. *The Thai Journal of Veterinary Medicine*, *41*(4), 417-424.

Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N. P., Phivthong-Ngam, L., ... & Chivapat, S. (2008). The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. *leaves. Journal of Ethnopharmacology*, 116(3), 439-446.

Cliff, R., Curran, J., Hirota, J. A., Brauer, M., Feldman, H., Carlsten, C., Thomson, E. M., Pal, S., Guénette, J., Wade, M. G., Atlas, E., Holloway, A. C., Williams, A., Vincent, R., Das, P., & Chatterjee, P. (2016). Effect of diesel exhaust inhalation on blood markers of inflammation and neurotoxicity: A controlled, blinded crossover study. Toxicological Sciences, 22(1), 343–349.

Coppin, J. P., Xu, Y., Chen, H., Pan, M. H., Ho, C. T., Juliani, R., Simon, J. E., & Wu, Q. (2013). Determination of flavonoids by LC/MS and anti-inflammatory activity in Moringa oleifera. *Journal of Functional Foods*, 5(4), 1892–1899.

Costa, E. A., & de Paula Ayres-Silva, J. (2023). Global profile of anaemia during pregnancy versus country income overview: 19 years estimative (2000–2019). *Annals of Haematology*, 102(8), 2025.

Coulibaly, A., Gnangoran, B. N., Oussou, J. B. N. G., & Bleyere, M. N. (2020). Evaluation of Moringa oleifera Lam leaves (Moringaceae) diets against induced anaemia in Wistar rats. *J. Nutr. Food Sci*, *2*(3), 101-106.

Csurhes S. and Navie S. 2010. Invasive plant risk assessment (Horseradish *tree-Moringa oleifera).* Department of Agriculture and Fisheries, Biosecurity Queensland.

Das, P., & Chatterjee, P. (2014). Assessment of haematological profiles of adult male athletes from two different air pollutant zones of West Bengal, India. *Environmental Science and Pollution Research,* 22(1), 343–349.

Das, R., Mitra, S., Tareq, A. M., Emran, T. B., Hossain, M. J., Alqahtani, A. M., ... & Simal-Gandara, J. (2022). Medicinal plants used against hepatic disorders in Bangladesh: A comprehensive review. *Journal of Ethnopharmacology*, 282, 114588.

de las Cuevas Allende, R., Díaz de Entresotos, L., & Conde Díez, S. (2021). Anaemia of chronic diseases: Pathophysiology, diagnosis and treatment. *Medicina Clínica (English Edition),* 156(5), 235–242.

De. S, Dey. Y. N, & Ghosh. A.K. (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuberof *Amorphaphallus paeoniifolius (Araceae). Int J Pharm Biol Res*.;1(5):150-7.

Di Renzo, G. C., Spano, F., Giardina, I., Brillo, E., Clerici, G., & Roura, L. C. (2015). Iron deficiency anaemia in pregnancy. Women's Health, 11(6), 891–900.

Dzuvor, C. K., Pan, S., Amanze, C., Amuzu, P., Asakiya, C., & Kubi, F. (2022). Bioactive components from Moringa oleifera seeds: production, functionalities and applications–a critical review. *Critical Reviews in Biotechnology*, 42(2), 271-293.

Effah-Yeboah, E., Dartey, E., Asare, E. A., Abraham, J. D., Kagya-Agyemang, J. K., Balali, G. I., & Aboagye, V. (2021). Effect of Kalanchoe crenata extract on renal and liver impairment, dyslipedemia and glycemia in streptozotocin induced diabetic rats. *Annual Research & Review in Biology*, 109-125.

Egba, G., Larbi, I. A., Nti, H., & Sciences, A. (2022). Anaemia prevalence and associated factors among school-age children in Accra and Kumasi metropolis in Ghana. April.

Ekpo, G., Ofem, E., Bassey, S., Abam, K., Arikpo, I., Enobong, J., ... & Ekam, V. (2019). Antipuritive Effect of Aqueous Root Extracts of Moringa oliefera on Alcohol-induced Haematoxicity in Wistar Rats. *International Blood Research & Reviews*, *9*(4), 1-11.

Ekundina, V. O., Ebeye, O. A., Oladele, A. A., & Osham, G. O. (2015). Hepatotoxic and nephrotoxic effects of Moringa oleifera leaves extract in adult Wistar rats. *Journal of natural sciences Research*, *5*(3), 1-9.

El-bakry, K., Toson, E. S., Serag, M., & Aboser, M. (2016). Hepatoprotective effect of Moringa oleifera leaves extract against carbon tetrachloride-induced liver damage in rats. *World J Pharm Pharm Sci,* 5(5), 76-89.

Elbarbary, M., Honda, T., Morgan, G., Guo, Y., Guo, Y., Kowal, P., & Negin, J. (2020). Ambient air pollution exposure association with anaemia prevalence and haemoglobin levels in Chinese older adults. *International Journal of Environmental Research and Public Health*, 17(9), 1–15.

El-behairy, D. S. A., Diaa Eldin, N. H., Elbadry, E. E., Mohamed, A. A., & Farrag, I. M. (2019). Hepato-Renal Toxicity of Titanium Dioxide Nanoparticles and The Protective Effects of Moringa oleifera Leaves Extract and Vitamin E in Male Albino Rats. *The Egyptian Journal of Hospital Medicine,* 75(2),

El-Gharabawy, R. M., Ahmed, A. S., & Al-Adhadh, T. I. (2019). Ameliorating effect of Moringa against liver and kidney injury induced by monosodium glutamate. *Annual Research & Review in Biology*, 1-10.

Elysium (2011). The global prevalence of anaemia in 2011.Who,1-48.

Engmann, C., Walega, P., Aborigo, R. A., Adongo, P., Moyer, C. A., Lavasani, L., Williams, J., Bose, C., Binka, F., & Hodgson, A. (2012). Stillbirths and early neonatal mortality in rural Northern Ghana. *Tropical Medicine and International Health*, 17(3), 272–282.

Escalante, C. P., Chisolm, S., Song, J., Richardson, M., Salkeld, E., Aoki, E., & Garcia Manero, G. (2019). Fatigue, symptom burden, and health related quality of life in patients with myelodysplastic syndrome, aplastic anemia, and paroxysmal nocturnal hemoglobinuria. *Cancer medicine*, *8*(2), 543-553.

Esmeeta, A., Adhikary, S., Dharshnaa, V., Swarnamughi, P., Maqsummiya, Z. U., Banerjee, A., ... & Duttaroy, A. K. (2022). Plant-derived bioactive compounds in colon cancer treatment: An updated review. *Biomedicine & Pharmacotherapy*, 153, 113384.

Farid, A. S., & Hegazy, A. M. (2020). Ameliorative effects of Moringa oleifera leaf extract on levofloxacin-induced hepatic toxicity in rats. *Drug and Chemical Toxicology*, *43*(6), 616-622.

Fernand, L. O. A., Bertrand, A. E. V., Pascal, O. A., Theophile, T. O. H. O. U. E. G. N. O. N., Anatole, L. A. L. E. Y. E., & Hyacinthe, A. H. I. S. S. O. U. (2017). Evaluation of antihyperglycemic, antiradical and acute oral toxicity activities of aqueous leaves extract of Moringa oleifera Lam (Moringaceae) from Benin in normal rats. *African Journal of Biotechnology*, 16(27), 1513-1519.

Fila, W. O., Johnson, J. T., Edem, P. N., Odey, M. O., Ekam, V. S., Ujong, U. P., & Eteng, O. E. (2012). Comparative anti-nutrients assessment of pulp, seed and rind of rambutan (Nephelium lappaceum). *Annals of Biological Research*, 3(11), 5151-5156.

Fowomola, M. A. (2010). Some nutrients and antinutrients contents of mango (Magnifera indica) seed. African Journal of Food Science, 4(8), 472-476.

Fowoyo, P. T., & Oladoja, E. O. (2015). Phytochemical screening, nutritional composition and antimicrobial activity of Moringa oleifera seed and leaf extract against selected gastrointestinal pathogens. *Journal of Pharmacy and Biological Sciences*, *10*(6), 116-124.

Fraser, I. S., Mansour, D., Breymann, C., Hoffman, C., Mezzacasa, A., & Petraglia, F. (2015). International Journal of Gynecology and Obstetrics Prevalence of heavy menstrual bleeding and experiences of affected women in a European patient survey. 128, 196–200.

Fucharoen, S., & Weatherall, D. J. (2016). Progress toward the control and management of the thalassemias. *Hematology/oncology Clinics*, *30*(2), 359-371.

Gaikwad, S. B., Mohan, D. G. K., & Reddy, K. J. (2011). Moringa oleifera leaves: Immunomodulation in wistar albino rats. Int. J. Pharm. Pharm. Sci, 3(5), 975-1491.

Gallaher, D. D., Gallaher, C. M., Natukunda, S., Schoenfuss, T. C., Mupere, E., & Cusick, S. E. (2017). Iron bioavailability from Moringa oleifera leaves is very low. *The FASEB Journal*, 31, 786-13.

Gamit, M. J., & Talwelkar, H. S. (2017). Survey of different types of anaemia. 6(3), 493–496.

Ganz, T. (2019). Anaemia of chronic disease. Molecular Haematology, 155–160.

Ghana Health Service. "Ghana: landscape analysis of anemia and anemia programming." (2016).

Ghebreselassie, D., Mekonnen, Y., Gebru, G., Ergete, W., & Huruy, K. (2011). The effects of Moringa stenopetala on blood parameters and histopathology of liver and kidney in mice. *Ethiopian Journal of Health Development*, 25(1), 51-57.

Ghersi-Egea, J. F., Strazielle, N., Catala, M., Silva-Vargas, V., Doetsch, F., & Engelhardt, B. (2018). Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. Acta neuropathologica, 135(3), 337-361.

Gonete, K. A., Tariku, A., Wami, S. D., & Derso, T. (2018). Prevalence and associated factors of anaemia among adolescent girls attending high schools in Dembia District, Northwest Ethiopia, 2017. *Archives of Public Health*, 76(1), 1-9

Gounden, V., Bhatt, H., & Jialal, I. (2018). Renal function tests.

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A., & Vernekar, S. N. (2010). Markers of renal function tests. *North American journal of medical sciences*, 2(4), 170–173

Gupta, A. (2017). Aetiology of Iron Deficiency in Children. In Nutritional Anaemia in Preschool Children (pp. 47-118). Springer, Singapore.

Gupta, A., Gautam, M. K., Singh, R. K., Kumar, M. V., Rao, C. V., Goel, R. K., & Anupurba, S. (2010). Immunomodulatory effect of Moringa oleifera Lam. extract on cyclophosphamide induced toxicity in mice.

Halaby, M. S., El-Din, M. M., & Emara, N. E. (2015). Influence of Moringa oleifera on non-alcoholic fatty liver in adult albino rats. *Middle East Journal of Applied Science*, *5*, 902-912.

Halli, S. S., & Biradar, R. A. (2020). Is distribution a problem in iron-folic acid consumption in India? An exploration of district level household survey. *The Open Family Studies Journal*, *12*(1).

Hally, K., Fauteux-Daniel, S., Hamzeh-Cognasse, H., Larsen, P., & Cognasse, F. (2020). Revisiting platelets and toll-like receptors (TLRs): at the interface of vascular immunity and thrombosis. *International journal of molecular sciences*, 21(17), 6150.

Hamza, R. G. (2016). Ameliorating Effect of Gamma-irradiated Moringa Leaves Against Arsenic-Induced Oxidative Damage in Rat Liver. *Pakistan Journal of Zoology*, *48*(2).

Hassan, F. A. (2020). Effect of Substituting Lentil Screening By-Product for Soybean Meal on Growth Performance, Nutrients Digestibility and Carcass Parameters of Growing Rabbits. *Egyptian Poultry Science Journal,* 40(2), 463-480.

Health Service, G. (2016). Ghana: Landscape Analysis of Anaemia and Anaemia Programming. www.spring-nutrition.org

Helmi, N., Bashir, M., Shireen, A., & Ahmed, I. M. (2017). Thalassemia review: features, dental considerations and management. Electronic physician, 9(3), 4003.

Honda, T., Pun, V. C., Manjourides, J., & Suh, H. (2017). Anaemia prevalence and haemoglobin levels are associated with long-term exposure to air pollution in an older population. *Environment International*, 101, 125–132.

Htut, T. W., Thein, K. Z., & Oo, T. H. (2021). Pernicious anaemia: Pathophysiology and diagnostic difficulties. *Journal of Evidence-Based Medicine,* 14(2), 161–169.

Idohou-Dossou, N., Diouf, A., Gueye, A., Guiro, A., & Wade, S. (2011). Impact of daily consumption of Moringa (Moringa oleifera) dry leaf powder on iron status of Senegalese lactating women. *African Journal of Food, Agriculture, Nutrition and Development*, 11(4), 4985–4999.

Ifeyinwa, S., Jane, C., & Esther, C. (2017). Effect of Moringa Leaf Powder Supplementation on Some Biochemical Indices of Rats. *International Journal of Research Studies in Biosciences*, 5(9), 48–58.

Jacob, M., Chappell, D., & Becker, B. F. (2016). Regulation of blood flow and volume exchange across the microcirculation. Critical care, 20(1), 1-13.)

Jagadeesan, G., Muniyandi, K., Manoharan, A. L., Nataraj, G., & Thangaraj, P. (2022). Understanding the bioaccessibility, α-amylase and α-glucosidase enzyme inhibition kinetics of Allmania nodiflora (L.) R. Br. ex Wight polyphenols during in vitro simulated digestion. Food Chemistry, 372, 131294.

Jayanthi, M., Garg, S. K., Yadav, P., Bhatia, A. K., & Goel, A. (2015). Some newer marker phytoconstituents in methanolic extract of Moringa oleifera leaves and evaluation of its immunomodulatory and splenocytes proliferation potential in rats. *Indian journal of pharmacology*, 47(5), 518.

Jiang, W., Gao, M., Sun, S., Bi, A., Xin, Y., Han, X., ... & Luo, L. (2012). Protective effect of L-theanine on carbon tetrachloride-induced acute liver injury in mice. Biochemical and biophysical research communications, 422(2), 344-350.

Jung, J., Rahman, M. M., Rahman, M. S., Swe, K. T., Islam, M. R., Rahman, M. O., & Akter, S. (2019). Effects of haemoglobin levels during pregnancy on adverse maternal and infant outcomes: a systematic review and meta-analysis. *Annals of the New York Academy of Sciences*, 1450(1), 69–82.

Kadhim, K. A., Baldawi, K. H., & Lami, F. H. (2017). Prevalence, Incidence, Trend, and Complications of Thalassemia in Iraq. Haemoglobin, 41(3), 164–168.

Kalra A, Yetiskul E, Wehrle CJ, et al. Physiology, Liver. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; January, 2023.

Karthivashan, G., Kura, A. U., Arulselvan, P., Isa, N. M., & Fakurazi, S. (2016). The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. PeerJ, 4, e2127.

Kashyap, P., Kumar, S., Riar, C. S., Jindal, N., Baniwal, P., Guiné, R. P., ... & Kumar, H. (2022). Recent advances in Drumstick (Moringa oleifera) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. Antioxidants, 11(2), 402.

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., & Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities.

Kassebaum, Nicholas. J., & GBD 2013 Anaemia Collaborators. (2016). The Global Burden of Anaemia GBD 2013 Anaemia Collaborators and Nicholas J Kassebaum. Haematology/Oncology Clinics of North America, 30(2), 247–308.

Kesharwani, S., Prasad, P., Roy, A., & Sahu, R. K. (2014). An overview on phytochemistry and pharmacological explorations of Moringa oleifera. *Pharmaceutical and Biosciences Journal,* 34-41.

Khalid, S., Arshad, M., Raza, K., Mahmood, S., Siddique, F., Aziz, N., ... & Aqlan, F. (2023). Assessment of hepatoprotective, nephroprotective efficacy, and antioxidative potential of Moringa oleifera leaf powder and ethanolic extract against PCOS□induced female albino mice (Mus Musculus). *Food Science & Nutrition*.

Kim, Y. J., & Kim, H. S. (2019). Screening Moringa species focused on development of locally available sustainable nutritional supplements. *Nutrition Research and Practice*, 13(6), 529-534.

Kocaoz, S., Cirpan, R., & Degirmencioglu, A. Z. (2019). The prevalence and impacts heavy menstrual bleeding on anaemia, fatigue and quality of life in women of reproductive age. *Pakistan Journal of Medical Sciences*, 35(2), 365–370.

Koralkova, P., Solinge, W. W. V. A. N., & Wijk, R. V. A. N. (2014). Rare hereditary red blood cell enzymopathies associated with hemolytic anaemia – pathophysiology, clinical aspects, and laboratory diagnosis. 388–397.

Kulczynska, K., Bieker, J. J., & Siatecka, M. (2019). A Krüppel-like factor 1 (KLF1) mutation associated with severe congenital dyserythropoietic anemia alters its DNA-binding specificity. *Molecular and cellular biology*.

Kumar, A., Sharma, E., Marley, A., Samaan, M. A., & Brookes, M. J. (2022). Iron deficiency anaemia: Pathophysiology, assessment, practical management. *In BMJ Open Gastroenterology* (Vol. 9, Issue 1).

Kumar, N., & Sharma, S. (2023). Pharmacology, Ethnopharmacology, and Phytochemistry of Medicinally Active Moringa oleifera: A Review. *The Natural Products Journal*, 13(8), 13-41.

Kumar, R., & Thakur, A. (2022). Younis Ahmad Hajam, Rahul Datta, Sonika, Ajay Sharma. Medical Microbiology, 93.

Lee, M. T., Lin, W. C., Yu, B., & Lee, T. T. (2017). Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals—A review. Asian-Australasian journal of animal sciences, 30(3), 299.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., & Aristil, J. (2015). Moringa oleifera Seeds and Oil: Characteristics and Uses for Human Health. *International Journal of Molecular Sciences,* 16(6), 12791-12800.

Ling, X. C., & Kuo, K. L. (2018). Oxidative stress in chronic kidney disease. Renal Replacement Therapy, 4(1), 1-9.

Lopez-Giacoman, S., & Madero, M. (2015). Biomarkers in chronic kidney disease, from kidney function to kidney damage. *World journal of nephrology*, 4(1), 57.

Lyons, G., Gondwe, C., Banuelos, G., Mendoza, C., Haug, A., Christophersen, O., & Ebert, A. W. (2015, November). Drumstick tree (Moringa oleifera) leaves as a source of dietary selenium, sulphur and pro-vitamin A. In *International Symposium on Moringa 1158* (pp. 287-292).

Macário, A. P. S., Ferraz, R. L. D. S., Costa, P. D. S., Brito Neto, J. F. D., Melo, A. S. D., & Dantas Neto, J. (2020). Allometric models for estimating Moringa oleifera leaflets area. *Ciência e Agrotecnologia*, *44*.

Madkhali, H. A., Alharthy, K. M., Asiri, M. A., Ganaie, M. A., Ansari, M. N., Rehman, N. U., & Hamad, A. M. (2019). Moringa oleifera Lam. (family Moringaceae) leaf extract attenuates high-fat diet-induced dyslipidemia and vascular endothelium dysfunction in Wistar albino rats. *Tropical Journal of Pharmaceutical Research*, 18(12), 2597-2604.

Madukwe, E.U., Ugwuoke, A.L., & Ezeugwu, J.O (2013). Effectiveness of dry Moringa oleifera leaf powder in the treatment of anaemia.

Mahmood, K., Zia, K. M., Zuber, M., Shahid, S. A., & Mahringer, A. (2018). Triterpenoids as potential anticancer agents. Phytotherapy Research, 32(11), 2164-2177.

Mahmood, S. K., Ahmed, N. S., Sultan, G. A., & Yousif, M. J. (2022). Histomorphological and carbohydrate histochemical study of the pancreas in native ducks (Anas Platyrhynchos). *Iraqi Journal of Veterinary Sciences*, *36*(4), 1103-1110.

Maina Hassan, I., Saidu, B., Abdullahi Ishaq, J., Dahiru, A., Abdulazeez, N., Ibrahim Yusuf, H., Abdulrahman Shekwaye, A., Bashir Bello, M., Abdul Abubakar, A., & Shuaibu Baraya, Y. (2020). Changes in Haematological Parameters Following Toxicity Study with 80% Methanol Extract of <i>Moringa oleifera</i> in Wistar Rats. *American Journal of Applied Chemistry,* 8(6), 143.

Masenga, S. K., Kabwe, L. S., Chakulya, M., & Kirabo, A. (2023). Mechanisms of Oxidative Stress in Metabolic Syndrome. *International Journal of Molecular Sciences*, *24*(9), 7898.

McCranor, B. J., Kim, M. J., Cruz, N. M., Xue, Q. L., Berger, A. E., Walston, J. D., ... & Roy, C. N. (2014). Interleukin-6 directly impairs the erythroid development of human TF-1 erythroleukemic cells. Blood Cells, Molecules, and Diseases, 52(2-3), 126-133.

Meteorological Service Department (2013). Annual report, Mampong Ashanti Municipal Assembly. Mampong -Ashanti, Ghana.

Mezban, N. A., & Hussein, A. M. (2015). Comparative study to some of the biochemical and histological effects of alkaloids extracts from Nerium oleander and Apium graveolens in females mice. *Mus musculus*.

Milla, P. G., Peñalver, R., & Nieto, G. (2021). Health benefits of uses and applications of Moringa oleifera in bakery products. Plants, 10(2), 318.

Mirza, F. G., Abdul-Kadir, R., Breymann, C., Fraser, I. S., & Taher, A. (2018). Impact and management of iron deficiency and iron deficiency anaemia in women's health. Expert review of haematology, 11(9), 727-736.

Mockenhaupt, F. P., Rong, B., Günther, M., Beck, S., Till, H., Kohne, E., Thompson, W. N. A., & Bienzle, U. (2000). Anaemia in pregnant Ghanaian women: Importance of malaria, iron deficiency and haemoglobinopathies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94(5), 477–483.

Mohamed, M., Thio, J., Thomas, R. S., & Phillips, J. (2020). EASILY MISSED? Pernicious anaemia.

Mohamed, O. H., Mohamed, S., Mohamed Elhag Ahmed, M., Ibrahim Osman, B., Mohammed, B. E., O, F. A., Osman, B., Elhassan, A. M., & KK, T. (2015). Assessment of acute toxicity and LD 50 of Moringa oleifera ethanolic leave extract in albino rats and rabbits Charaterization and fuctional properties of Gum producing Acacias of Sudan View project leishmenia treatment View project. *Journal of Medical and Biological Science Research*, 1(4), 38–43.

Moodley, I. (2017). Acute toxicity of Moringa oleifera leaf powder in rats Indres Moodley. *Journal of Medicinal Plants Studies*, 5(5), 180–185.

Muhammad, H. I., Asmawi, M. Z., & Khan, N. A. K. (2016). A review on promising phytochemical, nutritional and glycemic control studies on Moringa oleifera Lam. in tropical and sub-tropical regions. *Asian Pacific Journal of Tropical Biomedicine*, *6*(10), 896-902.

Mulyaningsih, T. R., & Yusuf, S. (2018). Determination of minerals content in leaves of Moringa oleifera by neutron activation analysis. Ganendra Majalah IPTEK Nuklir, 21(1), 11-16.

Munro, M. G. (2023). Heavy menstrual bleeding, iron deficiency, and iron deficiency anaemia: framing the issue. *International Journal of Gynecology & Obstetrics*, 162, 7-13.

Munro, M. G., Mast, A. E., Powers, J. M., Kouides, P. A., O'Brien, S. H., Richards, T., ... & Levy, B. S. (2023). The relationship between heavy menstrual bleeding, iron deficiency, and iron deficiency anaemia. *American journal of obstetrics and gynecology*.

Mutwedu, B. V. (2022). Investigation of the Pharmacological Effects of Moringa Oleifera Aqueous Extract on Oxidative Damages and Reproductive Performances Induced by Heat Stress in Female Rabbit (Doctoral dissertation, University of Nairobi).

Namadina, M. M., Aliyu, B. S., Yahaya, N., Aliko, A. A., Sani, L. A., Zakari, S. M., ... & Umar, A. M. (2019). Phytochemical and Acute Toxicity Study of Moringa Oliefera Root. *International Journal of Science for Global Sustainability*, *5*(2), 11-11.

Namadina, M. M., Aliyu, B. S., Yahaya, N., Aliko, A. A., Sani, L. A., Zakari, S. M., ... & Umar, A. M. (2019). Phytochemical and Acute Toxicity Study of Moringa Oliefera Root. *International Journal of Science for Global Sustainability*, 5(2), 11-11.

Nambiar, V. S., & Seshadri, S. (2001). Bioavailability trials of β-carotene from fresh and dehydrated drumstick leaves (Moringa oleifera) in a rat model. Plant Foods for Human Nutrition, 56, 83-95.

National Research Council. (2011). Guide for the Care and Use of Laboratory Animals (8th ed.). National Academies Press.

Navie, S., & Csurhes, S. (2010). Weed risk assessment horseradish tree Moringa oleifera. Queensland Government.

Nfambi, J., Bbosa, G. S., Sembajwe, L. F., Gakunga, J., & Kasolo, J. N. (2015). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. *Journal of basic and clinical physiology and pharmacology*, *26*(6), 603-611.

Nobossé, P., Fombang, E. N., & Mbofung, C. M. (2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh Moringa oleifera L. leaves. *Food science & nutrition*, *6*(8), 2188-2198.

Ntenda, P. A., Nkoka, O., Bass, P., & Senghore, T. (2018). Maternal anaemia is a potential risk factor for anaemia in children aged 6–59 months in Southern Africa: a multilevel analysis. BMC public health, 18(1), 1-13.

Nugraha, A. S., Damayanti, Y. D., Wangchuk, P., & Keller, P. A. (2019). Anti-infective and anti-cancer properties of the Annona species: Their ethnomedicinal uses, alkaloid diversity, and pharmacological activities. *Molecules*, *24*(23), 4419.

Nwabuko, O. C., Onwuchekwa, U., & Iheji, O. (2022). An overview of sickle cell disease from the socio-demographic triangle-a Nigerian single-institution retrospective study. *Pan African Medical Journal*, 41.

Nwamarah, J. U., Otitoju, O., & Otitoju, G. T. (2015). Effects of Moringa oleifera Lam. aqueous leaf extracts on follicle stimulating hormone and serum cholesterol in Wistar rats. *African Journal of Biotechnology*, 14(3), 181-186.

Odukoya, A. A., Adejumo, I. O., & Ologhobo, A. D. (2022). Chemical Constituents of Crude Extracts of Moringa oleifera (Lam) Leaf and Biochemical Response of Weanling Wistar Rats Administered Crude Extract. *Nigeria Agricultural Journal*, 53(1), 351-356.

Ogobuiro, I. & Tuma, F. (2023). Physiology, Renal. StatPearls. Treasure Island (FL) with ineligible companies. Disclosure: Faiz Tuma declares no relevant financial relationships with ineligible companies: StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC.

Okumu, M., Mbaria, J., Kanja, L., Gakuya, D., Kiama, S., Ochola, F., & Okumu, P. (2016). Acute toxicity of the aqueous-methanolic Moringa oleifera (Lam) leaf extract on female Wistar albino rats. *International Journal of Basic and Clinical Pharmacology*, 5(5), 1856–1861.

Olayemi, F. O., & Arowolo, R. O. (2012). The haematological and biochemical effects of methanol extract of the seeds of Moringa oleifera in rats. *Journal of Medicinal Plants Research*, *6*(4), 615-621.

Olusanya, R. N. (2018). The nutritional composition and acceptability of Moringa oleifera leaf powder (MOLP)-supplemented mahewu: a maize meal-based beverage for improved food and nutrition security (Doctoral dissertation).

Omodanisi, E. I., Aboua, Y. G., Chegou, N. N., & Oguntibeju, O. O. (2017). Hepatoprotective, antihyperlipidemic, and anti-inflammatory activity of Moringa oleifera in diabetic-induced damage in male wistar rats. *Pharmacognosy research*, *9*(2), 182.

Omodanisi, E. I., Aboua, Y. G., Chegou, N. N., & Oguntibeju, O. O. (2017). Hepatoprotective, antihyperlipidemic, and anti-inflammatory activity of Moringa oleifera in diabetic-induced damage in male wistar rats. *Pharmacognosy research*, *9*(2), 182.

Opoku, E., Crankson, S., & Anokye, N. K. (2021). Prevalence and Risk Factors of Anaemia Among Children Under Five Years in Ghana: Analysis from the Ghana Demographic and Health Survey.

Osman, H. M., Shayoub, M. E., & Babiker, E. M. (2012). The effect of Moringa oleifera leaves on blood parameters and body weights of albino rats and rabbits. *Jordan Journal of Biological Sciences*, 5(3), 147-150.

Osungbade, K. O., & Oladunjoye, A. O. (2012). Preventive treatments of iron deficiency Anaemia in pregnancy: A review of their effectiveness and implications for health system strengthening. *In Journal of Pregnancy* (Vol. 2012).

Ouédraogo, M., Lamien-Sanou, A., Ramdé, N., Ouédraogo, A. S., Ouédraogo, M., Zongo, S. P., Goumbri, O., Duez, P., & Guissou, P. I. (2013). Protective effect of Moringa oleifera leaves against gentamicin-induced nephrotoxicity in rabbits. Experimental and Toxicologic Pathology, 65(3), 335–339.

Oyedemi, S., Bradley, G., and A, Afolayan. (2010). Toxicological effects of the aqueous stem bark extract of Strychnos henningsii Gilg in Wistar rats. *J. Nat. Pharm*. 1(1). 33

Oyewo, E. B., Adetutu, A., Ayoade, A., Adesokan, A., & MA, K. (2013). Repeated oral administration of aqueous leaf extract of Moringa oleifera modulated immunoactivities in wistar rats. *J. Nat. Sci. Res*, 3(6), 100-109.

Oyewo, E. B., Adetutu, A., Ayoade, A., Adesokan, A., & MA, K. (2013). Repeated oral administration of aqueous leaf extract of Moringa oleifera modulated immunoactivities in wistar rats. *J. Nat. Sci. Res*, *3*(6), 100-109.

Padayachee, B., & Baijnath, H. J. S. A. J. O. B. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of Moringa oleifera. *South African Journal of Botany,* 129, 304-3

Pagadala, P., Shankar, M. S. V., Hemalatha, A., & Shashidhar, K. N. (2022). Effect of Moringa oleifera leaves on haematological profile of fluorosis affected rats. 18(1), 14–18.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2(5), 270-278.

Pasricha, S. (2014). Anaemia: a comprehensive global estimate. 123(5), 611–612.

Phimarn, W., Sungthong, B., & Wichaiyo, K. (2021). Efficacy and safety on Moringa oleifera on blood glucose and lipid profile: A meta-analysis. *Pharmacognosy Magazine*, *17*(74), 373-378.

Phimarn, W., Sungthong, B., & Wichaiyo, K. (2021). Efficacy and safety on Moringa oleifera on blood glucose and lipid profile: A meta-analysis. *Pharmacognosy Magazine*, *17*(74), 373-378.

Pobee, R. A., Setorglo, J., Klevor, M., & Murray-Kolb, L. E. (2021). The prevalence of anaemia and iron deficiency among pregnant Ghanaian women, a longitudinal study. PLoS ONE, 16(3 March).

Powell-Wiley, T. M., Poirier, P., Burke, L. E., Després, J. P., Gordon-Larsen, P., Lavie, C. J., ... & American Heart Association Council on Lifestyle and Cardiometabolic Health; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Epidemiology and Prevention; and Stroke Council. (2021). Obesity and cardiovascular disease: a scientific statement from the American Heart Association. Circulation, 143(21), e984-e1010.

Prabu, S. L., Umamaheswari, A., & Puratchikody, A. (2019). Phytopharmacological potential of the natural gift Moringa oleifera Lam and its therapeutic application: An overview. *Asian Pacific Journal of Tropical Medicine*, 12(11), 485-498.

Prasetio, D. B., Setyaningsih, Y., Suhartono, & Suroto. (2022). The Effect of Moringa Leaf Extract on Malondialdehyde Levels in Male Wistar Rats. *Journal of Hunan University Natural Sciences,* 49(4), 424–428.

Pullakhandam, R., & Failla, M. L. (2007). Micellarization and intestinal cell uptake of β-carotene and lutein from drumstick (Moringa oleifera) leaves. *Journal of medicinal food*, 10(2), 252-257.

Rana, A., Samtiya, M., Dhewa, T., Mishra, V., & Aluko, R. E. (2022). Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry*, *46*(10), e14264.

Rani, N. Z. A., Husain, K., & Kumolosasi, E. (2018). Moringa genus: A review of phytochemistry and pharmacology. *Frontiers in Pharmacology*, 9(FEB), 1–26.

Ranjan, S., Himani, D., & Mukopadayay, S. (2022). A review article on. *International Journal of Health Sciences,* 14(June), 11077–11088.

Rees, D. C., Robinson, S., & Howard, J. (2018). How I manage red cell transfusions in patients with sickle cell disease. *British Journal of Haematology*, 180(4), 607–617.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from Moringa oleifera Lam leaves. Industrial Crops and Products, 66, 246-254.

Rostam, M. A., Shajimoon, A., Kamato, D., Mitra, P., Piva, T. J., Getachew, R., ... & Little, P. J. (2018). Flavopiridol inhibits TGF-β-stimulated biglycan synthesis by blocking linker region phosphorylation and nuclear translocation of Smad2. *Journal of Pharmacology and Experimental Therapeutics*, *365*(1), 156-164.

Sa, F., Sj, B., Doree, C., Chowdhury, O., Gooding, S., & Dj, R. (2013). Oral deferiprone for iron chelation in people with thalassaemia (Review). 8.

Safiri, S., Kolahi, A. A., Noori, M., Nejadghaderi, S. A., Karamzad, N., Bragazzi, N. L., Sullman, M. J. M., Abdollahi, M., Collins, G. S., Kaufman, J. S., & Grieger, J. A. (2021). Burden of anaemia and its underlying causes in 204 countries and territories, 1990–2019: results from the Global Burden of Disease Study 2019. *Journal of Haematology and Oncology*, 14(1), 1–16.

Saleh, S. S., & Sarhat, E. R. (2019). Effects of ethanolic Moringa oleifera extract on melatonin, liver and kidney function tests in alloxan-induced diabetic rats. *Indian Journal of Forensic Medicine & Toxicology*, *13*(4), 1015-1019.

Sandoval, M. A. S., & Jimeno, C. A. (2013). Effect of malunggay (Moringa oleifera) capsules on lipid and glucose levels. Acta Medica Philippina, 47(3).

Santoyo-sánchez, A. (2016). Iron deficiency anaemia. 79(2), 88–97.

Sari, W. F., & Suwondo, A. (2022). A Literature Review of Effect of Moringa oleifera Leaf Extract Toward Lipid Profile Level in Hyperlipidemia Patients. *International Journal of Nursing and Health Services* (IJNHS), 5(3), 294-303.

Sharma, K., Kumar, M., Waghmare, R., Suhag, R., Gupta, O. P., Lorenzo, J. M., ... & Kennedy, J. F. (2022). Moringa (Moringa oleifera Lam.) polysaccharides: Extraction, characterization, bioactivities, and industrial application. International Journal of Biological Macromolecules, 209, 763-778.

Sharourou, A. S. A., Hassan, M. A., & Teclebrhan, M. B. (2018). Anaemia: Its Prevalence, Causes and Management. *The Egyptian Journal of Hospital Medicine*, 70(10), 1877–1879.

Shija, A. E., Rumisha, S. F., Oriyo, N. M., Kilima, S. P., & Massaga, J. J. (2019). Effect of Moringa oleifera leaf powder supplementation on reducing anaemia in children below two years in Kisarawe District, Tanzania. Food Science and Nutrition, 7(8), 2584–2594.

Shinde, M. B., Waghchoure, D. R., Bhutada, S. A., Dahikar, S. B., & Kshirsagar, R. V. (2021). Efficacy of Moringa oleifera Leaf Extract for the Treatment of Anaemia in Girls. *Journal of Advances in Medical and Pharmaceutical Sciences*, 23(5), 1–5.

Shubham, K., Anukiruthika, T., Dutta, S., Kashyap, A. V., Moses, J. A., & Anandharamakrishnan, C. (2020). Iron deficiency anaemia: A comprehensive review on iron absorption, bioavailability and emerging food fortification approaches. Trends in Food Science and Technology, 99(March), 58–75.

Shunmugam, L. (2016). Moringa oleifera crude aqueous leaf extract induces apoptosis in human hepatocellular carcinoma cells via the upregulation of NF-kB and IL-6/STAT3 pathway (Doctoral dissertation).

Simeon, J. O., Zubairu, S. A., & Tosin, J. O. (2021). Clinical evaluation of the potential benefits of taking Moringa oleifera on blood triglyceride and cholesterol level in patient taking Tenofovir/Lamivudine/Efavirenz (TLE) combination. *Journal of Pharmaceutical Sciences and Research*, 13(10), 623-629.

Singh, A. P., Kishore, P. S., Kar, S., & Dewanjee, S. (2022). Secondary Metabolites of Brassica juncea (L.) Czern and Coss: Occurence, Variations and Importance.

Soundarya, N. P. S. (2017). A review on anaemia – types, causes, symptoms and their treatments | Journal of science and technology investigation. *Journal of Science and Technology Investigation,* 1(April), 10–17.

Sriprasert, I., Pakrashi, T., Kimble, T., & Archer, D. F. (2017). Heavy menstrual bleeding diagnosis and medical management. Contraception and Reproductive Medicine, 2(1), 1–9.

Srole, D. N., & Ganz, T. (2021). Erythroferrone structure, function, and physiology: Iron homeostasis and beyond. Journal of cellular physiology, 236(7), 4888-4901.

Stevens, G. A., Finucane, M. M., De-regil, L. M., Paciorek, C. J., Flaxman, S. R., Branca, F., & Peña-rosas, J. P. (2012). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995 – 2011: a systematic analysis of population-representative data. 16–25.

Stevens, G. A., Paciorek, C. J., Flores-Urrutia, M. C., Borghi, E., Namaste, S., Wirth, J. P., Suchdev, P. S., Ezzati, M., Rohner, F., Flaxman, S. R., & Rogers, L. M. (2022). National, regional, and global estimates of anaemia by severity in women and children for 2000–19: a pooled analysis of population-representative data. *The Lancet Global Health*, 10(5), e627–e639.

Stohs, S. J., & Hartman, M. J. (2015). Review of the safety and efficacy of Moringa oleifera. *Phytotherapy Research*, 29(6), 796–804.

Su, B., & Chen, X. (2020). Current status and potential of Moringa oleifera leaf as an alternative protein source for animal feeds. *Frontiers in veterinary science*, 7, 53.

Sultana, S. (2020). Nutritional and functional properties of Moringa oleifera. Metabolism open, 8, 100061.

Sung, S. H., Kim, K. H., Jeon, B. T., Cheong, S. H., Park, J. H., Kim, D. H., ... & Moon, S. H. (2012). Antibacterial and antioxidant activities of tannins extracted from agricultural by-products. *Journal of Medicinal Plants Research*, *6*(15), 3072-3079.

Sung, S. H., Kim, K. H., Jeon, B. T., Cheong, S. H., Park, J. H., Kim, D. H., ... & Moon, S. H. (2012). Antibacterial and antioxidant activities of tannins extracted from agricultural by-products. *Journal of Medicinal Plants Research*, *6*(15), 3072-3079.

Suprihartini, C., Ulilalbab, A., & Budiman, F. A. (2023). The effect of giving moringa tempeh flour on hemoglobin levels and body weight of Wistar Rats. *Jurnal SAGO Gizi dan Kesehatan*, *4*(2), 297-302.

Swallah, M. S., Bondzie-Quaye, P., Wu, Y., Acheampong, A., Sossah, F. L., Elsherbiny, S. M., & Huang, Q. (2023). Therapeutic potential and nutritional significance of Ganoderma lucidum–a comprehensive review from 2010 to 2022. Food & Function.

Taher, A. T., Weatherall, D. J., & Cappellini, M. D. (2018). Thalassaemia. *The Lancet,* 391(10116), 155–167. https://doi.org/10.1016/S0140-6736 (17)31822-6

Tang, Y., Choi, E. J., Han, W. C., Oh, M., Kim, J., Hwang, J. Y., ... & Kim, E. K. (2017). Moringa oleifera from Cambodia ameliorates oxidative stress, hyperglycemia, and kidney dysfunction in type 2 diabetic mice. *Journal of medicinal food,* 20(5), 502-510.

Thomas, A. (2017). Investigation and management of anaemia. Medicine (United Kingdom), 45(4), 209–213. https://doi.org/10.1016/j.mpmed.2017.02.015

Thom, C. S., Dickson, C. F., Gell, D. A., & Wiess, M. J. (2013). Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harbor prerspective in medicine, 3(3), a011858.

Thomson, E. M., Pal, S., Guénette, J., Wade, M. G., Atlas, E., Holloway, A. C., Williams, A., & Vincent, R. (2016). Ozone inhalation provokes glucocorticoid-dependent and -independent effects on inflammatory and metabolic pathways. *Toxicological Sciences,* 152(1), 17–28.

Thurber, M. D., & Fahey, J. W. (2009). Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "Diffusion of innovations" theory. *Ecology of food and nutrition*, 48(3), 212–225.

Udikala, M., Verma, Y., Sushma, S. L., & Lal, S. (2017). Phytonutrient and pharmacological significance of Moringa oleifera. *International Journal of Life-Sciences Scientific Research*, 3(5), 1387-1391.

Unissa, R., Monica, B., Konakanchi, S., Darak, R., Keerthana, S. L., & Kumar, S. A. (2018). Thalassemia: a review. *Asian Journal of Pharmaceutical Research*, *8*(3), 195-202.

Unung, H., Yakubu, D., Dagare, J., Oyedapo, F., and R, Gobe. (2019). Effects of varying protein levels on the haematology and serum biochemical components of non-descript rabbits. *Int. J. Res. Agric. Forest.* 6(5):11-15

Upadhyay, P., Yadav, M. K., Mishra, S., Sharma, P., & Purohit, S. (2015). Moringa oleifera: A review of the medical evidence for its nutritional and pharmacological properties. *International Journal of Research in Pharmacy & Science,* (2).

Urso, E., & Maffia, M. (2016). Behind the link between copper and angiogenesis: established mechanisms and an overview on the role of vascular copper transport systems. *Journal of vascular research,* 52(3), 172-196.

Varghese, S., Kareem, S. N., Purushothaman, B. P., & Mukkadan, J. K. (2022). Prevalence of anaemia among college going students in India: a systematic review. *International Journal of Community Medicine and Public Health*, 9(9), 3576.

Vaschenko, V. I., & Vil'yaninov, V. N. (2019). Eryptosis (quasi-apoptosis) the human red blood cells. Its role in medicinal therapy. *Reviews on Clinical Pharmacology and Drug Therapy,* 17(3), 5–38.

Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants, 6(4), 1–13.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., (2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Indust. Crops Prod. 44, 566–571.

Weiss, G., Ganz, T., & Goodnough, L. T. (2019). Anaemia of inflammation. Blood, 133(1), 40–50.

WHO Prevalence of anaemia (2017). https://www.who.int/data/gho

Wijayanti, H. N., Fadhilah, Y. N., Yuniarti, W. M., Lukiswanto, B. S., Arimbi, A., Suprihati, E., & Kurnijasanti, R. (2023). Protective effect of Moringa oleifera leaves extract against gentamicin induced hepatic and nephrotoxicity in rats. *Iraqi Journal of Veterinary Sciences*, 37(1), 129-135.

Williams, T. N., & Weatherall, D. J. (2012). World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harbor perspectives in medicine*, *2*(9).

Woldekidan, S., Mulu, A., Ergetie, W., Teka, F., Meressa, A., Tadele, A., ... & Tolcha, Y. (2021). Evaluation of antihyperglycemic effect of extract of moringa stenopetala (Baker f.) aqueous leaves on alloxan-induced diabetic rats. Diabetes, Metabolic Syndrome and Obesity, 185-192

Wonkam, A., & Makani, J. (2019). Sickle cell disease in Africa: an urgent need for longitudinal cohort studies. The Lancet Global Health, 7(10), e1310–e1311.

World Health Organization. (2008). Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia.

World Health Organization. (2008). Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia.

Xie, J., Wang, Y., Jiang, W. W., Luo, X. F., Dai, T. Y., Peng, L., Song, S., Li, L. F., Tao, L., Shi, C. Y., Hao, R. S., Xiao, R., Tian, Y., & Sheng, J. (2018). Moringa oleifera Leaf Petroleum Ether Extract Inhibits Lipogenesis by Activating the AMPK Signaling Pathway. *Frontiers in Pharmacology,* 9(December).

Ye, A., Pf, A., & Om, O. (2019). Effects of Graded Levels of Moringa oleifera Leaf-Meal in Albino Rat Diet on Some Haematological Parameters. 1(2), 37–46.

Zaher, H. A., Alawaash, S. A., Tolba, A. M., Swelum, A. A., Abd El-Hack, M. E., Taha, A. E., & Abdelnour, S. A. (2020). Impacts of Moringa oleifera foliage substituted for concentrate feed on growth, nutrient digestibility, haematological attributes, and blood minerals of growing goats under Abu Dhabi conditions. Sustainability, 12(15), 6096.