EFFECT OF *DIALIUM GUINEENSE* ON IRON AND HAEMATOLOGICAL PROFILES USING RAT MODELS

THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF DIETETICS, UNIVERSITY OF GHANA, LEGON

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN DIETETICS

BY

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OCTOBER, 2020

DECLARATION

I, Ama Baduwa Dadson, hereby declare that this thesis is the result of the original research work personally done by me under the supervision of Dr. Matilda Asante and Prof. George Asare for the award of Máster of Science (MSc.) degree in Dietetics at the University of Ghana, Legon. All references to other people's work have been duly acknowledged, and the thesis has not been submitted in part or whole for the award of a degree elsewhere.

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ABSTRACT

Background: Iron deficiency anaemia is a consequence of suboptimal dietary iron intake or inefficient dietary iron absorption. *Dialium guineense* also known as black velvet tamarind (BVT) has a rich combination of both iron as well as vitamin C, coupled with low levels of antinutrients. Traditionally, the fruit pulp of BVT is used to treat iron deficiency anaemia in certain parts of the Ghana. The efficacy and effectiveness of these interventions however need a wider exploration.

Aim: To determine the effect of *Dialium guineense* pulp on iron and haematological profile levels of rats.

Methods: A total of twenty SD rats were weight-matched into 4 groups of 5 rats each (Groups 1-4). Group 1 was fed on a normal diet and groups 2 - 3 fed on BVT fruit pulp in the form of fruit juice extract with different concentrations for 90 days. The rats in group 2 (low dose) were fed with 2.5 g of BVT fruit juice, with 5 g and 10 g of BVT juice for group 3 (medium dose) and group 4 (high dose) respectively. The doses of BVT were administered orally to the SD rats via gavage. Blood samples from the animals were collected by cardiac puncture at the end of the experiment. Serum iron profile (serum iron, total iron binding capacity- TIBC, percentage saturation) was performed. Additionally, haematological profile (red blood cell (RBC), haemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), and white blood cell differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils) were assayed using an automated haematological analyser at the end of the 90-day period.

Results: The study revealed a dose dependent increase of serum iron among treatment groups, with the high dose recording the highest mean (51.93 ± 0.58 mmol/L) against the control (47.08 ±

0.58 mmol/L). There was a general decrease in TIBC among treatment groups with the medium dose group recording the lowest mean value (88.00 ± 0.50 mmol/L). A general increase in %SAT was also observed across the treatment group with the high dose group recording (52.38 ± 0.93mmol/L) against the control group (38.45 ± 0.93 mmol/L).These observations were however not statistically significant. The study showed no significant change in nearly all the haematological parameters of the research group, compared to the control group. An increase was observed in RBC with the high dose group recording ($7.44 \pm 0.76 \times 10^{12}$/L) in contrast to the control group ($7.22 \pm 0.76 \times 10^{12}$/L), however the difference was not statistically significant. A general decrease was observed in HB across experimental groups with the high dose group having (13.98 ± 0.42 g/dL) against the control (14.18 ± 0.42 g/dL). Furthermore a decrease was observed across WBC values with the control having ($14.18 \pm 0.33 \times 10^{9}$/L) against the high dose group ($13.60 \pm 0.33 \times 10^{9}$/L). For WBC differentials, there was a general increase except for lymphocytes and basophils. All these differences were however, not statistically significant. A significantly strong negative correlation was observed between percent saturation and TIBC ($r= -0.85$, $p<0.01$). A significantly strong positive correlation was also observed between RBC and HBG ($r= 0.72$, $p= 0.01$) as well as MCV and MCH ($r= 0.82$, $p <0.01$).

Conclusion: The findings of this study showed that *Dialium guineense* did not significantly increase the serum iron levels and haematological indices of the rats, although serum iron increased in a dose dependent manner.

DEDICATION

To my beloved mother, Ms. Cecilia Acquah

ACKNOWLEDGEMENTS

Absolute gratitude goes to the Almighty God who supplied me with all that I needed and has seen me through this programme.

My heartfelt appreciation goes to my supervisors, Dr Matilda Asante, Head of Department (HOD), of Dietetics, and Prof. George Asare, Department of Chemical Pathology Unit (CPU, DMLS), for their cautious admonitions, encouragement, firm dedication, keen and sustained interest.

Special thanks go to Dr. Samuel Adjei, Department of Animal Experimentation, NMIMR, who also made inputs, constructive criticisms and provided academic direction. Profound gratitude to Dr. Konadu and laboratory technicians of Department of Animal Experimentation, NMIMR, for helping me with my laboratory work. Thanks also to Mrs. Portia Nkumsah-Riverson, Mrs. Eunice Nortey, Department of Dietetics, for their support.

Sincere thanks to my Mum, Ms. Cecilia Acquah and my siblings: Mrs. Olivia Asamoah, and Mr. Ekow Dadson for their prayers, motivation and financial support.

My deep sense of gratitude to Pastor and Mrs. Julius Gloryfilled-ChristLord, Mr. and Mrs. Fredrick Gloryfilled-ChristLord, Ms. Josephine Monique Nsiah Amponsah, Dr George Amoanoo Nino and Mr. Dennis Faith for their prayers, Inspiration and valuable guidance for this research’s success. I owe a great deal of gratitude to my friends and loved ones, who have been very supportive. Your precious commitment is priceless. Many thanks to you.

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LIST OF ABBREVIATIONS

Bas	Basophiles
BVT	Black Velvet Tamarind
d/l	Decilitre
DMRT	Duncan Multiple Range Test
DPP	Diphenyl-1-picrylhydrazyl
Eso	Eosinophils
fL	Femtolitre
GAFCO	Ghana Agro Food Company
HB	Haemoglobin
HCL	Hydrogen Chloride
Lym	Lymphocytes
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
ML	Millilitres
Mon	Monocytes
Neu	Neutrophils
NMIMR	Noguchi Memorial Institute for Medical Research

pg	Picogram
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
SD	Sprague-Dawley
TIBC	Total Iron Binding Capacity
µg	Microgram
VOL	Volume
WBC	White Blood Cell
WHO	World Health Organization
%SAT	Percentage Saturation
QoL	Quality of Life
BWT	Body Weight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The World Health Organization (WHO) defines anaemia as a deficiency in which the number of red blood cells or their oxygen-carrying capacity is inadequate to achieve physiological needs that differ in age , sex, elevation of residence, smoking habits and pregnancy (WHO, 2018). Anaemia is defined as a haemoglobin concentration of less than 130g/L and less than 120g/L in men and women respectively (WHO, 2011). Iron deficiency due to suboptimal iron intake or inefficient iron absorption has been the most frequent cause of anaemia worldwide. Other factors, such as folate, vitamin A deficiency, vitamin B12, bacterial infections, chronic inflammation, and hereditary disorders, may all cause anaemia (WHO, 2018).

Globally, 1.62 billion people which is 24.8% of the total population are affected by anaemia. The uppermost prevalence is in pre-school children (47.4%) and the lowest in men (12.7%). Sub-Saharan Africa recorded the highest prevalence of anaemia globally with the elevated numbers in pre-school children (67.6%) and pregnant women (57.1%). In Ghana, the 2014 Demographic Health Survey revealed that 66% of children under 5 years had variable forms of anaemia with 42% of women 15-49 years also being anaemic (Ghana Statistical Service & Ghana Health Service, 2015).

Anaemia is prevented or treated with vitamins or mineral supplements and diet. The dietary regimen includes iron-rich foods and other micronutrients such as vitamin C, vitamin B_{12}, and folate. Promoting intake of essential nutrients, including indigenous foods, from local foods that are currently underexplored, have also been proposed to help reduce micronutrient malnutrition (Dewey & Vitta, 2013). *Dialium guineense* also known as black velvet tamarind (BVT) in English is a widely consumed wild fruit in tropical Africa. It is commonly known as *yooy*i in Ghana and in other parts of Africa, it is called *Icheku* (Igbo), *Assonswe*n (Fon; Benin) *Awin* (Yoruba) *Tsamiyar Kurmi* (Hausa) (Aiyeloja & Bello, 2006; Gnansounou *et al*., 2014). It is a shrubby tree with densely leafy crown that grows to about 15-30m high. The fruit is usually globose and flattened with about 6mm stalk. Black Velvet Tamarind fruit has a black velvety bristle shell and a reddish-brown, sweet-tasting pulp with about 1 or 2 seeds embedded inside (Asoiro *et al.*, 2017; Osanaiye *et al*., 2013; Oyegoke & Oladiji, 2018). It is an annual fruit eaten fresh as a snack by people of all ages. It has been used by food industries to make products like non-alcoholic drinks, candies and jam (Asoiro *et al.,* 2017b). Its roots, bark, leaves and seeds have also been recognized as having Therapeutic value are used to treat a range of health conditions and such as genital infection, diarrhoea, severe cough and malaria (Bero *et al.*, 2009).

Proximate analysis of BVT in some studies showed that the pulp is a rich source of micronutrients especially iron, magnesium, calcium and vitamin C (Gnansounou *et al.,* 2014; Osanaiye *et al.*, 2013). Gnansounou *et al.* (2013) and Osanaiye *et al*. (2013) reported iron and vitamin C content of BVT as 14.75±0.25 mg/100g and 1.43±0.01 mg/l00g respectively. Compared to raw lean beef which is an excellent source of iron, the iron content of BVT is higher, Williams *et.al.*, (2007) reported that a 100g of raw beef contained 1.8 mg iron while the liver contained 5.8 mg and the liver of lamb contains 9.5 mg. These values are however expected to reduce upon cooking with the extent of iron loss or retention dependent on the cooking method (Pereira *et al.*, 2014; Pourkhalili *et al.,* 2013; Turhan *et al.*, 2004). Bioavailability of the iron in BVT is questionable because it is non-heme iron. Nonetheless, non-heme iron absorption can be augmented by either increasing vitamin C intake or by decreasing the content of antinutrients or absorption inhibitors such as phytates and tannins (Zijp, *et al.*, 2000). The rich balance of both iron and vitamin C in BVT combined with low levels of anti-nutrients specifically cyanide, tannins, phytate and oxalate (Niyi, 2015) makes its iron absorption potentially effective and hence a possible good source of iron that may be effective in dietary interventions to curb anaemia in Africa. Although the nutrient composition of BVT has been largely ascertained, its influence on haemoglobin levels in humans or animals needs further investigation.

1.2 Problem Statement

Micronutrient malnutrition especially iron deficiency anaemia is still a nutrition related problem in Ghana despite several nutrition interventions such as supplementation and food fortification. In recent times there have been suggestions to promote intake of indigenous fruits to help curb this situation (Dewey & Vitta, 2013). Some researchers proposed enhanced intake of BVT and fortification of infant meals with BVT pulp powder (Ewedje & Tandjiekpon, 2013). These suggestions were based on the nutritional composition of BVT which has received much attention lately. The fruit is rich in iron and vitamin C and low in anti-nutrients (Gnansounou *et al.*, 2014; Niyi, 2015; Osanaiye *et al.*, 2013). Scientific evidence on the true potential of BVT to raise haemoglobin levels is limited. Currently, there is only one published study that has tested BVT on haematological parameters of rats. Findings of the study did not show any significant effect of BVT on blood composition however the fruit was found to be toxicologically safe for consumption (Oyegoke & Oladiji, 2018). This study however administered BVT as a meal-based diet which contained corn starch, soybeans, vitamins and minerals, sucrose, cellulose, soybean oil, D-Methionine and different proportions of BVT pulp powder to the rats. Interaction between the

BVT and the other meal constituents is unknown. This warrants further studies to explore the effect of BVT on haematological parameters.

1.3 Significance of Study

In a study to assess the toxicity of *Dialium guineense* fruit pulp, (Oyegoke & Oladiji, 2018) observed no significant difference ($p<0.05$) among treatment groups fed with *Dialium guineense* fruit pulp meal based diet. The findings indicated that *Dialium guineense* could express structural toxicity on selected organs (liver and kidney), but depicted little functional toxicity signs that are within the tolerable range.

Findings of this research will provide evidence based data on *the* ability of *Dialium guineense* to boost haemoglobin levels of animals. It will also provide useful information which may help in dietary management of iron deficiency anaemia. The food industry will also have evidence-based information to guide them in the development and fortification of food products. The research can serve as a baseline for further studies.

1.4 Hypothesis

- H_{01} *Dialium guineense* fruit pulp will not improve iron and haematological profiles of rat

1.5 Aim

The aim of this study was to determine the effect of *Dialium guineense* pulp on iron and haematological profile levels of rat models.

1.6 Specific Objectives

1. To assess the effect of *Dialium guineense* pulp powder on iron profiles of rat models.
2. To assess the effect of *Dialium guineense* pulp powder on haematological profiles of rat models.
3. To determine the relationship between iron, total iron binding capacity, percentage saturation and haematological profiles of rat models.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Anaemia

One-third of the global population is affected by anaemia with the prominent cause being IDA. According to the 2016 Global Health Observatory data, pre-school children (< 5 years), have the most prevalence of IDA; with prevalence rates of up to 41.7%. 32.8% and 40.1%, are found in females of reproductive age and pregnant women respectively. This prevalence translates to 273.2 million (95%) children, 496.3 million (95%) non-pregnant females, and 32.4 million (95%) pregnant females, providing a total of 528.7 million (95%) females of a reproductive age with anaemia globally in 2011. Of these, 9.6 million (95%) Children, 19.4 million (95%) non-pregnant females and 0.8 million (95%) pregnant females had severe anaemia, resulting in a total of 20.2 million (95%) reproductive-age females (WHO, 2011). In the elderly population, IDA is more complicated to treat and only accounts for about 30 % of cases of anaemia, as there may be other forms of anaemia that exist (Busti, *et al.*, 2014).

In the African region, children accounted for 62.3% being the largest percentage of people affected by severe anaemia in 2011 (WHO, 2011). About 50% of cases of anaemia are considered to be due to iron deficiency (WHO, 2011).

In Ghana, anaemia is prevalent in rural areas, poor families and in the Northern Belt, 35.6% of children had anaemia, 21.5% with iron deficiency, 12.2% had anaemia with iron deficiency. 21.7% non- pregnant women were anaemic, 13.7% were iron deficient, and 8.9% had iron deficiency anaemia (Wegmü Ller Id *et al.*, 2020).

2.2 Nutritional Anaemias

2.2.1 Folate-Deficiency Anaemia

Folate deficiency can lead to anaemia (megaloblastic) primarily through their role in thymidylate and DNA synthesis, thus leading to ineffective erythropoiesis (Al Khatib *et al.*, 2006). This deficiency is passive in essence and most instances does not cause clinically occurring anaemia (Alaliwi, *et al.*, 2018).

2.2.2 Vitamin B12 Deficiency Anaemia

Vitamin B12 deficiency is another causative of nutritional anaemia. Predominant cases are due to malabsorption. Any cause of vitamin B12 deficiency must continue for years, before any symptoms are noticed as body stores are high, vitamin B12 deficiency will rarely be attributed to reduce dietary intake as average adults have around 2,500 μg stores, rendering insufficiency very unlikely during dietary intake. Vitamin B12 can only come from animal products, such as meat, fish, liver, and eggs, and in adults, its daily requirement is around 1 μg. For the absorption of vitamin B12, the intrinsic factor is essential and induces absorption in the ileum. (Alaliwi *et al.*, 2018).

2.2.3 Iron Deficiency Anaemia

Iron deficiency is refers to the decrease in total body iron reserves, resulting in a long-term negative iron balance (Cappellini, *et al.*, 2020 & WHO, 2011). Iron deficiency anaemia can occur in instances of increased demand, reduced intake, reduced or malabsorption, or chronic blood loss. Increased demand is typically physiological and is frequently found in infants, pre-school children, teenage growth spurts and pregnancy (typically second and third trimesters) (Cappellini *et al.*, 2020).

Functionally, the lack of mobilizable iron stores would ultimately lead to a measurable improvement in classical laboratory tests, including haemoglobin concentration, mean corpuscular haemoglobin concentration, mean corpuscular volume, total iron binding capacity, saturation of transferrin, and zinc-erythrocyte protoporphyrin. (WHO, 2001).

Iron is an essential dietary mineral that is associated with many functions of the body, such as blood oxygen transport. The features of iron deficiency anaemia are inadequate haemoglobin synthesis, which results in microcytic and hypochromic red blood cells. The capacity of the blood to carry oxygen to all cells and tissues of the body is decreased due to inadequate haemoglobin (Aldallal, 2016).

Severe anaemia is significantly correlated with increased mortality, behavioural and functional results, ranging from 0.9% to 1.5% in children and females in 2011 (WHO, 2011). IDA adversely affect the cognitive capacity, motor actions and physical growth of babies, as well as the health and growth of preschool and school-aged children. During pregnancy IDA elevates the perinatal risk for mothers and new born and surges the overall mortality rate of babies (WHO, 2001), the increased risk of preterm delivery, low neonatal weight and perinatal complications. Increased infant and maternal mortality is also associated with extreme iron deficiency anaemia due to reduced tolerance for excessive blood loss during childbirth and increased risk of infection (Mirza *et al.*, 2018).

Fig. 2.1 illustrates the Clinical effects of anaemia due to iron deficiency

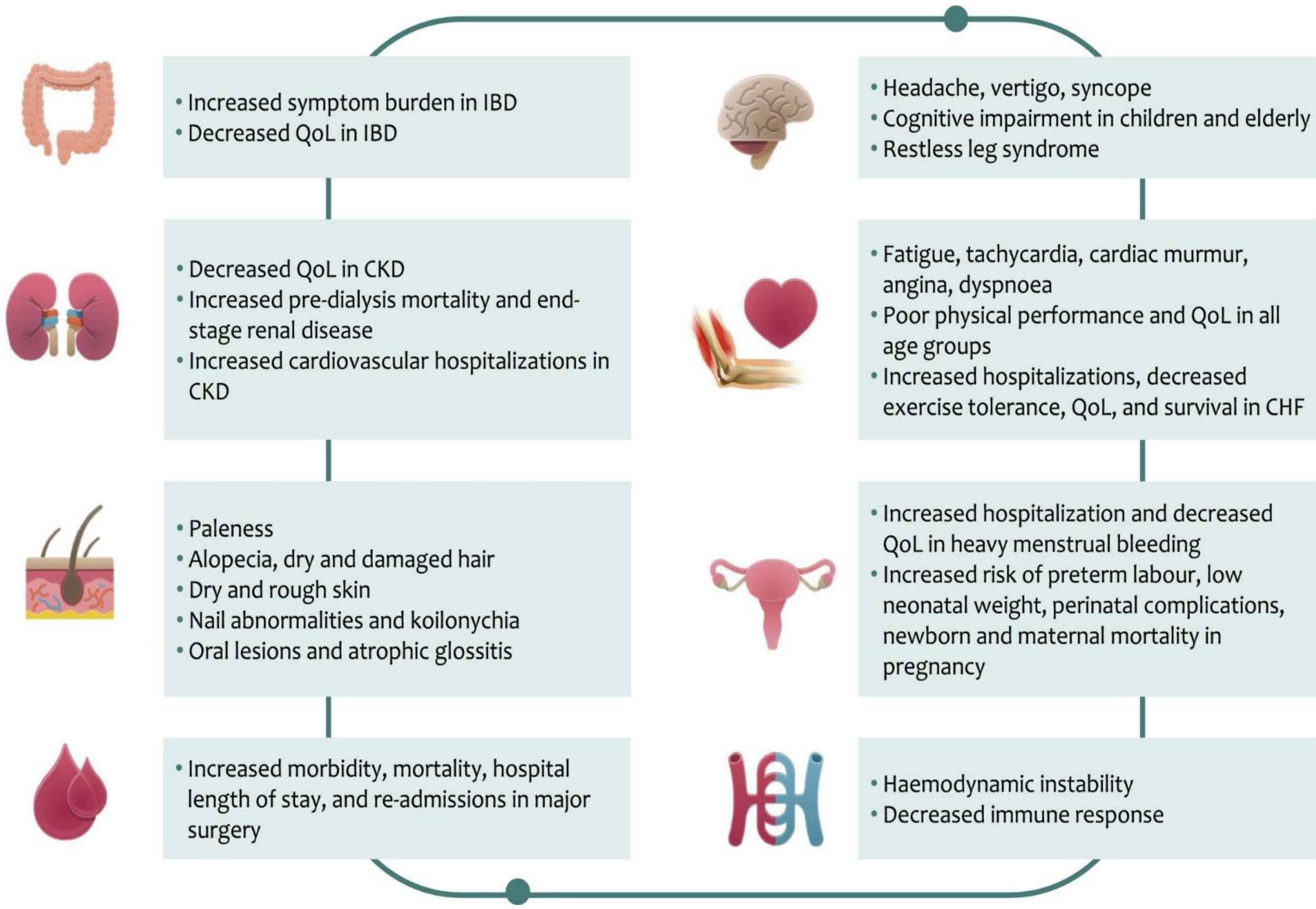


Figure 2.1: Clinical effects of anaemia due to iron deficiency.

Source: Cappellini *et al.*, 2020

2.4 Treatment of Iron Deficiency Anaemia.

The main goals of the treatment of the treatment of nutritional anaemia are to: replace or increase iron stores, treat the underlying condition that causes anaemia, prevent complications of anaemia such as heart or nerve damage, and to relieve symptoms and improve quality of life (AlDallal, 2016). These goals are achieved with the use of vitamins and mineral supplements and diet

2.4.1 Dietary Approaches for management of Iron Deficiency Anaemia

Approaches based on food are the most preferable and feasible technique to prevent malnutrition and micronutrients deficiencies. Such methods are intended to boost dietary consumption of micronutrients. Including foods that are high in iron to meals alongside with enhancers of iron bioavailability such as vitamin C helps treat IDA (WHO, 2011).

2.4.2 Food Fortification

There is a general agreement that the fortification of food is an efficient long-term strategy for enhancing populations' iron status. It is a cost-effective and sustainable way once a fortification program is developed. This involves, a suitable food vehicle that meets the target population, is processed locally and is commonly accessible and consumed by susceptible population groups in comparatively predictable quantities. In food fortification, it is imperative that the end product is not significantly altered in terms of its organoleptic quality, price or shelf life. The food should be acceptable to the target group (WHO, 2011).

2.5 Enhancers of Iron Bioavailability

The bioavailability of dietary iron is the amount of iron that is actually accessible to the body for absorption and use (Aggett, 2010). The higher bioavailability of dietary iron can be achieved by improving the quality of food components that maximize the absorption of iron or by decreasing the inhibitor quality (Hallberg *et al.*, 1989).

Ascorbic acid, as well as amino acids such as cysteine and histidine, are known to be able to reduce Fe3+to Fe2+ and, additionally, the key reducing activity is due to duodenal haemoprotein found on the enterocyte's apical membrane, which uses ascorbate to promote ferrireductase activity (Teucher *et al*., 2004). Ascorbic acid is known as the most active iron enhancer. Ascorbic Acid works by creating an iron chelate at a low pH of the stomach that is retained in the intestine (Blanco-Rojo & Vaquero, 2019). This inhibits iron from interacting with other ligands, such as phytates, (one of the main inhibitor of iron in plant based diet) , which bind iron to higher pH levels (Blanco-Rojo & Vaquero, 2019).

Animal tissue, such as fish, poultry or meat has an increased effect on the absorption of iron. This is a recognised cause, although the principle of this mechanism of action needs further studies Bæch *et al.*, (2003) in a study evaluating the bioavailability of iron in young women: the addition of pork meat (50 g or 75 g) to a meal believed to have poor iron bioavailability (containing 220 mg of phytic acid and 7.4 mg of ascorbic acid) substantially improved iron absorption. Another research found that iron absorption doubled by adding 60 g of lean fish to a basic meal consisting of boiled vegetables, rice and curry (Hallberg, *et al.*, 1978). Several of these experiments were conducted using low-fat meat or fish, however Navas-Carretero *et al*., (2008) examined the potential effect of adding salmon fish to high-phytate foods. The research was intended to replicate a meal, red-kidney beans (80 g) with or without salmon (100 g), which was spiked with stable isotopes and given to iron-deficient women. The fish contained about 20% protein, expected to benefit bioavailability, and 20% fat, part of which was unsaturated fat, which was reported to induce alterations in cell membranes and haemolysis at high doses (Pérez-Granados, *et al.*, 1995). Findings reported that the portion of salmon has substantially improved the absorption of iron from bean meal, indicating that an effective combination of enhancers and inhibitors influences the final effect on the bioavailability of iron (Navas-Carretero *et al.*, 2008).

2.6 Inhibiters of Iron Bioavailability

High intake of a repetitive diet of grains and legumes are deficient in bioavailable iron due to the presence of phytic acid and certain polyphenols that are inhibitors of the bioavailability of iron. (Blanco-Rojo & Vaquero, 2019 & Tako, 2018). Furthermore, current findings also indicates that cellular structures of legumes, such as cotyledon cell walls, may also be a key factor restricting the absorption of iron from legume (Tako, 2018). Micronutrient deficiencies or decreased bioavailability are more often characterized by low dietary quality than by inadequate energy intake (Tako, 2018). Most types of tea, wine, caffeine, chocolate, herbs and spices are examples of iron inhibitors (Hurrell & Egli, 2010; Navas-Carretero *et al.*, 2007; Petry, *et al.*, 2015).

2.7 Role of Indigenous Foods in the Nutrition Landscape

There is a growing discussion on finding means of sustainable nutrition worldwide with emphasis on strategies that are less expensive and acceptable to communities and less burdening to the environment. One identified strategy is the use of an ecosystem approach that focuses on encouraging the use of traditional and indigenous food (Ghosh-Jerath, *et al.*, 2015). Indigenous foods are foods that originate in and are characteristic of a particular region or country. They are locally accessible without having to purchase them. Some common indigenous foods in Africa including Ghana are *Moringa oleifera*, African Star Apple (*Chrysophyllum albidum*) and Black Velvet Tamarind (*Dialium guineense).*

Although indigenous foods grow spontaneously in an ecosystem and used to be the main foods during the hunter and gatherer era (Bharucha & Pretty, 2010), they are currently highly underutilised especially among the younger generation compared to the older ones. An ethnobotanical survey carried out in the Eastern Cape Province in South Africa to document the wild vegetables growing in the Amathole District municipality and to assess the state of the communities' knowledge, revealed that compared to older women, the younger generation and men had little knowledge of indigenous foods (Bvenura & Afolayan, 2014). Majority of the study population preferred intake of conventional vegetables to wild or indigenous ones. Globally, there have been less recognition for indigenous foods in food and nutrition policies (Cousins & Witkowski, 2015). Notwithstanding, the South African food based dietary guidelines have currently recognised and are promoting the utilization of indigenous foods (Vorster *et al.*, 2013).

Indigenous food studies and nutritional activities related to these foods are gaining ground in recent times. This may be due to the possibility of these foods to provide excellent nutrition due to their diversity, which is important for the maintenance of a holistic health status through natural and less expensive means. It could also be due to the ability of indigenous foods to provide a buffer against the increasing patronization of marketed, processed foods (Ghosh-Jerath *et al.*, 2015). Indigenous foods may be a powerful source of nutrients and could potentially contribute to the global nutrition basket. Kruger *et al.* (2015), have suggested that indigenous foods can play a key role in improving dietary quality and food and nutrition protection. Similarly, Dewey & Vitta (2013) have proposed promoting intake of key nutrients from local foods including indigenous foods that are currently underutilized to help reduce micronutrient malnutrition. There is evidence from studies that indigenous foods are indeed nutritious and healthy for consumption. Uvsiku *et al.* (2010) discovered from their study that most healthy sources of micronutrients such as vitamin A, C, iron, zinc and magnesium are available in African leafy vegetables and provide substantial amounts of micro-nutrients that are important for health. In an exploratory cross-sectional study, Ghosh-Jerath *et al*. (2015) studied the quantitative nutritional importance of indigenous foods in Oraon Tribal community of Jharkhand, India. The researchers carried out nutrient analysis in laboratories and also used data from the Indian food composition database where available. They found that majority of the over 130 varieties of indigenous foods identified in the region contained an appreciable amount of micronutrients especially iron, vitamin C, beta carotene, calcium, thiamine etc. Another investigative cross-sectional study with a longitudinal dietary intake assessment component was carried out to determine the influence of indigenous foods to nutritional status and nutrient intakes of 151 women (15-49 years) from 151 different households in Jharkhand, India (Ghosh-Jerath *et al.*, 2016). The Researchers discovered that although a widespread variety of indigenous foods were reported by participants, dietary recalls showed low intake. In the past two days, women who ate indigenous foods had a substantially higher calcium and iron intake than those who did not. This implies that although the population had rich knowledge of the availability of indigenous foods in the region, this did not translate into utilization probably due to lack of awareness of the nutritive value of these foods.

2.8 Black Velvet Tamarind

2.8.1 Botanical description

Dialium guineense, also known as black velvet tamarind belongs to the sub-family of Caesalpinioideae and family of Fabaceae and. BVT tree is about 30 m of height with densely leafy crown, greyish smooth bark (Osanaiye et al., 2013). It has hairy finely leaves, with a common stem about 5 to 13 cm long, generally alternate leaflets, the lower leaflet being slightly smaller, elliptical to broadly elliptical, Occasionally somewhat oval, blunt at the apex, symmetrical and rounded or slightly curved at the base; leathery, globous above and with a short acuminate base (Besong *et al.,* 2016). Flowers are mostly white in a broad terminal, or sometimes axillary, with about 30 cm panicles; branches spread widely (horizontally) (Szolnok, 1985).

The fruits are very noticeable and unique. Fruits are usually plentiful, less circular and flattened. (Aiyeloja & Bello, 2006). Fruits have a diameter about 2.5 cm, densely velvety and black. Each fruit has about 6 mm long stalk with a little collar near the apex, and a brittle shell enclosing 1 seed (or unusually 2), embedded in a dry, brownish, sweetly acidic, pulp (Aiyeloja & Bello, 2006). Edible fruits are mostly in season between January and May; but between March and April is the peak period for harvest (Okafor, 1975).

2.8.2 Ecology

The velvet tamarind grows in dense savannah forests, shady canyons and galley forests. It is found on the southern border of the Sahel, from Senegal to Sudan. It is found naturally in wet, often black soils with average annual rainfall of less than 2100-2600 mm (Besong *et al.*, 2016).

2.8.3 Distribution

It is also present in West African countries such as Ghana, Guinea-Liberia, Benin, Burkina Fasso, Mali, Senegal, Sierra Leone, Cameroon, Central African Republic, Chad, Ivory Coast, and Togo. It is often referred to as "*icheku*" among the Igbo of Nigeria, as *"awin"* among the Yorubas, and as "*tsamiyar kurm*" among the Hausas of Nigeria (Nwosu, 2000 & Akinpelu *et al.*, 2011). In Ghana, it is also referred to as *"yoyi",* Sierra Leone "tamarind" and "*tamarinier noir*" in French.

Fig. 2.2; depicts from left, BVT tree, BVT fruit shield, BVT fruit edible pulp-unshelled.

BVT tree

BVT fruit-shelled

BVT fruit edible pulp- unshelled

Figure 2.2: BVT tree, fruit-shelled and edible pulp-unshelled

Source: (Gnansounou *et al*., 2014)

2.8.4 Proximate Analysis

Proximate analysis of BVT shows that the pulp is a rich source of micronutrients especially iron, magnesium, vitamin C and calcium (Osanaiye *et al.,* 2013; Gnansounou *et al.,* 2014;). Studies by Gnansounou *et al.,* (2014) to analyse the chemical composition of BVT from three different localities in Benin, revealed the presence of magnesium, calcium, iron, sodium, potassium and iodine in varying quantities with not much difference in composition among the fruits from the three localities (table 2.1). Considerable variations exist in nutrient composition of the same foods due to their biological origin by virtue of factors such as degree of maturity, genetic influence, temperature, sunshine and nature of soil (Gnansounou *et al.,* 2014). However, nutrient composition of BVT as determined by Gnansounou *et al*., (2014) were not far different from BVT from Nigeria determined by Adepoju (2009).

While the bioavailability of plant nutrients are usually challenged by anti-nutritional factors such as tannins, cyanide, phytate and oxalate, Niyi (2015) found that BVT had relatively lower levels of these anti-nutritional factors (table 2.2). More specifically, their analysis showed that cyanide which is capable of binding to haemoglobin to form cyano-haemoglobin and cause serious disorders in the blood system was very low and hence consumption of BVT will not cause any negative disorder in the blood.

Table 2.1: Nutrient composition of BVT

Nutrient composition of BVT	
Nutrient	**Amount (mg/100 g BVT)**
Vitamin C	04.50± 0.01
Iodine	04.34±0.12
Iron	14.75±0.25
Magnesium	30.84±5.60

Source: (Gnansounou *et al*., 2014)

Table 2.2: Anti nutritional factors BVT pulp

Anti-nutritional factors BVT pulp	
Nutrient	**Amount (mg/100g BVT)**
Phytate	112.8±0.02
Oxalate	2.251±0.01
Tannin	0.076±0.06
Cyanide	0.338±0.04

Source: (Gnansounou *et al*., 2014)

2.8.5 Ethnomedicinal Uses of *Dialium guineense*

Traditionally, various part of the plant have been used in treating diseases such as headache, fever, cancer, haemorrhoids and among others. There is literature on the ability the saponin in BVT plant to provide a cleansing effect of the teeth, which helps prevent dental caries and plaques (Alo *et al.*, 2012) The fruits of black velvet tamarind are chewed by most females in South-eastern Nigeria to check genital infection and improve lactation (Asoiro, *et al.,* 2017). Literature records that, the bark is used to treat stomach ache and also as a local tooth brush to maintain oral hygiene (Ogu & Amiebenomo, 2012; Akinpelu *et al*., 2011). The leaves are used as a therapeutic drug in fever, prenatal pains and oedema; the fruit in treating diarrhoea (Arbonnier, 2004).

The stem bark and leaves are used as traditional therapies for illnesses such as extreme cough, haemorrhoids, bronchitis, burns, malaria, diarrhoea, stomach ache jaundice and ulcers (Bero *et al*., 2009). The antioxidant properties and vitamin C makes it a good food nutrient to improve the body's immunity. Findings of Okegbile *et al*., (1990) affirms the high content of vitamin C and other micronutrients in wild fruits compared to other fruits such as oranges, pineapples, paws and commercially grown fruits. It is used in folk medicine for the prevention of heart disease. Research states that additionally to its antioxidant action, the tannin portion possesses excellent cardio-protective properties (Lawrence *et al.,* 1997 as cited in David, A. A *et al.,* 2011). Fruit pulp is made up of more than 13% of dietary fiber, which improves bowel movement and helps prevent constipation. The fiber also binds to toxins in food to aid defend the mucus membrane of the colon from chemical cancer. Additionally, it binds to bile salt and decreases their re-absorption in the colon, thus helping to remove “bad” cholesterol (Besong *et al.,* 2016).

2.8.6 Biological Activities of *Dailium guineense*

Diverse sections of the plants have notable therapeutic activities that can help this plant’s traditional use in the therapy of certain disorders. The therapeutic characteristics and pharmacological actions of *Dialium Guineense* are reported in several ways, based on contemporary scientific research.

2.8.6.1 Anti-ulcer Activities

The aqueous extract from *Dialium Guineense* was assessed with ethanol / HCL and indomethacin as ulcerative for its anti-ulcer activity. Furthermore, the impact of the extract on gastric mucous secretion was explored. The sample was given orally to the experimental groups at doses of 100 and 200 mg/kg bwt, with the control and treatment groups receiving distilled water (5 ml/kg) and cimetidine (32 mg/kg), respectively. The magnitude of healing was determined by decreasing the index of ulcer. The results showed that the extract reduced the ulcer index significantly from 4.75±0.17 to 0.20±0.12 and from 3.95±0.19 to 0.14±0.09 in ulceration-induced ethanol / HCL and indomethacin, respectively. The decoction also boosted the gastric mucus secretion significantly. The research revealed that aqueous extract of *Dialium guineense* has anti-ulcer impact that may be due to its capacity to boost the secretion of gastric mucus. The results of this research also warrant the use of *Dialium guineense* in folkloric gastric ulcer therapy (Balogun *et al.*, 2013).

2.8.6.2 Anti-microbial Activity

Ogu *et al.* (2012) determined the antimicrobial activity of *Dialium guineense* in a study using the leaf decoction against clinical isolates of six species of bacterial (*Pseudomonas aeruginosa*, *Streptococcus mutans*, *Bacillus cerus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* infection) and four fungal species (*Microsporum gypseum*, *Trichophyton rubrum Candida albicans* and *Trichophyton mentagrophytes*) Extract-formed inhibition zones were likened to the norms: ciprofloxacin and griseofulvin. The leaf extract was efficient against *S. mutans.* The findings also indicated that in comparison with the reference medication, significant diameter inhibition zones were achieved. In general, the most sensitive organisms were discovered to be the Gram-positive bacteria, followed by the fungi and the Gram-negative bacteria. The *Dialium* *guineense* leaf extract proved antimicrobial activity outcomes against the test organisms. In various ailments, this research supports its common and broad traditional applications.

2.8.6.3 Antioxidant Activity

The methanolic leaf decoction of *Dialium guineense in vitro* antioxidant has been researched with 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and energy reduction. Furthermore, it was also evaluated for the complete phenolic content. The findings of the extract's DPPH scavenging activity showed concentration-dependent antioxidant activity with peak scavenging activity at a concentration of 250 ug/ml and similar to ascorbic (95.75%) and garlic acids (93.67%). The decoction's decreasing potential (0.07±0.03 nm) was similar to that of garlic acid (0.08±0.02 nm), whereas the complete phenolic content was equal to 69.45±0.02 mg/g garlic acid. It was discovered in this research that the extract had antioxidant activity *in vitro* (Ogu *et al*., 2012).

2.8.6.4 Anti-diarrheal Activity

In a research conducted by Ogu *et al.*, (2012), the stem bark of BVT was studied on diarrheal-induced rats. The impact of the extract on castor oil-induced diarrhoea, gastrointestinal motility (charcoal meal) and castor oil-induced intestinal fluid accumulation in rats at oral doses of 50-200 mg / kg body weight were examined. The findings showed a substantial dose-dependent decrease of the extract in the watery nature and frequency of faecal dropouts over 4 hours, while lope amide lowered gastrointestinal motility by 85.8%, the extract also decreased the tiny intestine travel time of charcoal meal and intestinal fluid quantity in a way similar to 5mg / kg of each atropine. This finding validates its use in the treatment of infections such as diarrhoea as folk remedies.

2.8.6.5 Anti-vibrio Activity

In the assessment of the anti-vibrio activity of the *Dialium guineense* leaf decoction, Akinpelu *et al.*, (2011) conducted a study on 18 strains of *vibrio*. Bioactivity against 14 out of 18 environmental strains of *vibrio* species tested at a final concentration of 20 mg / ml was found. On the other hand, standard antibiotics used for ampicillin inhibited the growth of 15 of the 18 strains tested for vibrio species. The inhibition zones of the decoction against the isolates tested ranged between 12 and 20 mm. The inhibition zones shown by ampicillin against the isolates tested ranged from 7 to 40 mm. *Dialium guineense* leaf decoction demonstrated a minimum inhibitory concentration of between 0.31 and 5.0 mg/ml against *vibrio* isolates, while the minimum bactericidal concentration reported was between 0.63 and 10 mg / ml for leaf extract.

2.8.6.6 Anti-haemorrhoidal Activity

Dialium guinese decoction was found to have an astringent effect that helps to repair mucous membranes and exposed tissues and causes contraction and firmness of tissue. Herbal treatment for haemorrhoid therapy requires the use of astringents. This astringent effect is caused by tannins that have increased the lubrication of precipitating proteins. It is suggested that this astringent herb performs anti-haemorrhoid function by inducing protein coagulation in the perianal skin cells or the anal canal liner. They plug minute leaks and holes in the veins and capillaries, encourage the elasticity of the veins and act as vasoconstrictors in the perianal zone. This increases the skin's dryness, which in turn will help burn relief, scratching and pain associated with haemorrhoids (Odukoya *et al.*, 2009).

Complete blood count is one of the most often requested routine blood tests; it offers key indices such as haemoglobin and the amount of white cell differentials and offers information to help diagnose anaemia (Blann, 2014). Red blood cells (also referred to as erythrocytes) are the most common blood cells that supplies oxygen to body tissues through the cardiovascular system (Hajjawi, 2013). The production of red blood (erythropoiesis) cells demands nutrients, such as iron, vitamin B12 and folic acid (Hajjawi, 2013).

Haematological parameters used in the assessment of anaemia are haemoglobin and red blood cell indices (Sgnaolin *et al.,* 2013). The indices for red blood cells include the cell count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) (Sgnaolin *et al.*, 2013). The most common parameter used is the haemoglobin concentration as an indicator of the pathophysiological consequences of anaemia.

White blood cells (leucocytes) acts as the body's defence system against viruses, bacteria and parasites (Blann, 2014 & Hajjawi, 2013). White blood cells consist of five differentials. Neutrophils amount up to 70% of white cell counts, is responsible to identify, target and kill bacteria. Lymphocytes are the second most abundant white blood cell (approximately 20-25% of the white cell count) are divided into two groups – B lymphocytes produce antibodies, whereas T lymphocytes kill virus-infected cells. Monocytes: are responsible for the removal of bacteria, and are involved in inflammation and tissue repair. Eosinophils and basophils: these cells play a role in allergy and hypersensitivity (Blann, 2014)

CHAPTER THREE

3.0 METHODS

3.1 Study Design

An experimental design was employed for this study.

3.2 Study Site

This study was conducted at the Department of Animal Experimental at the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. The Department breeds and supplies pathogen-free laboratory animals for research, taking cognisance of their humane care and use and in line with the 3Rs (Reduction, Refinement & Replacement) and the 5Fs (Freedom from hunger or thirst, Freedom from discomfort, Freedom from pain, injury or disease, Freedom to express (most) normal behaviour and Freedom from fear and distress). The Department houses the secretariat of the University of Ghana Institutional Animal Care in NMIMR and the University of Ghana and Use Committee (UG-IACUC), which ensures the proper care and humane use of animals for research, testing and teaching programmes. The laboratory tests were conducted at the Liberty Medical Laboratory Services, Korle-Bu.

3.3 Sample Size

A total of twenty (20) Sprague-Dawley (SD) male rats were used for the study. The rule of 3Rs (replacement, reduction and refinement) was used alongside similar works done in other studies (Ferdowsian, & Beck, 2011).

3.4 Procedure for Data Collection

3.4.1 *Dialium guineense* feed

Powdered fruit pulp was purchased from AGROMYX Ghana, an agro-food producer and supplier of a wide range of food items. Fruit pulp powder was purchased from this company due to their high safety precautions during production and the high quality of their product. Nutritional contents of the fruit pulp is shown in Table 3.1.

Table 3.1: Nutritional Composition of the BVT Fruit Pulp Powder

SERVING SIZE	**130g**	**% DAILY VALUE**
Amount Per Serving Calories	280 Calories	
Sodium	80 g	3%
Total Caborhydrate	72 g	24%
Dietary Fiber	48 g	192%
Sugars	16 g	
Vitamin C	162%	
Calcium	32%	
Iron	32%	
Magnesium	2.176 mg	62%

Source: AGROMYX

3.4.2 Rat Chow Diet

The animals were provided with normal feed and water and fed *ad libitum*. The feed with standardization was obtained from the Ghana Agro Food Company (GAFCO). Ghana Agro Food Co. Ltd. (GAFCO) is located in Accra, Ghana. It is a company in the agro food industry that processes agricultural products for sale to the public

3.4.3 Laboratory animals

Animals were obtained from the Department of Animal Experimentation, NMIMR of the University of Ghana. Rats were acclimatized for 7 days by keeping them in a well-ventilated animal house (temperature: 22 ± 3^{o}C; photoperiod: 12 hrs light and 12 hrs dark; humidity: 40 - 45%) with free access to tap water and rat pellets. Animals (150 – 200 g) were randomized and divided into four groups of five animals each, comprising a control group (Group 1) and three Treatment groups (Groups 2 – 4). Rats were marked, coded and housed in groups (weight-matched and grouped into five per cage). At the end of seven days of acclimatization rats were placed on the *Dailium guineense* fruit juice (apart from the control group) in addition to a normal diet for 90 days, the 90- day study provides information on the possible efficacy of the BVT fruit pulp to raise haemoglobin levels and also possible health hazards likely to arise from repeated exposure over a prolonged period of time. Furthermore, animals were weighed weekly for the 90 days period.

3.4.4 Animal groupings and feeding

Fruit Juice was prepared in 3 different concentrations, as shown in Table 3.2, it was administered orally by the use of a gavage.

Table 3.2: Animal Groupings and Feed Composition

GROUP	FRUIT PULP (g)	CODE
1	Normal rat palettes	Control
2	2.5 g/kg/bwt	Low Dose
3	5 g/kg/bwt	Medium Dose
4	10 g/kg/bwt	High Dose

Rats from each group were sacrificed after the period of study. Blood was obtained by cardiac puncture after rats were anaesthetized using ethyl ether. Animals were then euthanizated and organs harvested thereafter. Two milliliters of blood was discharged into EDTA tubes for haematological analysis, and about 3 mls into iron-free tubes for the serum iron profile.

3.4.5 Rats Organ Collection

The abdomen and thoracic cage was dissected to harvest the spleen, heart, kidney, liver. These organs were rinsed with 0.9% NaCl solution (Seo *et al.*, 2012), and then dried on filter paper and electronically weighed separately (LAB-KITS, 2010) for further analysis. Organs were kept in buffered formalin solutions to be histologically examined later (which is beyond the scope of this work).

3.4.5 Determination of Haematological Profiles

Whole blood was used for haematological indices determination. The Automated haematology analyser, Sysmex, KX-21 (Japan) was used to determine concentrations of haemoglobin (Hb), red blood cells (RBC), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Mean Cell Volume (MCV) and White Blood Cell (WBC).

3.4.6 Determination of Iron and Total Iron Binding Capacity

Blood collected into iron-free tubes were allowed to clot at room temperature. The samples were centrifuged at 3000g for 10 min to separate the serum. Aliquots of sera were stored in Eppendorf tubes, labelled and stored in the laboratory freezer at −80°C until use.

3.4.7 Reagents Composition

R5 Saturating solution	Iron solution	500 µg/dL
R6 Precipitating solution	Magnesium bicarbonate	500 µg/dL
Ref. 30270	Iron Ferrozine	

3.4.8 Reagent Preparation and Stability

Reagents were in their ready to use state.

3.5 Test Procedure for iron concentration

Serum (0.5ml) and 1.0ml of R5 Saturating solution were pipetted into test tubes. They were mixed well and incubated for 10 mins at room temperature (15-25C). 3 spoonful of R6 Precipitating agent was added to each test tube, mixed well and incubated for 10min, at room temperature. Samples were then centrifuged for 15min, at 3000r.p.m. The supernatant was carefully collected and iron concentration was measured.

3.6 Calculation of TIBC and %SAT

TIBC = serum iron + unsaturated iron

$$\%SAT = \frac{\text{serum iron}}{\text{TIBC}} \times 100$$

3.7 Data Analysis

Data was entered in Microsoft excel and exported into SPSS version 23.0 for analysis. The findings were reported as mean ± standard deviation. Kruskal-Wallis test was to determine mean differences between groups. Spearman's correlation test was performed to determine the relationship between iron, TIBC, percent saturation and haematological indices of rats. Statistical significance was set at $p<0.05$.

3.8 Ethical Issues

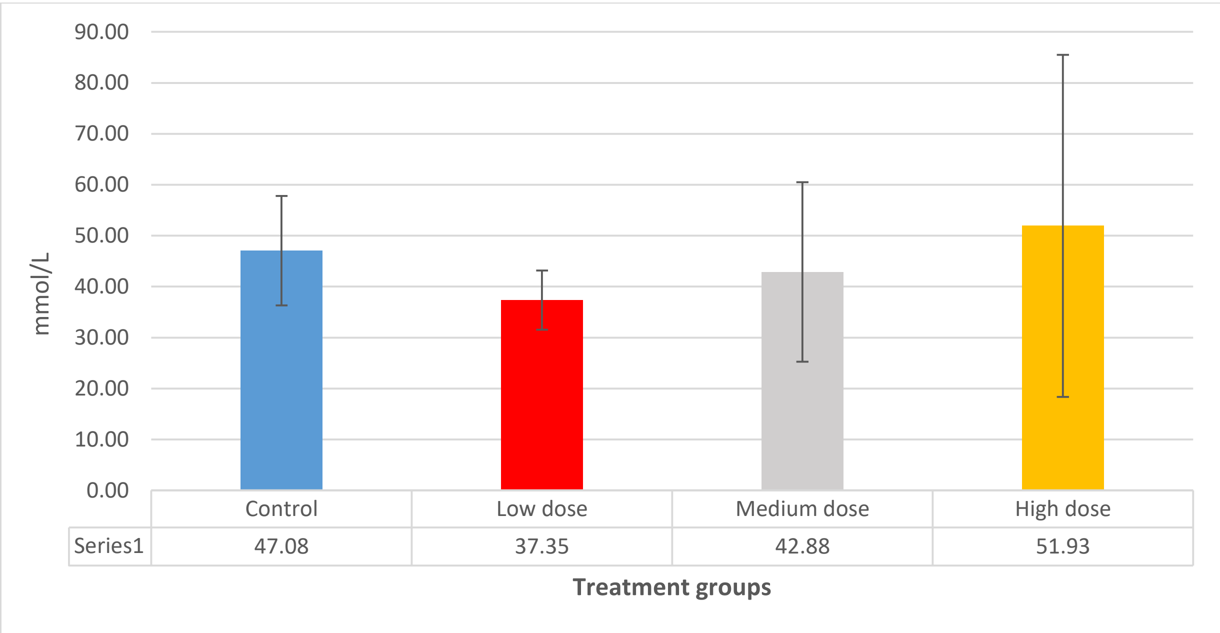
Ethical clearance was obtained from the Ethics and Protocol Review Committee of the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC). This experiment was carried out in accordance with the protocol for good laboratory practice. All methods and techniques used in this study were consistent with the 3Rs (Reduction, Refinement & Replacement) and the 5Fs (Freedom from hunger or thirst, Freedom from discomfort, Freedom from pain, injury or disease, Freedom to express (most) normal behaviour and Freedom from fear and distress).

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of *Dialium guineense* fruit pulp on serum iron of rats.

There was a slight dose dependent increase of serum iron from the low dose group to the high dose group after the 90 days of administering the BVT fruit pulp. It is noteworthy, however, there was no statistically significant difference among the groups (Fig 4.1).



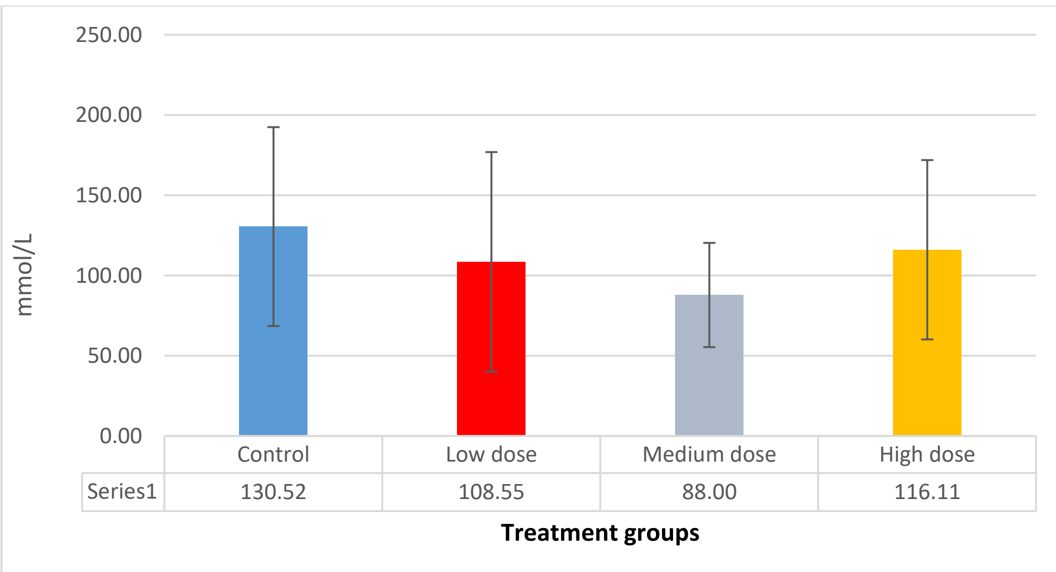
Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.1: Effect of *Dialium guineense* fruit pulp on serum iron of rats

An increase was observed in the high dose group (51.93 ± 0.58 mmol/L) as compared to the control group (47.08 ± 0.58mmol/L) Observations were however not significant.

4.2 Effect of *Dialium guineense* fruit pulp on TIBC of rats.

TIBC recorded a general decreased in the treatment groups compared to the control, with the medium dose recording the least mean. However the observed difference was not statistically significant (Fig 4.2).



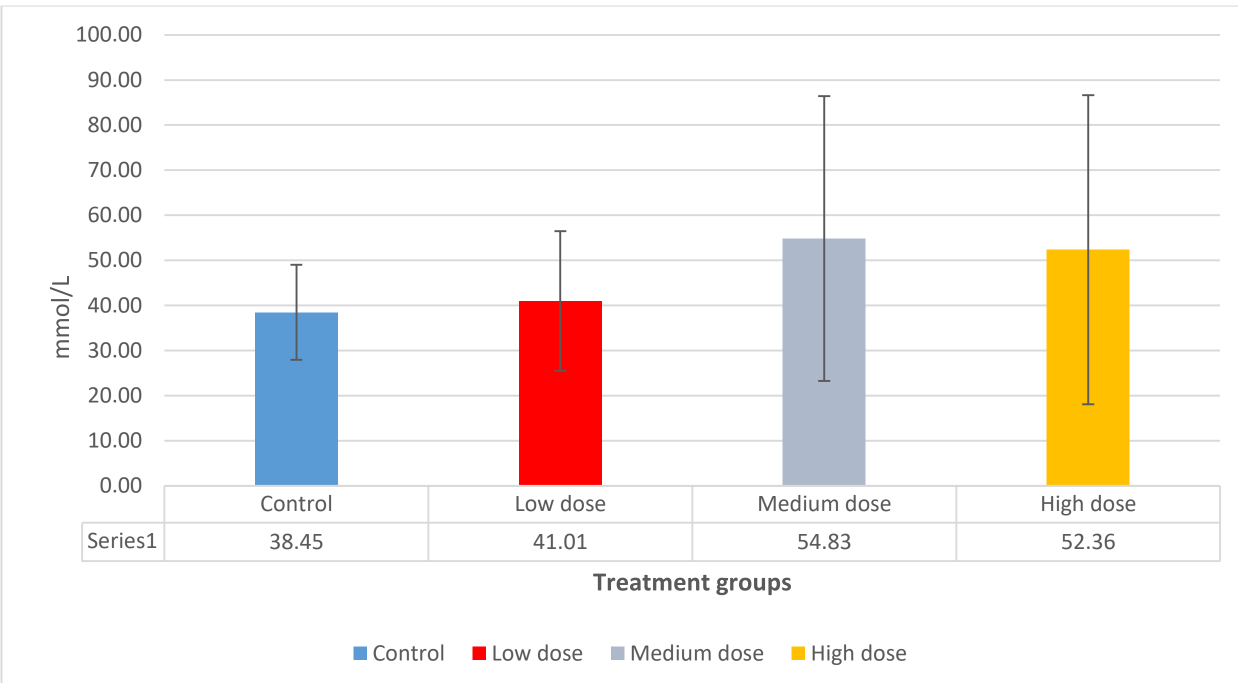
Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.2 Effect of *Dialium guineense* fruit pulp on TIBC of rats

There was a general decrease in TIBC across the treatment groups, the medium dose group recorded the least (88.00 ± 0.51mmol/L) against the control group (130.52 ± 0.576mmol/L). These differences were however not significant

4.3 Effect of *Dialium guineense* fruit pulp on %SAT parameters of rats

The mean differences in %SAT after the study is presented in Fig 4.3. An increased was observed across the experimental groups with the medium dose recording the highest mean as compared with the control. Despite the difference in means, the increase was not statistically significant.



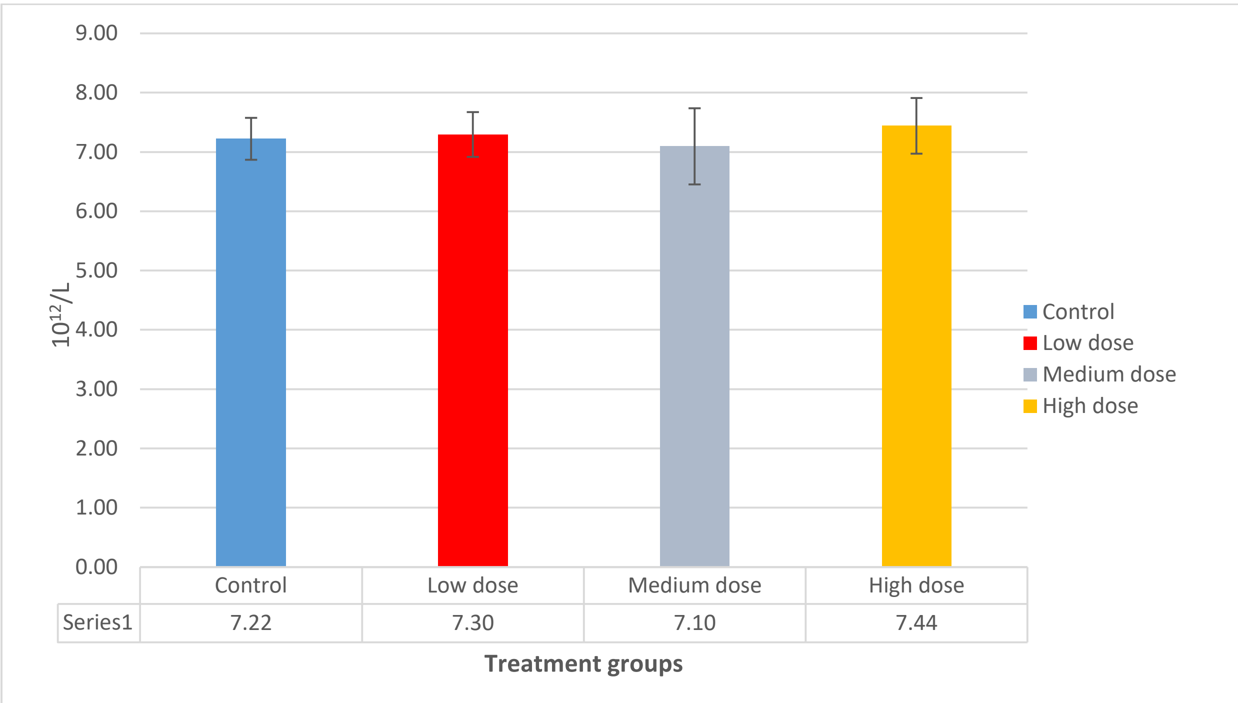
Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.3 Effect of *Dialium guineense* fruit pulp on %SAT parameters of rats

There was a general increase in %SAT across the treatment groups, with the medium dose (54.83 mmol/L) recording the highest as compared with the control group (38.45 ± 4.71 mmol/L)

4.4 Effect of *Dialium guineense* fruit pulp on RBC of rats

A dose dependent increase was observed in the RBC of rats with the high dose recording the highest mean. The increase was however, not statistically significant (Fig 4.4).



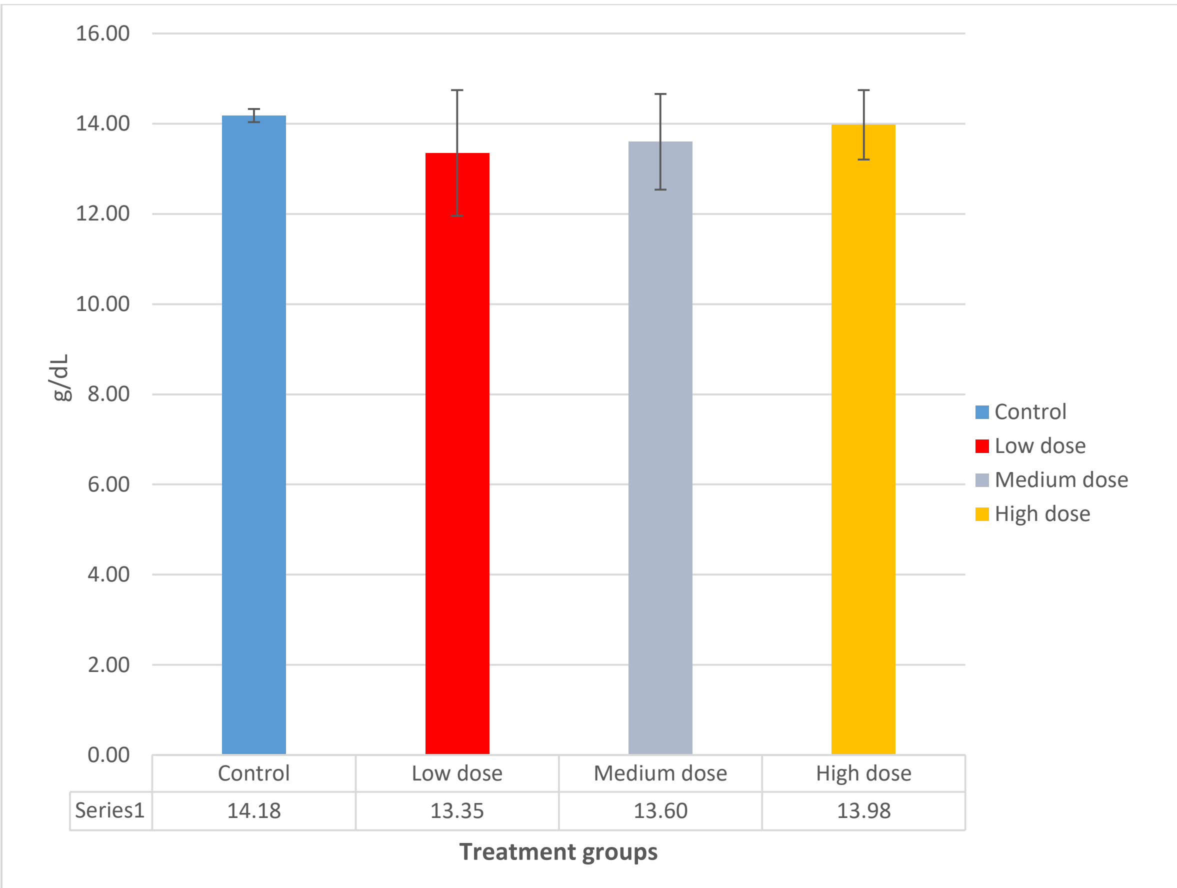
Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.4: Effect of *Dialium guineense* fruit pulp on RBC of rats

Figure 4.5 outlines the mean serum HB levels of rats. A decrease was observed across treatment groups. The low dose group recorded the lowest mean ($7.30 \pm 0.19 \times 10^{12}$/L) against the control group ($14.18 \pm 0.16 \times 10^{12}$/L).However this was not statistically significant.

4.5 Effect of *Dialium guineense* fruit pulp on HB of rats

Figure 4.5 outlines the mean serum HB levels of rats. A decrease was observed in the treatment group as compared to the control after the 90-day period. The observed differences were however not statistically significant.



Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.5: Effect of *Dialium guineense* fruit pulp on HB of rats

A decrease was observed in Hb across treatment groups. The low dose group recorded the lowest mean (13.60 ± 0.69 g/dL) against the control group (14.18 ± 0.07 g/dL). However the decrease was not statistically significant.

4.6 Effect of *Dialium guineense* fruit pulp on RBC parameters of rats

The effect of *Dialium guineense* fruit pulp on red blood cell (RBC) parameters are shown in Table 4.1. Serum, MCV and MCH levels decreased after administration of *Dialium guineense* fruit pulp. The control group had 58.82 ± 2.59 fL and 19.66±0.88 pg respectively, while the high dose group had 55.68 ± 1.93 fL and 18.78 ± 0.49 pg. Mean MCHC levels increased slightly among the high dose group (33.44 ± 0.57 g/dL). The observed differences were not statistically significant.

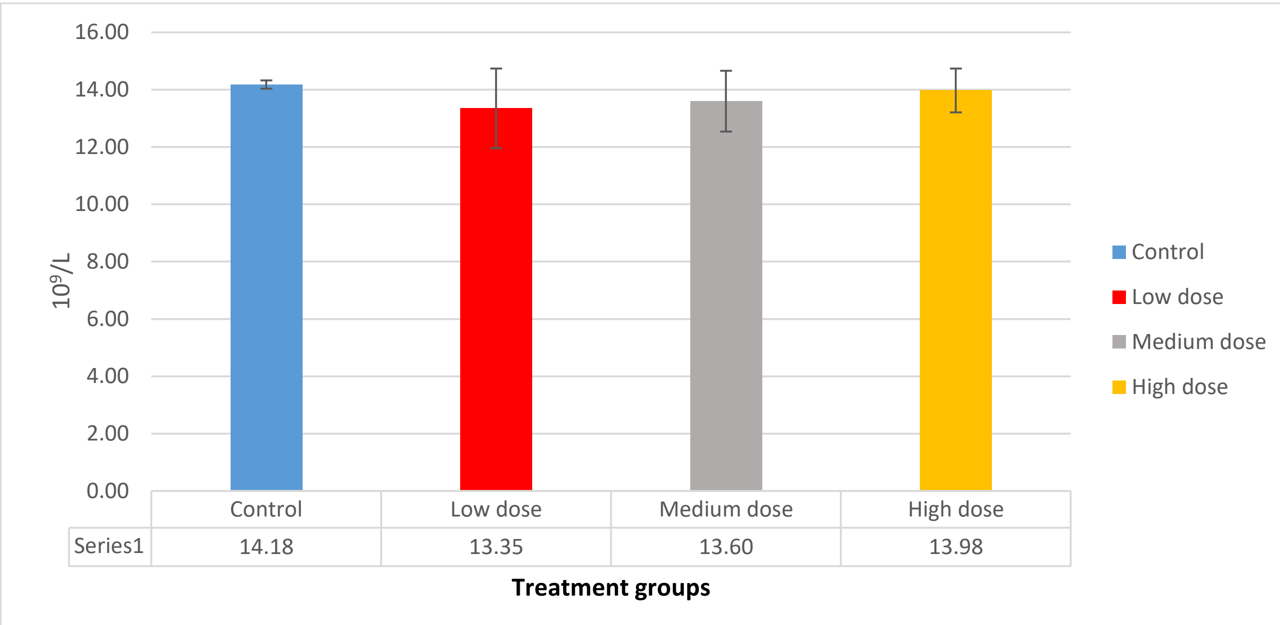
Table 4.1: Effect of *Dialium guineense* fruit pulp on RBC parameters of rats

RBC Parameters	**Treatment Groups**				
	Control **Mean±SD**	**Low Dose** **Mean±SD**	**Medium Dose** **Mean±SD**	**High Dose** **Mean ±SD**	**p-value**
MCV (fL)	58.82±2.59	54.65±3.19	57.38±1.94	55.68±1.93	0.11
MCH (pg)	19.66±0.88	18.28±1.06	19.18±0.32	18.78±0.49	0.15
MCHC (g/dL)	33.44±0.69	33.48±0.13	33.40±0.35	33.73±0.57	0.66

Kruskal-Wallis test, *fL: femtolitre, pg: pictogram. Data is presented as mean ± SEM. p is significant at < 0.05.*

4.7 Effect of *Dialium guineense* fruit pulp on WBC levels of rats

A decrease was observed in WBC among treatment groups with the low dose recording the least mean. This decrease was however not statistically significant



Data is presented as mean ± SEM. p is significant at < 0.05.

Fig 4.6: Effect of *Dialium guineense* fruit pulp on WBC levels of rats

Compared to the control, there was a general decrease in WBC among treatment groups with the high dose group recording a mean of ($13.96 \pm 0.33 \times 10^9$/L) as compared to the control ($14.18 \pm 0.33 \times 10^9$/L), however this decrease was not statistically significant, Fig 4.5.

4.8 Effect of *Dialium guineense* fruit pulp on WBC parameters

There was a general increase in white blood cell indices except for lymphocytes and basophils. These changes were not statistically significant (Table 4.2).

Table 4.2: Effect of *Dialium guineense* fruit pulp on WBC parameters

WBC Parameters	**Treatment Groups**				
	Control	**Low Dose**	**Medium Dose**	**High Dose**	**p-value**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean ±SD**	
Neu (10^9/L)	1.57±0.81	1.44±1.03	1.82±0.83	1.42±0.83	0.78
Lym (10^9/L)	8.49±3.78	4.52±2.19	5.75±1.33	4.33±1.33	0.29
Mon (10^9/L)	0.36±0.49	0.05±0.04	0.58±0.44	0.63±0.44	0.13
Eso (10^9/L)	0.04±0.04	0.51±0.99	0.04±0.03	0.03±0.03	0.91
Bas (10^9/L)	0.07±0.05	0.03±0.02	0.05±0.02	0.04±0.02	0.38
%Neu	15.88±5.53	23.27±11.56	21.18±6.36	18.05±6.36	0.64
%Lym	65.46±35.20	75.18±11.86	70.55±4.29	68.45±4.29	0.44
%Mon	3.88±1.19	0.85±0.15	7.25±2.48	7.63±3.43	0.22
%Eso	0.22±4.27	0.30±0.30	0.45±1.81	0.40±1.81	0.23
%Bas	0.56±0.21	0.40±0.22	0.58±0.13	0.65±0.13	0.53

Kruskal-Wallis test, *Data is presented as mean ± SEM. p is significant at < 0.05.*

4.9 Relationship between iron, %SAT, TIBC and haematological profiles of rats

A significantly strong negative correlation was observed between percent saturation and TIBC (r= -0.85, p<0.01). A significantly strong positive correlation was also observed between RBC and HBG (r= 0.72, p= 0.01) as well as MCV and MCH (r= 0.82, p <0.01).

Table 4.3: Correlation between Iron, TIBC, %SAT and Haematological Parameters ([a]r, [b]p)

	Iron	TIBC	%SAT	RBC	HBG	MCV	MCH	MCHC	WBC
Iron		0.02	0.40	0.18	0.23	-0.09	-0.34	0.01	0.43
		0.99	0.09	0.49	0.39	0.71	0.19	0.99	0.08
TIBC			[a]-0.60	0.02	-0.02	0.25	0.25	0.19	0.18
			[b]0.05*	0.95	0.94	0.33	0.34	0.45	0.49
%SAT				0.06	-0.02	-0.32	-0.38	-0.11	-0.06
				0.81	0.94	0.21	0.13	0.68	0.82
RBC					[a]0.72	-0.43	-0.27	-0.38	0.03
					[b]0.01*	0.08	0.29	0.14	0.89
HBG						0.34	0.16	-0.40	0.29
						0.19	0.55	0.11	0.32
MCV							[a]0.82	-0.46	0.33
							[b]<0.01*	0.06	0.19
MCH								0.01	0.12
								0.96	0.64
MCHC									-0.24
									0.35

Spearman's correlation, statistical was set at $p<0.05$

CHAPTER FIVE

5.0 DISCUSSION, RECOMMENDATIONS AND CONCLUSION

Iron deficiency anaemia (IDA) is one of the most common nutritional deficiencies, and accounts for about one-third of cases of anaemia. It is prevalent in many developing countries and, in most cases, can be diagnosed using full blood examination (complete blood count) and serum iron levels. The Recommended Dietary Allowance (RDA) for iron is: 11 mg/day for infants < 1 year of age, 7-10 mg/day for children, 8-11 mg/day for males, 8-18 mg/day for females and 27 mg/day during pregnancy (García-Casal, *et al.,* 2013). The aim of this study was to determine the effect of *Dailium guineense (*African Black Velvet Tamarind*)* on serum iron, TIBC, percent saturation and haematological parameters using rat models.

The findings of this study revealed an overall increase in the mean serum iron levels of the rat modules, with the high dose group recording highest. These changes were however not statistically significant. This may be attributed to interaction between animal feed and the fruit pulp which may have interfered with the bioavailability of the iron. This result agrees with findings of a cross-sectional study conducted by Stuetz *et al*., (2019), to assess nutritional and micronutrient status (iron and vitamin A) and their associations with the diet of female small-scale farmers in the sub-humid Kilosa, in India. In their study, they found out that women who consumed dark green leafy vegetable with wholemeal millet met the recommended daily micronutrient intakes, although there was an increase in serum iron status, the results was statistically not significant.

Total iron binding capacity (TIBC) is an important test used to diagnose iron deficiency anemia and other iron metabolism disorders. Iron binding capacity is transferrin's ability to bind with iron (Faruqi *et al.,* 2020). In assessing iron deficiency anaemia, TIBC test further arguments the results compared to iron, TIBC values will be high, when relative transferrin content decreases (Faruqi *et al.*, 2020). Percentage Saturation represents the percentage of transferrin that transport iron and thus a decrease (< 20%) indicates an iron deficiency, either absolute or functional (Bermejo & García-López, 2009). In this current study, although not significant, there was a general decrease in TIBC and increase Percentage Saturation. Contrarily, a study conducted in Iran by Naghii & Mofid, (2017) to consider the efficacy of the use of iron fortified ready-to-eat cereal and pumpkin seed kernels as two sources of dietary iron on status of iron nutrition and response of haematological characteristics of females at reproductive ages. The results of the study revealed a substantial difference between pre-and post-consumption phases for higher serum iron 60 ± 22 vs. 85 ± 23 μg/dl), higher transferrin saturation percent (16.8 ± 8.0 vs. 25.6 ± 9.0%), and lower TIBC (37 ± 31 vs. 34±31 μg /dl). After intake, most individuals had higher serum iron levels, a significant positive correlation (r=0.98, p=0.00) between the differences in serum iron levels and differences in transferrin saturation percentages and a significant negative correlation (r=-0.92, p<0.01) between the differences in serum iron levels and differences in TIBC was found, as well. These variations could be due to the fact that their study was carried out in human.

Red blood cells (RBC) also known as erythrocytes function as a transporter of haemoglobin an iron-rich protein that binds to oxygen in the lungs to transport to animal tissue for the oxidation of consumed food in order to release energy for the functions of the body (Ugwuene, 2011; Omiyale *et al*., 2012; Soetan *et al*., 2013; Isaac *et al*., 2013). This study depicted that, there was no significant difference in the mean red blood cell levels and haemoglobin concentrations of the rats, which was in accordance with the observations of Oyegoke & Oladiji, (2018). They reported no significant difference (p>0.05) between red blood cell (RBC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV), of rats fed with *Dialium guineense* fruit pulp meal-based diet at all inclusion levels (5%-40%) compared to rats in the basal diet (control) group. This means that there is no likely decrease in the amount of oxygen that will be delivered to the tissues. This also suggests that there would be no signs of anaemia.

These findings are consistent with a study carried out in the Eastern region of Ghana by Boateng, *et al.*, (2019) to determine the Impact of complementary foods fortified with Moringa oleifera leaf powder on the concentration of haemoglobin and the development of infants. 273 infants, aged 8–12 months were randomly assigned to receive one of three study foods; Weanimix a cereal-legume blend formulated with *Moringa* (*Moringa* with *Weanimix-35g*), Moringa leaf powder sprinkled on infants' usual complementary foods (*Moringa* as Sprinkles-*5g*) and the control food Weanimix without *Moringa*. Findings of this study revealed that feeding infants with a 5 g daily dose of MLP, either as part of a cereal-leguminous blend or as a supplement to infants' normal 4-month complementary foods, did not significantly increase infants' haemoglobin concentrations.

Again observations are similar to the report by Schoorl *et al.*, (2012) in their pursuit to determine Impact of iron supplementation on the content of red blood cells and haemoglobin throughout pregnancy, conducted a study with 25 participants suspected with iron deficiency. The researchers observed a significant increase in Hb but not in RBC.

Furthermore, Schaffer *et al.*, (2014) recorded similar accounts in their study to assess the impact of conventionally bred, high-iron rice on plasma iron, haemoglobin, and red blood cell variables of early-weaned piglets during a 33-day feed study. Plasma iron, haemoglobin and red blood cell variables were assessed in days 8, 16, 23, 30 and 33. There were no significant differences in the iron-related parameters examined between group 1 piglets (low-iron rice + low-iron supplementary feed) and group 2 piglets (high-iron rice + low-iron supplementary feed) except for red blood cells.

Mean Corpuscular Volume (MCV) measures the average size of Red Blood Cells, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). These gives the average amount of hemoglobin found in a single red blood cell (Ganong, 2001). NseAbasi *et al*., (2014) asserted that these three parameters indicate the conditions of the blood level. The negligible change in MCV results indicates that the size of the red blood cells is normal since an abnormally small-sized red blood cell would either signify a system failure to synthesize RBCs or a lack of available haemoglobin to be integrated into RBCs to complete the synthesis process (Oyegoke & Oladiji, 2018). Low MCHC means that a unit packed RBC contains less haemoglobin than normal and high MCHC means that there is more haemoglobin in an RBC unit. A low MCH value generally indicates the existence of iron deficiency anaemia and high MCH indicates a folate deficiency anaemia (Ganong, 2001). Consequently, their insignificant differences in this research indicate that their functions were not altered. This is line with the findings of Oyegoke & Oladiji, (2018).

In a study conducted in Karawang by Thaha, (2018) to assess the effect of fortified rice containing Fe and Zinc elements on the children's blood profile. It was observed that non-anaemia, including expiremental and control group had the impact of Fe and Zinc through ingestion of fortified rice. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCHC) levels increased significantly. There was no substantial difference in haematocrit, MCV, MCH, and MCHC among case and control groups. The authors recommended that, an increase in consumption of vitamin C foods and reducing intake of iron-inhibiting foods such as tea to facilitate iron absorption. In similar studies conducted by Adom *et al.*, (2010) in

Ghana to assess the impact of iron fortified maize and cowpea on growth and anaemia status of children (56, aged 6-18month). They reported, improved iron status in the study group, significant differences were observed in haemoglobin concentration (1.08±1.43 compared with -0.40±1.72 g/dl, p=0.01), and the risk of developing anaemia was about three times less likely among this group compared to the non-fortified group.

Considering that there was no significant difference between the RBC (erythrocytes) and its related indices (Hb, MCV, MCH and MCHC) of rats fed with BVT fruit pulp juice during the study period depicts that the *Dialium guineense* fruit pulp did not interfere with the metabolism of the red blood cell components (Ganong, 2001). This may be a suggestion that there was no destruction of mature RBCs and no alterations in the rate of erythrocyte development.

White blood cells (leukocytes) are classified as granulocytes or non-granulocytes. Granulocytes include neutrophils, eosinophils and basophils, while non-granulocytes are lymphocytes and monocytes (Egbi *et al.*, 2018). They are responsible to fight infection, protect the body from invasion by foreign species, and to generate or transport and disperse antibodies in the immune response (Al-Dulaimi *et.al.*, 2018). Animals with low white blood cells count are exposed to a high risk of infections, whereas those with high counts have the capacity of producing antibodies to the phagocytosis process and have a high degree of disease tolerance (Soetan *et al*., 2013). Some studies have found that during iron deficiency, several immune parameters are affected, particularly cell-mediated immunity and neutrophil granulocyte bactericidal activity (Chandra, 1973; Dallman, 1987). In another research aimed to assess the relationship between markers of iron status and white blood cell counts in the North-West province of South Africa, a total of 556 school children (aged 7–10 years). The study generally showed no relationships between iron status and WBC counts in this population. This findings showed no association between the measured iron markers and some immune variables (differentiated WBC). This observation was attributed to the possibility that sample was taken from a subpopulation of children with low iron deficiency prevalence (Onabanjo *et al*., 2012).

In this research, there was a general decrease in white blood cell and a general decrease among the differentials across treatment groups. Even though there was an increase in neutrophils in some treatment groups, knowing that the body's defence is centred on these. The overall results was however not statically significant, this confirms that the defense mechanism was not compromised. Again the observations in white blood cell counts, implies that the *Dialium guineense* (Balck Velvet Tamarind) fruit is not likely to pose any risk of contamination/toxicity (Oyegoke & Oladiji,. 2018).

A significantly strong negative correlation was observed between percent saturation and TIBC ($r = -0.85$, $p < 0.01$), the relationship between these two parameters is that, an increase in TIBC connotes that the amount that is already saturated is low (decreased %SAT) indicating the presence of iron deficiency and vice versa. Secondly, a significantly strong positive correlation was observed between RBC and HB, ($r = 0.72$, $p = 0.01$), Hb is an essential component of RBC as such an increase or decrease will affect the concentration of RBC (i.e.: as Hb increase, RBC increases also). Again, there was a significantly strong positive correlation between MCV and MCH ($r = 0.82$, $p < 0.01$). MCV is a measure of the average size of the red blood cells. MCH measures the average mass of heamoglobin per RBC, thus the results of MCH tend to reflect the results of MCV. This is because larger red blood cells usually contain more hemoglobin, whereas smaller red blood cells appear to have less.

5.2 Conclusion

There was a general increase in the serum iron and percent saturation levels of the rats after they were fed with the *Dialium guineense* fruit pulp for 90 days. For TIBC, a general decrease was observed among the treatment groups. All changes were however not statistically significant. With regards to the haematological indices, with exception of red blood cells and lymphocytes, there was a general increase in white blood cell indices of the rats. However, the observed differences were not statistically significant between the groups. The findings of this study showed that *Dialium guineense* did not significantly increase the serum iron levels and haematological indices of the rats, although slight variations were observed.

5.3 Limitations of the Study

However, the findings of this present research should be interpreted with the following limitations in the understanding of the preliminary analysis;

1. The fruit pulp was sourced from an already processed one, in which the proximate contents may have been affected during the production chain.

2. This study was carried out in animal models with few (20) population sample used for this study.

3. Researcher did not assess the proximate analysis of the fruit pulp powder to establish its actual nutritional content.

4. There is paucity of data with regards to haematology and iron profiles and animal work in literature. Hence this diacussion focused on medicinal plants used to boost haemoglobin levels.

5.4 Recommendations

1. Future studies should consider milling the fruit pulp themselves rather than purchasing the already processed one.

2. Future studies should consider adding ferritin and transferrin analysis to further assess the potential of BVT to increase iron levels.

3. Future research should consider increasing the sample size to have a wide overview of the impact of the fruit pulp on iron and haematological profiles.

4. Future studies should consider assessing the interaction between rat meal and *Dialium guineense* fruit pulp and its impact on iron bioavailability.

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26/10/2020

ETHICAL CLEARANCE
(UG-IACUC 007/19-20)

Your protocol for an ethical clearance has been reviewed by the University of Ghana Institutional Animal Care and Use Committee and has been approved as follows:

TITLE OF PROTOCOL: Effect of *dialium guineense* on iron and haematological profiles using rat models

PRINCIPAL INVESTIGATOR: Ama Baduwa Dadson

Please note that the final review report must be submitted to the Committee at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to UG-IACUC for review and approval prior to implementation.

Please report all serious adverse events related to this study to UG-IACUC within seven (7) days verbally and in writing within fourteen (14) days.

This certificate is valid till 1st November, 2021. You are to submit annual reports for continuing review.

Signature of Chairperson
Prof. Major (Rtd.) George A. Asare

APPENDICIES

Table 1: Effect of *Dialium guineense* fruit pulp Serum Iron of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	47.08 ± 4.80	36.40	60.76	
Low Dose (Treatment)	37.35 ± 2.91	28.35	42.50	
Medium Dose (Treatment)	42.88 ± 7.89	28.35	73.14	0.58
High Dose (Treatment)	51.93 ± 15.01	32.68	111.18	

Table 2: Effect of *Dialium guineense* fruit pulp Serum TIBC of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	130.52 ± 27.71	70.80	223.32	
Low Dose (Treatment)	108.55 ± 34.19	73.41	211.11	
Medium Dose (Treatment)	88.00 ± 14.51	63.60	144.57	0.50
High Dose (Treatment)	116.11 ± 24.97	57.18	205.59	

Table 3: Effect of *Dialium guineense* fruit pulp %Saturation of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	38.45 ± 4.71	24.64	51.41	
Low Dose (Treatment)	41.01 ± 7.74	20.13	55.36	
Medium Dose (Treatment)	54.83 ± 14.13	23.98	103.83	0.93
High Dose (Treatment)	52.36 ± 15.33	16.83	96.31	

Table 4: Effect of *Dialium guineense* fruit pulp RBC of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	7.22±0.16	6.83	7.65	
Low Dose (Treatment)	7.29±0.19	6.84	7.76	
Medium Dose (Treatment)	7.09±0.32	6.48	8.00	0.76
High Dose (Treatment)	7.44±0.23	6.95	8.00	

Table 5: Effect of *Dialium guineense* fruit pulp HB of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	14.18±0.07	14.00	14.40	
Low Dose (Treatment)	13.35±0.69	11.40	14.70	
Medium Dose (Treatment)	13.60±0.53	12.80	15.10	0.42
High Dose (Treatment)	13.98±0.38	13.40	15.10	

Table 6: Effect of *Dialium guineense* fruit pulp WBC of Rats

Animal Groupings	Mean ± S.D	Minimum	Maximum	p-value
Control	14.18 ± 1.89	5.10	14.23	
Low Dose (Treatment)	13.35 ± 1.51	3.45	9.96	
Medium Dose (Treatment)	13.60 ± 1.04	10.59	8.20	0.33
High Dose (Treatment)	13.98 ± 1.51	9.23	6.42	