EFFECT OF CRUDE METHANOL EXTRACTS, ALKALOID AND FLAVONOID FRACTIONS OF *Carica papaya* Linn LEAF AND SEED ON ALUMINIUM CHLORIDE-INDUCED ANAEMIC WISTAR RATS

BY

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ABSTRACT

Anaemia is a haematological disorder characterized by a reduction in red blood cells or an inadequate oxygen-binding capacity of haemoglobin molecule to transport oxygen to different epithelial tissues and satisfy physiological needs. This study assessed the efficacy of the methanol extracts, alkaloid and flavonoid fractions of the leaves and seeds of *Carica papaya* in reversing aluminium chloride-induced anaemia and associated complications. Phytochemical, acute toxicity and haematological parameters were evaluated using standard procedures. In the first stage, thirty-five rats were divided into nine groups of three rats each. Groups 1, 2 and 3 were administered with distilled water, $AlCl_3$ and the $FeSO_4$ respectively, groups 4, 5 and 6 were treated with 100 mg/kg, 300 mg/kg and 500 mg/kg methanol (leaves) extract, while groups 7, 8 and 9 were treated with 100 mg/kg, 300 mg/kg and 500 mg/kg methanol (seeds) extract respectively. In the second stage of the study, thirty-five rats were divided into eleven groups of three rats each. Groups 1, 2 and 3 were administered with distilled water, $AlCl_3$ and $FeSO_4$ respectively, groups 4 and 5 were treated with 75 mg/kg and 150 mg/kg alkaloid (seeds), groups 6 and 7 were treated with 75 mg/kg and 150 mg/kg flavonoid (seeds), groups 8 and 9 were treated with 75 mg/kg and 150 mg/kg alkaloid (leaves), while groups 10 and 11 were treated with 75 mg/kg and 150 mg/kg flavonoid (leaves) respectively. Methanol extracts revealed the presence of alkaloids, flavonoids, phenols, saponins and tannins. Saponins had the highest concentrations (1249.83±13.05 and 723.65±0.39 mg/g) for the leaves and seeds respectively, followed by phenols (261.34±1.07 and 171.45±0.91 mg/g) and alkaloids (67.75±1.06 and 35.42±0.50 mg/g) and tannins (45.25±0.46 and 40.67±0.50 mg/g) and flavonoids (34.89±0.66 and 25.80±0.99 mg/g). The methanol extract had LD_{50} value >5000 mg/kg bodyweight in rats. The results obtained showed that the methanol leaves and seeds extracts of *C. papaya* significantly improved red blood cell count (10.88±0.50 and 10.66±0.85 10^{12}/L), haemoglobin (14.35±0.45 and 13.65±0.15 g/dL), packed cell volume (47.00±0.50 and 40.00±0,00 %), White blood cell count (9.40±1.00 and 6.50±0.10 10^9/L), platelet count (423.50±40.00 and 556.50±85.50 10^9/L), mean cell volume (58.00±6.00 and 51.00±0.00 fL), mean cell haematocrit (18.00±0.00 and 16.50±1.50 pg) and mean cell haematocrit count (34.50±1.50 and 32.50±2.50 g/dL) respectively compared to the non-treated group red blood cell count (5.05±1.25 10^{12}/L), haemoglobin (8.55±1.25 g/dL), packed cell volume (26.50±3.50 %), White blood cell count (2.50±0.60 10^9/L), platelet count (434.00±30.00 10^9/L), mean cell volume (52.00±1.00 fL), mean cell haematocrit (16.00±1.00 pg), and mean cell haematocrit count (31.00±1.00 g/dL). The result obtained in the second stage showed that the flavonoid fractions of *C. papaya* seeds had no positive effect on haematopoietic system of anaemic rats. Rats treated with alkaloid fraction of *C. papaya* seeds significantly improved red blood cell count (7.27±0.12 10^{12}/L), haemoglobin (11.93±0.32 g/dL), and packed cell volume (36.00±0.58 %) compared to the non-treated group red blood cell count (5.05±1.25 10^{12}/L), haemoglobin (8.55±1.25 g/dL), and packed cell volume (26.50±3.50 %). Treatment with alkaloid and flavonoid fractions of *C. papaya* leaves significantly improved red blood cell count (8.76±0.26 and 7.26±0.78 10^{12}/L), haemoglobin (13.46±0.84 and 10.93±0.93 g/dL), and packed cell volume (41.33±2.40 and 35.44±1.55 %) compared to the non-treated group red blood cell count (5.05±1.25 10^{12}/L), haemoglobin (8.55±1.25 g/dL), and packed cell volume (26.50±3.50 %). From the results of this study, it can be concluded that the alkaloid and flavonoid obtained from crude

methanol extract of *C. papaya* leaves may contribute to the observed haematopoietic effects in the experimental animals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

$AlCl_3$	Aluminium Chloride
ALK	Alkaloids
ANOVA	Analysis of Variance
EDTA	Ethylene Diammine Tetraacetic Acid
FLV	Flavonoids
Hgb	Haemoglobin
LYM	Lymphocyte
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Count
MCV	Mean Cell Volume
METOH	Methanol
NVRI	National Veterinary Research Institute
PCV	Packed Cell Volume
PLT	Platelets
RBC	Red Blood Cells
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Science
WB	Weighing Balance
WBC	White Blood Cells

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Anaemia is a haematological and pathological conditions characterized by the decrease in the haemoglobin level, red blood cell count or packed cell volume count (Pingali *et al.,* 2015). It includes the decreased oxygen-binding capacity of each haemoglobin molecule due to deformity or lack in numerical development of haemoglobin (Halterman *et al*., 2016; Qaseem *et al.,* 2018). During this condition, there is reduction in the ability of the blood to transport oxygen to various epithelial tissues to meet the physiological body needs. However, this disorder result into significant morbidity (lowered resistance) and mortality (Pingali *et al.,* 2015). The disease state is classified based on red blood cell morphology and etiologic mechanisms.

Haemolytic anaemia is a type of anaemia that lead to the destruction of red blood cells (Qaseem *et al.,* 2018). It is caused by the inability of red blood cells to function properly leading to spherocytosis, elliptocytosis, fauvism, sickle cell disease, and thalassaemia. It can also be induced externally with chemicals like phenyl-hydrazine, dapsone, hydroxylamine, divicine and aluminum chloride (Tata *et al.,* 2016; Telford *et al.,* 2017). The most common signs of hemolytic anaemia are early fatigue and exhaustion, others include weakness, pale mucosal membrane and pale skin and nail bed, irregular or fast heartbeat, short nail, chest pain, lightheadedness or mild vertigo, numbness or coldness of the extremities, and headache (Lippi *et al.,* 2016). It could be so mild at early stage that its symptoms are not noticed, but as the anaemia continued to progress, its symptoms become severe.

Insomnia is also a symptom of haemolytic anaemia (Lippi *et al.,* 2016). It is the type of anaemia characterized by increased bilirubin and haematopoiesis in bone marrow and jaundice, due to the destruction of red blood cell (Haemolysis). Other pathological conditions such as renal impairments, splenomegaly, hepatomegaly, disturbed spermatogenesis, gallstones, and renal hypertension are also detected in this type of illness (Sharda *et al.,* 2016).

The occurrence of anaemia is highest in developing countries, this is as a result of malnutrition, blood feeding parasites like plasmodium, trypanosomes and helminthes infestation and regular use of drugs (Pingali *et al.,* 2015). Socially, anaemia has adverse consequence among the adults which result in reduced life quality and depression. According to Abdullah *et al*. (2019), the prevalence of anaemia in Nigeria among adults was estimated to be about 36.2 %. The anaemia occurrence remains high in Africa, with an overall rate of 64.6 % in children, 55.8 % among pregnant women and 44.4 % among young girls (WHO, 2008), and the risk factor was associated with Malaria. According to Ogbe *et al.* (2018), there are several types of anaemia which are rare but, in all cases, higher proportion of lower circulating red blood cell is common than normal number of circulating red blood cells. Presently, more than half of the world's population experience different forms of anaemia during their life time (Duff, 2018).

Aluminum (Al) is a trivalent metallic cation, it exists in form of ions in most animal and plant tissue, it also exists in natural waters everywhere (Domingo *et al*., 2016; Geyikoglu *et al*., 2018). Its biological role is unknown, but when accumulates in the body it can induce some clinical disorders such as neurotoxicity and hepatotoxicity (Akah *et al.,* 2015), bone

diseases and anemia (Martínez *et al.,* 2017). Aluminum also has a direct effect on hematopoiesis (Wills and Savory, 2013; Martínez *et al.,* 2017) and its high levels in serum of hemodialysis patients were associated with impaired erythropoiesis and iron-deficiency anemia (Wills and Savory, 2013; Mahieu *et al*., 2015). Aluminium contributes to hemolytic oxidative stress, by generating reactive oxygen species (ROS) which cause damage to various proteins, DNA and membrane lipids (Ogbonnia *et al*., 2019).

The effect of aluminum on iron metabolism is very direct as it controls iron absorption in the intestine, it also inhibits the iron's transport in blood serum and displace iron by binding transferring (Vota *et al.,* 2014; Mostighie, 2016). Aluminum produces peroxidative changes in the erythrocyte's membrane, which result to hemolysis. Therefore, the reduced red blood cell count in animals intoxicated with aluminum might be the outcome of both the hemolytic action of aluminum and the reduced time of survival of red blood cell (Lemire *et al.,* 2014; Renuka, 2017).

Immense benefits have been derived by man from making used of medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs (UNESCO, 2018). The increasing discovery of more medicinal plants demand for increased scientific scrutiny of their bioactivity and to generate data that will be helpful to physicians and patients. This will guide both physician and the patient to make wise decision before they make used of them.

Carica papaya (family: *Caricaceae*) is commonly known as pawpaw with potential medicinal values and its cultivation is very common in most tropical countries Sudgakar and Vidhyar (2014). *C. papaya* is considered as a good rejuvenator and has been utilized as antioxidative (Mehdipour *et al.,* 2016; da Silva *et al.* (2018), antiplasmodial Ayoola and

Adeyeye (2017), antisickling (Imaga *et al.,* 2015), antifertility (Poharkar *et al.,* 2016), antibacterial (Doughari *et al.,* 2017; Emeruwa *et al.,* 2017 and Leite *et al.,* 2018). antifungal (Giordiani *et al.,* 2014), antihypertensive (Koffi *et al.,* 2019) and immunomodulator (Mike *et al.,* 2015). A wide range of active principles such as alkaloids, flavonoids, tannins, saponins, phenols, phytates, and cardiac glycosides have been isolated from this species. The extracts and metabolites from this plant have been known to possess pharmacological properties.

C. papaya (Linn.) contains a wide range of chemical constituents which includes antioxidant nutrients (e.g., carotenes, vitamin C, and flavonoids), the B vitamins (e.g., folate and pantothenic acid), minerals (e.g., potassium, iron and magnesium), and fibre (Edible diet index, 2012). Papaya plant is laticiferous as they contain specialized cells known as laticifers. The lactifiers secrete latex and are dispersed throughout most plant tissues. The papaya-latex is well known for being a rich source of the four cysteine endopeptidases namely papain, chymopapain, glycyl endopeptidase and caricain (Azarkan *et al.*, 2016). Papaya leaves contain alkaloids, carpaine, pseudocarpaine, vitamins C, vitamin E, and corpocid, also contain minerals such as potassium, calcium, magnesium, copper, iron, zinc, and manganese Ayoola and Adeyeye (2010).

C. papaya is reported in the literature for its various biological activities such as a traditional remedy for gastrointestinal functional disorders, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2015). Papaya leaves have shown the presence of anti-dengue, anti-plasmodial, anti-cancer, antibacterial, hepatoprotection, anti-inflammatory and Antioxidant properties in-vitro and in-vivo studies (Nugroho *et al.,* 2017). This plant has been traditionally used for kidney failure, low sperm

count, dental care, heart problems, natural memory enhancer, and remedy for fibroids in uterus (Uduak *et al.,* 2015).

Due to well documented reports implicating the role of oxygen-based free radicals in Al-induced anemia and presence of a large array of phytoconstituents in the leaves and seed of *C. papaya*, this study aims at finding out the potential of *C. papaya* in the prevention and treatment of anaemia.

1.2 Statement of the Research Problem

There are various drugs for the management of anaemia, however, due to high-cost of modern health care and orthodox medicine, they are not affordable to many poor people especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the management of anaemia, so they depend heavily on plants and herbal products for the management of many diseases including anaemia.

The most current strategies used in the management and treatment of anaemia especially iron deficiency induced anaemia are oral or intravenous administration and supplementation with iron (ferrous) (Iwalewa *et al.,* 2015). Other techniques used are bone marrow transplantation and blood transfusion and they are very expensive.

Long usage of fersolates may expose the patient to mutagenicity, iron overload and other fatal risks (Sharda *et al.,* 2011). Haemochromatosis is an iron overload disorder that if left untreated it can lead to cancer and heart diseases. Damage to the pancrease could arise as a result of excessive storage of iron which could result to diabetes Kimetu and Lemhann (2010).

The present study was designed to determine the efficacy of *Carica papaya* leaves and seeds extracts in alleviating the toxicity of aluminium chloride ($AlCl_3$) in induced anaemia using certain hematological parameters in Albino rats, with a view to formulating new cost-effective therapies that are easily accessible, inexpensive, non-toxic and have the tendency to alleviate anaemia and its complications.

1.3 Aim and Objectives of the Study

The aim of this research was to evaluate the effect of crude methanol extracts, alkaloid and flavonoid fractions of *Carica papaya* leaves and seeds on Aluminum chloride-induced anaemic Wistar rats.

The objectives of this study were to:

i identify and quantify some phytochemical constituents of the crude extracts of *C. papaya* leaf and seed

ii determine the LD_{50} of the crude *Carica papay*a leaf and seed extracts

iii assess the haematopoietic potential of the crude methanol extracts, alkaloid and flavonoid fractions of *C. papaya* leaves and seeds on haematological parameters of Aluminium-chloride induced anaemic rats.

iv determine the effect of *C. papaya* leaves and seeds extracts on bodyweight of Aluminum chloride-induced anaemic rats

1.4 Justification for the Study

Nature remains the major source of therapeutic compounds as tremendous chemical diversity is found in millions of species of plants (Bhanot *et al.,* 2017). Phytonutrients are

known to be very important in management and amelioration of many ailments by modulating metabolic pathways and promoting health and well-being of humans (Muanza *et al.,* 2015).

Phytochemicals are known to possess biological activities, including anti-parasitic, antimicrobials, anti-ulcer, anti-inflammation and antioxidants (Lawal *et al*., 2015). Phytochemical studies of *C. papaya* revealed the presence of important phytonutrients in appreciable concentration Ayoola and Adeyeye (2010). *C. papaya* has also been reported to be rich in minerals such as iron, magnesium, calcium and potassium.

The availability of iron in appreciable amount in *C. papaya* may be relevant in the treatment and management of some ailment resulting from iron deficiency. Food fibres aids absorption of trace elements in the gut and reduce absorption of cholesterol Leveille and Sanberlich (2016). The fibre content of *C. papaya* was reported to be similar to that of *T. occidentalis* (Ugu leaf) Leveille and Sanberlich (2016) which have been reported to possess anti-anaemic potential (Ogbe *et al*., 2018).

The high fiber content may be an essential nutrient whose consumption may facilitate the normal biosynthesis of hemoglobin; hence may serve as protective therapy against iron deficiency anaemia.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Carica papaya*

Pawpaw (*Carica papaya* Linn) is a widely grown perennial plant, which grows on erect having a branchless trunk (Figure 2.1) (Lohiya *et al*., 2017). *C. papaya* tree had a leaves that are large, 50-70 cm in diameter, having up to 7 lobe that are deeply palmate (Plate I). The scientific (taxonomic) classification of *C. papaya* from kingdom to specie is depicted above (Lohiya *et al*., 2017). Its fruit are melonlike and is known by different names in different parts of the world and these include fruta bomba (in Cuba), lechoza (in Venezuela, Puerto Rico, the Philippines and the Dominican Republic) and papaw (Sri Lankan) (Lohiya *et al*., 2017). In Nigeria, it is also known by different local names depending on the tribe. For example, among the Yoruba (South-West Nigeria) it is known as Ibepe and sigun, gwanda among the Hausa (Northern Nigeria), ojo and okwere among the Igbo (South-East, Nigeria), etihi-mbakara among the Efik (South-South Nigeria). The ripe fruit is edible and is usually eaten raw, without the skin or seeds. The unripe green fruit (which has high vitamin A content) can be eaten when cooked, it is regularly serve in curries, salads and stews in Thailand cuisine (Lohiya *et al*., 2014).

Several species of *Caricaceae* have been used as remedy against a variety of many diseases' ailments (Munoz *et al*., 2017; Mello *et al*., 2018). *C. papaya* is presently distributed over the whole tropical region in the world. In particular, *C. papaya* fruit circulates widely, and it is accepted as food or as a quasi-drug (Otsuki *et al*., 2010). Many scientific researches have been carry-out to evaluate the biological activities of numerous

parts of *C. papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex (Otsuki *et al*., 2015).

Figure 2.1: *Carica papaya* (Pawpaw Tree)

Source: Lohiya, (2017**)**

2.1.1 Taxonomic classification of *Carica papaya* (linn.)

Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Violales
Family:	Caricaceae
Genus:	Carica
Species:	Carica papaya

Source: Yogirag *et al.* (2017)

2.1.2 Chemical composition of *Carica papaya*

Papaya is a melonlike fruit that are very rich in fatty acids, the most important one being octanoate (Kim *et al*., 2015). It consists of *cis*-9- and *cis*-11-hexadecenoate, *cis*-9-, *cis*-11- and *cis*-13- octadecenoate. Papaya seeds contain a fixed oil that are made up of myristic, palmitic, stearic, arachidic, behenic and unsaturated fatty acids Singh and Ali (2011). Acording to Puangsri *et al*. (2015), Papaya seeds contain phospholipids, carpaine, benzylisothiocyanate, benzyl glucosinolate, glucopaeolin. Papaya seeds also contain Hentriaontane, sitosterol, caricin (sinigrin) and myosine (Rossetto *et al*., 2018). The papaya seeds have been shown to consist of chemicals asimitrin (an adjacent hydroxylated ring of *bis*-tetrahydrofuran acetogenin) and 4-hydroxytrilobin (an adjacent *bis*-tetrahydrofuran ring which contains two flanking hydroxyl groups and an-unsaturated - lactone with a 4-hydroxyl group). These chemicals are said to have selective cytotoxicity aga inst prostate adenocarcinoma (PC-3) and colon adenocarcinoma (HT-29) cell lines; thus, may get to be a useful chemotherapeutic chemical compound for these types of cancer (Kim *et al*., 2015). The leaves also consist of a toxic annonaceous chemical compound (acetogenins), this makes them impalatable to most insects except the zebra swallowtail butterfly (*Eurytides marcellus*). Studies have shown that the organism (*Eurytides marcellus*) larvae feed on the leaves of various species of *Asimina,* these confers it protection from predatory organisms throughout the butterfly's life time. The presence of trace amounts of acetogenins makes them unsuitable to birds and other predators (Martin *et al*., 2019).

The bark of pawpaw trees contains other acetogenins, including asimin, asiminacin and asiminecin, which have been shown to be potent inhibitors of mitochondrial NADH: ubiquinone oxidoreductase. Thus making *A. triloba* promising source of pesticide and anti-tumour compounds (Zhao *et al*., 2014).

Other compounds found in the parts of *C. papaya* are nicotine, tannins and flavones (Atlas, 2014).

2.1.3 Other constituents of *Carica papaya*

Papaya is considered one of the most important fruits because it is a rich source of antioxidant nutrients (e.g., carotenes, vitamin C, and flavonoids), the B vitamins (e.g., folate and pantothenic acid), minerals (e.g., potassium and magnesium), and fibre (EDI, 2012). Papaya plant is laticiferous as they contain specialized cells known as laticifers. The lactifiers secrete latex and are dispersed throughout most plant tissues. The papaya-latex is well known for being a rich source of the four cysteine endopeptidases namely papain, chymopapain, glycyl endopeptidase and caricain (Azarkan *et al.*, 2015) and the content of latex may vary in fruit, leaves and roots. As the papaya fruit ripen, the amount of laticifers cells that produces latex decreases (Organisation for economic co-operation and developmemt, 2005). Therefore, ripe papaya contains less latex and other constituents. The richness of enzymes in papaya juice has been known (Witmann, 2018). The most important enzyme papain was characterized (Drenth *et al.*, 2013). The enzymes chymopapain and papaya protease III were characterized in the 1980s of the last centuries (Zucker *et al.*, 2015; Jacquet *et al.*, 2019). These two important compounds like papain and chymopapain are supposed to aid in digestion and therefore they are widely used to cure the digestive disorders (Huet *et al.*, 2016). In addition, papain is used in meat tenderizing, pharmaceuticals, beauty products, and cosmetics (EDI, 2012). Besides, it has been used in brewing and wine making, and in the textile and tanning industries. It is also used to treat arthritis. It is important to note that the level and amount of the chemical compounds vary in the fruit, latex, leaves, and roots. In addition, plant parts from male and female trees differ in the quantity of the compounds. For example, phenolic compounds tend to be

higher in male trees than female trees. The amount of fresh papaya latex and dry latex (crude papain) also vary with the gender and age of the tree. Female and hermaphrodite trees yield cruder papain than the male trees and the older fruit yields more than the younger fruit. However, the activity of the papain is higher in the extracts from the younger fruit than the older fruit. Cultivars also vary in the quantity of the compounds. A recent study has reported that the green, yellow and brown leaves of papaya contain various phytochemicals, vitamins and minerals composition Ayoola and Adeyeye (2010). Therefore, the papaya leaves can be seen as a potential source of useful food and drug items.

2.1.4 Traditional uses of *Carica papaya*

Papaya fruits, seeds, latex and extracts have been used traditionally to treat various ailments in humans across the world. According to the folk medicine, papaya latex can cure dyspepsia and also applicable for external burns and scalds. Seeds and fruits are excellent anti-amoebic (Okeniyi *et al.,* 2017). Dried and pulverized leaves are sold for making tea; also, the leaf decoction is administered as a purgative for horses and used for the treatment of genetic-urinary system (Adebiyi *et al.,* 2016). Papain is popularly used as a chewing gum additive and beer clarifier plus for a large number of additional medical, cosmetic and industrial purposes. Carapine, an alkaloid present in papaya, can be used as a heart depressant, amoebicide and diuretic. The fruit and juice are eaten for gastrointestinal ailments; a fresh leaf poultice is used to treat sores. The fresh root with sugarcane alcohol can be taken orally or as a massage to soothe rheumatism. A flower decoction is taken orally for coughs, bronchitis, asthma and chest colds. In some countries, the seeds are used as an abortifacient and vermifuge (Orwa *et al.,* 2019). Leaves have been poulticed into

nervous pains, elephantoid growth and it has been smoked for asthma relief among tropical tribal communities. The stem and bark may be used in rope production (Rawani *et al.*, 2016).

2.1.5 Nutritional value of *Carica papaya*

The nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and an excellent source of vitamin C (ascorbic acid). Nutritive value of leaves of *Carica papaya* can also be used as a nutraceutical. It contains carbohydrates, minerals and vitamins, lipids and proteins terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids (Eleazu *et al.*, 2018). It can be used for dual purpose as a nutritional agent and medicinal agent. It has been observed to contain these compounds in varying proportions. Carbohydrates 8.3 %, ascorbic acid 38.6 %, protein 5.6 %, minerals like magnesium 0.035 %, iron 0.0064 % and phosphoric acid 0.225 % per 100 gm of edible portion Ayoola and Adeyeye (2010). These components are responsible for its role in coagulation of blood, proper functioning of the heart and nervous system and the normal contraction of muscles, antibacterial, metabolism of water, promoting digestion, assimilation, osmosis, functioning of the pituitary gland, the pineal gland and the brain, promoting hepato-renal function, combating anaemia, helping in normal growth Ayoola and Adeyeye (2010).

2.1.6 Phytochemical constituents of *Carica papaya*

From previous studies, the phytochemical components of the leaves of *C. papaya* Linn. showed the presence of saponins, cardiac glycosides and alkaloids in the green leaves, yellow leaves and brown (dry) leaves Ayoola and Adeyeye (2010). In addition, Okoye, (2015) reported the presence of alkaloids, flavonoids, tannins, saponins, phenols, phytates,

carbohydrates, hydrogen cyanide, fat, proteins, fibre and steroids in the seeds. In addition, two important compounds, papain and chymopapain are found in the milky sap found in the unripe fruit and leaves (Teixeira da Silva *et al.,* 2017). Likewise, the methanol extract of the leaves was found to contain alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, phlobatanins and saponins (Imaga *et al.,* 2015); while the unripe fruit contains cardenolides and saponins (Oloyede, 2005).

Papain and chymopapain were found to contain active ingredients that help in healing of wounds. The biological functions of flavonoids include protection against allergies, inflammations, platelets aggregation, microbes, ulcer, vineses and tumours Okwu and Okwu (2014). Flavonoids represent the common and widely distributed group of plant phenolics. Flavonoids are free radical scavengers and super antioxidants which prevent oxidative cell damage and have strong anticancer activity (Salas *et al.,* 2019). As antioxidants, flavonoids provide anti-inflammatory action (Okwu, 2012). This may be the reason behind the use of seed of C. *papaya* in herbal medicines. The saponins constituents are responsible for the possession of hemolytic property. Alkaloids are the most efficient therapeutically significant plant substance. Tannins have astringent properties which hasten the healing of wounds and inflamed mucous membrane. The presence of tannins in the seed of *C. papaya* can support its strong use for healing of wounds, ulcers, hemorrhoids, frost-bites and burns in herbal medicine (Igboko, 2013; Maduiyi, 2015).

2.1.7 Biological activity of *Carica papaya*

Digestive system disorders, papain extract is used as a treatment for certain intestinal and digestive problems. Ingredients of the papaya fruit and the processed fruit have been associated with a beneficial impact on digestion or diseases (Marotta *et al.,* 2011; Somanah

et al., 2012; Forstner, 2016; Aruoma *et al.*, 2017; Ghoti *et al.*, 2018). The fruit is considered as a traditional remedy for gastrointestinal functional disorders in countries with papaya plants. However, only little evidence has been produced with reference to its physiological effect in humans and the proof of efficacy. In line with these, Muss *et al.* (2013) studied the clinical effects of the papaya preparation called Caricol in a double-blind placebo-controlled study design and found that the (Caricol) contributes to the maintenance of digestive tract physiology. It ameliorates various functional disturbances, like constipation, heartburn, and symptoms of irritable bowel syndrome (IBS). Nevertheless, the mechanism of this digestive tract physiology support is discussed. The tea, prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2015).

2.1.7.1 *Antisickling activity of Carica papaya*

Folk medicine reportedly uses papaya as an herbal remedy for the management of sickle cell anaemia. The results indicate that the previously reported anti-sickling properties of papaya may be due to the inherent antioxidant nutrient composition, thus supporting the claims of the traditional healers and suggests a possible correlation between the chemical composition of the papaya plant and its uses in traditional medicine as an anti-sickle cell anaemia agent (Imaga *et al.*, 2015). In addition, Oduola *et al.* (2016) has described the anti-sickling activity of unripe papaya extracts that the anti-sickling and reversal of sickling activities reside in the ethyl acetate fraction that prevents the sickling of haemoglobin of the sickle cell patients.

2.1.7.2 *Anxiolytic and antioxidant activity of Carica papaya*

Papaya has been used in the Ethiopian traditional system of medicine to relieve stress and other disease conditions. Therefore, a study was undertaken to evaluate the anxiolytic and sedative effects of 80 % ethanolic papaya pulp extract in mice. The papaya pulp extract 100 mg/kg showed significant anxiolytic activity without altering locomotor and sedative effects and this study authenticated the traditional usage of papaya as an anxiolytic medicinal plant Kebebew and Shibeshi (2013). Similarly, a study was designed to explore the toxicological and antioxidant potential of dried *C. papaya* juice in vitro and in vivo. In vivo examination was performed after oral administration of dried papaya juice to rats for 2 weeks at doses of 100, 200 and 400 mg/kg. Blood thiobarbituric acid reactive substance and ferric reducing antioxidant properties assays were used to determine the potential of the juice to act against oxidative stress. The acute toxicity test (LD_{50}) demonstrated that papaya juice is not lethal up to a dose of 1500 mg/kg after oral administration and thus is considered nontoxic. In treated groups, no sign of toxicity was observed. In vitro evaluation of the antioxidant effects of papaya showed that the highest antioxidant activity (80 %) was observed with a concentration of 17.6 mg/mL. This preliminary study indicates the safety and antioxidative stress potential of the juice of papaya, which was found to be comparable to the standard antioxidant compound alpha-tocopherol (Mehdipour *et al.,* 2016). The study conducted by da Silva *et al.* (2018), further supports the notion that papain, the compound isolated from the latex of unripe *C. papaya* is a promising source of potential antioxidant.

2.1.10 Bioactive moieties in *Carica papaya*

Numerous biologically active moieties are present in papaya. Papaya latex is a sap that is exuded from the point of plant damage caused either mechanically or by insect herbivory

(Kotaro, 2016), and has been known to contain strong lipase activity. It is rich in cysteine endopeptidases having glycyl endopeptidase, cysteine proteinases, serine proteinase inhibitor, glutaminyl cyclase caricain, class II chitinase, papain, and chymopapain. (Azarkan *et al.*, 2015; Huet *et al.,* 2016). Recent studies report the identification of putative homologous lipase (a hydrolase and naturally immobilized biocatalyst) that is liable for the vital lipolytic activity of papaya latex (Dhouib *et al.*, 2018). *C. papaya* lipase (CPL) has emerged as a protease having versatile biocatalytic properties; (Domínguez de María *et al.*, 2016). it finds abundant applications, such as fats and oils modification, facilitating a wide array of acids and alcohols as substrates for esterification and inter-esterification reactions and asymmetric resolution of different non-steroidal anti-inflammatory drugs (NSAIDs) and non-natural amino acids. Four types of cysteine proteases are present in papaya proteases, i.e., papain (less than 10 %), chymopapain A and B (26–30 %), glycyl endopeptidase III and IV (23–28 %), and caricain (14–26 %). These form 69–89 % of its total protein content Barrett and Rawling (1998). These proteases find wide application in medicine and the food industry. The method of three phase partitioning (TPP) can be effectively utilized for the extraction of proteases from papaya peels (Chaiwut *et al.*, 2017). Proteases extracted from papaya exhibit a broad specificity and thermo stability thus utilized in the meat industry for meat tenderization. Papaya proteases are of medicinal significance especially for gastroenterology, wound healing, anti-inflammatory, antitumoral, anthelmintic, neurosurgery, ophthalmology, urology, and phlebology properties (Salas *et al.,* 2018; Pendzhiev, 2019; Seki *et al.*, 2020).

Anti-inflammatory properties of papaya proteases help to reduce pain and suffering from arthritis, edema, and osteoporosis. Papain is a non-specific thiol protease with an action similar to that of pepsin in gastric juice, an excellent aid to digestion and pepsin

dilapidation. (Huet *et al.*, 2016; Pendzhiev; 2019) Endopeptidase is a minor constituent (5–8 %) (Huet *et al.*, 2016).

2.2 Phytochemical Mechanism of Action

Phytochemicals are biologic ally active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Ilodibia *et al*., 2016). Phytochemicals are secondary metabolites produced by plants. They give plants their colour, flavour, smell and are part of a plant's natural defense system (Agte *et al.*, 2017). These compounds have been linked to human health by contributing to protection against degenerative diseases (Anderson, 2014; Liu, 2016). Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These plants include fruits, seeds, herbs and vegetables (Okwu, 2014). Epidemiological studies have shown that the consumption of fruits and vegetables is associated with reduced risk of chronic diseases (Doughari *et al*., 2017). Different mechanisms have been suggested for the action of phytochemicals. They may act as antioxidants, or modulate gene expression and signal transduction pathways (Doughari *et al*., 2017). They may be used as chemotherapeutic or chemo preventive agents (Doughari *et al*., 2015). These bioactive compounds usually present in relatively small quantities in higher plants, include the terpenoids, flavonoids, saponins, tannins, alkaloids, phenols, and many others.

2.2.1 Flavonoid contents

Flavonoids are low molecular weight polyphenolic antioxidants naturally present in fruits, vegetables, and beverages such wine and Astea. Flavonoids are believed to have various therapeutic values. Flavonoids have been reported to have antihyperglycemic effect

Adewunmi and Sofowora (2013). Flavonoids are known to improve cardiac function, decrease anginas and lowers cholesterol levels. These compounds act by regulation of inflammation mediators. Flavonoids have also been shown to reduce production of pathogenic thrombosis in mice models (Arika *et al*., 2015). A supplement of sea buckthorn which contains high amounts of flavonoids has been shown to restore cardiac function and improve blood circulation in patients with coronary heart disease (Piero *et al*., 2015). Flavonoids have been used in the treatment of chronic cardiac insufficiency and hypertension as they block the activation of necrosis factor kappa-B (Nyamai *et al*., 2015).

2.2.2 Saponins contents

Saponins are plant compounds that occur either as steroid alkaloids, glycosides of triterpenoids or steroids Holst and Williamson (2018). These phytochemicals are known to have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties (OBrien *et al*., 2016). The hypoglycemic effect of saponins is believed to due to stimulation of pancreatic β-cells, inhibition of glucose transport across the brush border cells of the small intestines and suppression of transfer of glucose from the stomach to the small intestines (Dholi *et al*., 2011). Saponins are also reported to inhibit gastric emptying in a dose dependent manner. Saponins lower cholesterol level by forming large micelles that are then excreted in bile (OBrien *et al*., 2016). These compounds are said to lower serum levels of low-density lipoproteins-cholesterol and decrease absorption of cholesterol in the intestines Holst and Williamson (2018). Saponins are believed to act as adjuvants in enhancing antibody production and in the stimulation of cell mediated immune system (Aggarwal *et al*., 2016). These compounds are reported to interact with antigen-presenting cells and induce interferon and interleukin production thus mediating immunostimulant

effects (Harshal *et al*., 2014). Saponins inhibit tumor cell growth by apoptosis in leukemia cell line and by cell cycle arrest in breast cancer cell line. They also exert antiproliferative active to prostate carcinoma cells by inducing apoptosis and cell cycle arrest at G1 phase. Saponins induce apoptosis by stimulation of cytochrome c-caspase pathway. The structure of the sugar portion in saponins influences the tumor specificity of cytotoxic action. Saponins are believed to lower the risk of cancer and other chronic diseases (Aggarwal *et al*., 2016). These compounds are effective for both hormone dependent and non-hormone dependent cancer (Aggarwal *et al*., 2016; Kahal *et al*., 2016). Saponins are also believed to antifungal and hypocholesterolemic effects (Wuju *et al*., 2013). These effects are believed to be due to combination with bile acids to form micellar aggregates (Wuju *et al*., 2013). Saponins prevent hyperlipemia and liver injury induced by lipid peroxidation (Mike *et al*., 2015). The mechanism of action of these compounds in this case is through inhibition of lipid peroxide peroxidation and inhibition of lipid peroxide production (Mike *et al*., 2015). Saponins are also believed to inhibit HIV infection *in vitro in* addition to having antitumor properties (Mike *et al*., 2015). This effect can be attributed to the prevention effect of HIV-induced cell fusion but have no direct effect on reverse transcriptase activity of the virus (Mike *et al*., 2015; Gerald *et al*., 2014). Saponins have been reported to have superoxide scavenging effect on oxygen radicals that are implicated in the development and initiation of several diseases (Wali *et al*., 2015). This pro-oxidative activity makes saponins to act as hydrogen abstractor leading to initial reaction of lipid oxidation Wali and John (2016).

2.2.3 Tannins contents

Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species (Zhao *et al*., 2014). It is found in abundance in the tree bark, wood,

fruit, fruit pod, leaves and roots and also in plant gall (Sharma *et al*., 2017). Tannins can be classified into two broad groups: hydrolysable tannins and condensed tannins. Clinically, all forms of tannins may participate in the management of glucose level in blood. Tannin has been shown to stimulate the receptor cells to utilize carbohydrate. Tannins are known to reduce viability, proliferation and trigger apoptosis of MOLT-4 human leukaemia cells (Romani *et al*., 2014). Tannins are also known to effectively inhibit skin tumorgenesis in mice (Romani *et al*., 2014).

2.2.4 Alkaloid contents

Alkaloids are phytochemicals that contain nitrogen and are derived from various amino acids. Alkaloids are known to have blood glucose lowering activity (Andre *et al*., 2017). Alkaloids tetrandine and berberine have been reported to demonstrate antioxidant activity responsible for various biological activities associated with *Carica papaya* including antidiabetic activity Holst and Williamson (2018). Alkaloid fractions have shown hypoglycemic potential in mice (Hwang *et al*., 2010). The alkaloids l-ephedrine of *Ephedra distachya* herbs have shown hypoglycemic effect in diabetic mice due to restoration and regeneration of atrophied pancreatic islets that induces the secretion of insulin (Appendino, 2016). Alkaloids with therapeutic effects mainly act by affecting chemical transmitters of the nervous system like dopamine, γ-aminobutyric acid, acetylcholine and serotonin (Kang *et al*., 2017). Alkaloids are also known to have anti-arrythmic effects, antihypertensive effects, anticancer and antimalarial activity (Appendino, 2016). Alkaloids are believed to have neuro-protective, cholinergic and antioxidant activities in Alzheimer's disease (Aggarwal *et al*., 2016). These compounds have memory and cognitive-enhancing activities on Alzheimer's patients (Grace *et al*., 2016). The therapeutic effect of these compounds is

believed to be by restricting oxidative stress and inflammatory reactions, enhancing cholinergic transmission, elevating estrogen and other neurotropic agents and preventing β-amyloid toxicity- formation (Dholi *et al*., 2011). These compounds inhibit acetylcholinesterase enzyme (Dholi *et al*., 2011). Inhibition of this enzyme enhances acetylcholine activity which is one of the main strategies in the management of Alzheimer's disease (Dholi *et al*., 2011). Tetramethyl pyrazine, an amide alkaloid and is known to elicit hypotensive effects by inhibiting platelet aggregation and vasoconstriction (Dhatmalchi *et al*., 2016). This alkaloid is also believed to cause inotropic and chronotropic responses on isolated atria. Tetramethyl pyrazine is used in the treatment of occlusive cerebral arteriolar diseases due to its vasodilatory effects. Alkaloids have also been reported to have antimicrobial, cytotoxic and trypanocidal activity. These compounds act by intercalating DNA thus impairing replication and transcription causing frame-shift mutations Tan and Vanitha (2014). Alkaloids are also believed to elicit antimicrobial and trypanocidal activity by inhibition of protein biosynthesis and by interaction with neuroreceptors (OBrien *et al*., 2016).

2.2.5 Phenolic acids contents

Phenolic acids are aromatic plant secondary metabolites that are widely spread. Phenolic acids that occur naturally can be divided into two main categories; cinnammic acid derivatives (ferulic acid and caffeic acid) and benzoic acid derivatives (Muriithi *et al*., 2015). Phenolic acid such as ferulic acid is known to have a wide range of therapeutic effect against diseases like diabetes, cancer, neurodegenerative, cardiovascular and inflammatory diseases (Abdirahman *et al*., 2017). These therapeutic effects are believed to be attributed partly to the antioxidant activity of this phenolic acid. Ferulic acid prevents

lipid peroxidation and scavenges superoxide free ion radical. The structural characteristics of phenolic acids help them confer the antioxidant properties (Mukundi *et al*., 2015).

These compounds have a phenolic nucleus and an unsaturated side chain that can form a resonance stabilized phenoxy group. Reactive radicals collide with these compounds gaining a hydrogen atom and forming a phenoxy radical (Mukundi *et al*., 2015). Phenolic acids and their ester derivatives reduce the level of inflammatory mediators like tumor necrosis factor-alpha, prostaglandin E2 (Wang *et al*., 2017). Ferulic acid derivatives have been reported to suppress the activity of cyclooxygenase-2 promoter activity in human colon cancer cells through the β-galactosidase reporter gene assay system. Phenolic acids are also reported to protect proteins, DNA and lipids from oxidative stress thus exerting anticancer properties (Mohammad *et al*., 2016). These compounds also act on pathways that regulate induction to apoptosis, response to oxidative stress and regulation of proliferation. Phenolic acids have been reported to inhibit occurrence of pulmonary cancers in mice, inhibit mutagenesis and decrease urinary N-nitrosoproline levels in humans (Lim *et al*., 2016). Its compounds restore normal homeostasis by inducing apoptosis in cancer cells, absorb UV radiation forming a stable phenoxyl radical radiation thus terminating free radical chain reaction (Majundar *et al*., 2019). These compounds preserve the physiological integrity of cells by scavenging deleterious radicals and chain reactions and suppress radiation-induced oxidative reactions. Phenolic acids are believed to provide protection against alcohol induced toxicity and also enables the body to overcome deleterious effects of alcohol. Phenolic acids preserve the integrity of cells exposed to alcohol stress by quenching and scavenging free radicals (Benkhayal *et al*., 2019).

The mechanism of action is believed to be by abstraction of H^+ by hydroperoxyl and hydroxyl radicals from a free phenolic substrate to form a phenoxyl radical which then forms products that are excreted in bile (Ngugi *et al*., 2014). Alzheimer's, a neurodegenerative disease is characterized by free radical mediated oxidative stress in brain cells. This oxidative stress mainly caused by reactive nitrogen species and reactive oxygen species can lead to neuronal dysfunction, RNA and DNA oxidation and lipid peroxidation. Phenolic acids are reported to prevent oxidative modification of proteins by reducing the chances of oxidative attack on them Joseph and Jini (2018). Nicotine causes oxidative cellular injury by increasing lipid peroxidation. This is believed to be a major cause of several smoking-related diseases (Pathirana *et al*., 2017). Phenolic acids increase the endogenous antioxidant defense system, reverses the damage caused by nicotine and protects cells from oxidative damage. These compounds protect the membrane by quenching the free radicals, improve the antioxidant status and inhibit the leakage of marker enzymes into circulation (Kang *et al*., 2015).

2.3 Haematological Parameters

Blood accounts for 7 % of the human body weight Yakubu and Afoloayan (2019) with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3 Xie and He (2015). The average adult has a blood volume of roughly 5 litres which is composed of plasma and several kinds of cells (Wong *et al*., 2016). Blood is one of the most common biological samples whose constituents are used for diagnosing a large number of diseases (Wiedenfeld, 2013; Chaparro, 2015).

Hematology refers to the study of the numbers and morphology of the cellular elements of the blood. The red cells (erythrocytes), white cells (leucocytes), and the platelets

(thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al.,* 2014; Chaparro, 2015). Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment Ovuru and Ekweozor (2016) and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Abah *et al.,* 2014; Mmereole, 2018; Isaac *et al.,* 2014). Haematological parameters are good indicators of the physiological status of animals Khan and Zafar (2015). Haematological parameters are those parameters that are related to the blood and blood forming organs (Waugh *et al.,* 2001; Bamishaiye *et al.,* 2009). (Figure 2.2); represent the red blood cell formation, stem cells located in the red bone marrow which multiply and become specialized and eventually to mature blood cells. Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions (Olafedehan *et al.,* 2018). As reported by Isaac *et al.* (2013) animals with good blood composition are likely to show good performance. Laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body. The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2014; Doyle, 2017).

According to Olafedehan *et al.* (2018) examining blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals. Blood constituents change in relation to the physiological conditions of health (Togun *et al.,*

2014). These changes are of value in assessing response of animals to various physiological situations Khan and Zafar (2015). According to Afolabi *et al.* (2018), changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors.

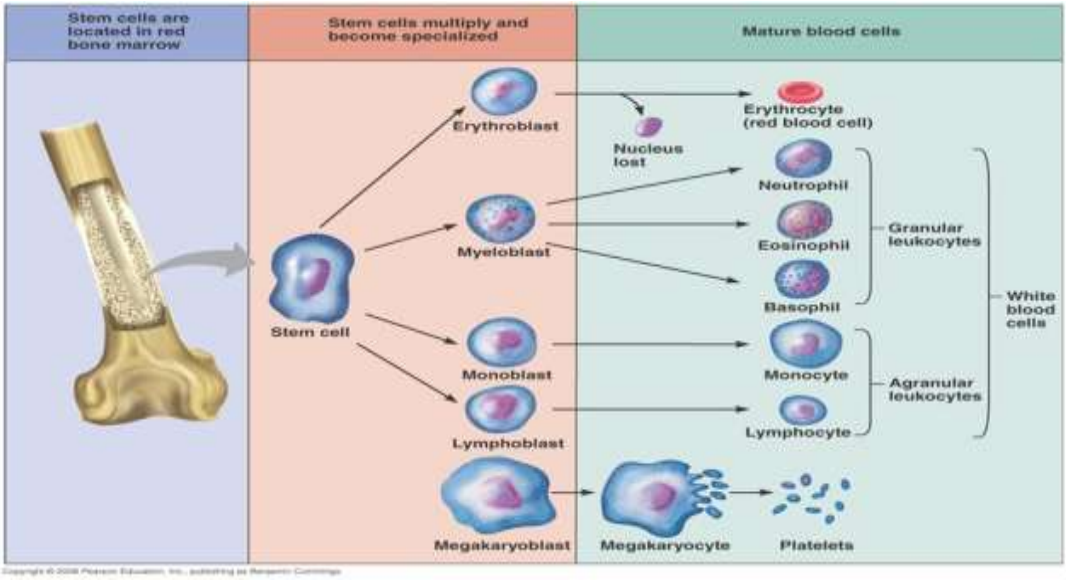

Figure 2.2: Red Blood Cells Formations

Sources: Chaparro, (2015).

2.3.1 Constituents of blood

2.3.2 Red blood cells

Red blood cells (RBCs) are the most common type of blood cells and the body's principal means of delivering oxygen (O_2) to the body tissues by the blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries (Price *et al*., 2017; Chaparro, 2015). The cytoplasm of erythrocytes is rich in haemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red colour of the cells. The cell membrane is

composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network in humans. Mature red blood cells are flexible and oval biconcave disks; they lack a cell nucleus and most organelles in order to accommodate maximum space for haemoglobin as depicted in (Figure 2.3). The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells (Price *et al*., 2017). Research has shown that RBC of rodents such as mice, rats, hamsters, and gerbils range from thirty-three to fifty-five RBC 3.5 – 7.0 x 10^9/µL (Karen, 2012). Red blood cells are formed in the red bone marrow of bones. Stem cells in the red bone marrow called hemocytoblast give rise to all of the formed elements in the blood. If a hemocytoblast commits to becoming a cell called proerythroblast, it will develop into a new red blood cell (Chaparro, 2015; Wilkins, 2018).

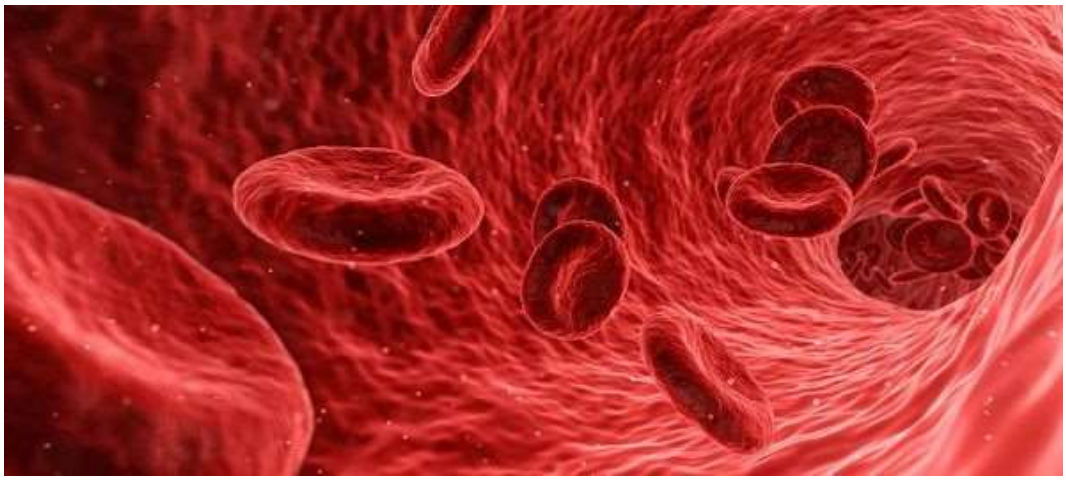

Figure 2.3: Red Blood Cells

Source: Chaparro, (2015).

2.3.3 White blood cells

White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders. All leukocytes are produced and derived from a multi-potent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton *et al*., 2014). (Figure 2.4) depict five different and diverse types of leukocytes which are distinguished by their physical and functional characteristics Lafleur and Brooks (2018). Monocytes and neutrophils are phagocytic (Figure 2.4). The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count is usually between 4 and 11 × 10^9/L. In the US this is usually expressed as 4,000–11,000 white blood cells per microliter of blood. They make up approximately 1 % of the total blood volume in a healthy adult. An increase in the number of leukocytes over the upper limits is called leucocytosis, and a decrease below the lower limit is called leukopenia. Research has shown that WBC of rodents such as mice, rats, hamsters, and gerbils range from 5.5 - 11 x10^9/µL (Karen, 2012).

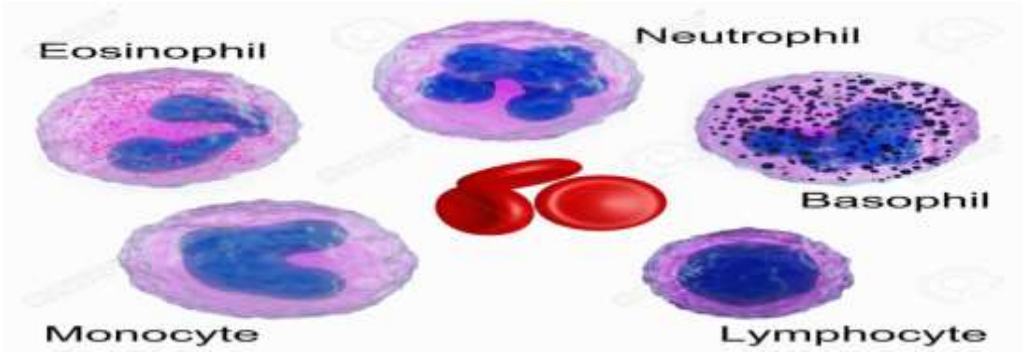

Figure 2.4: White Blood Cells

Source: Chaparro, (2015).

2.3.3 Platelets count

Platelets, also called "thrombocytes", are a component of the blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries (Walker *et al*., 2012). They are fragments of the cytoplasm which are derived from the megakaryocytes (Machlus *et al*., 2014; Chaparro, 2015) of the bone marrow which enters the circulation and they have no cell nucleus as presented in the (Figure 2.5). Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells. The main function of platelets is to contribute to hemostasis (the process of stopping bleeding at the site of interrupted endothelium). They gather at the site and unless the interruption is physically too large, they plug the hole. Firstly, platelets attach to substances outside the interrupted endothelium (adhesion). Secondly, they change shape, turn on receptors and secrete chemical messengers (activation). Thirdly, they connect to each other through receptor bridges (aggregation) (Yip *et al*., 2015). Formation of this platelet plug (primary hemostasis) is associated with activation of the coagulation cascade with resultant fibrin deposition and

linking (secondary hemostasis) (Chaparro, 2015). Low platelet concentration called thrombocytopenia is due to either a decreased production or increased destruction. Elevated platelet concentration called thrombocytosis is either congenital, reactive (to cytokines), or due to unregulated production. Research has shown that platelet of rodents such as mice, rats, hamsters, and gerbils range from 300 - 750 $x10^3/\mu L$ (Karen, 2012)

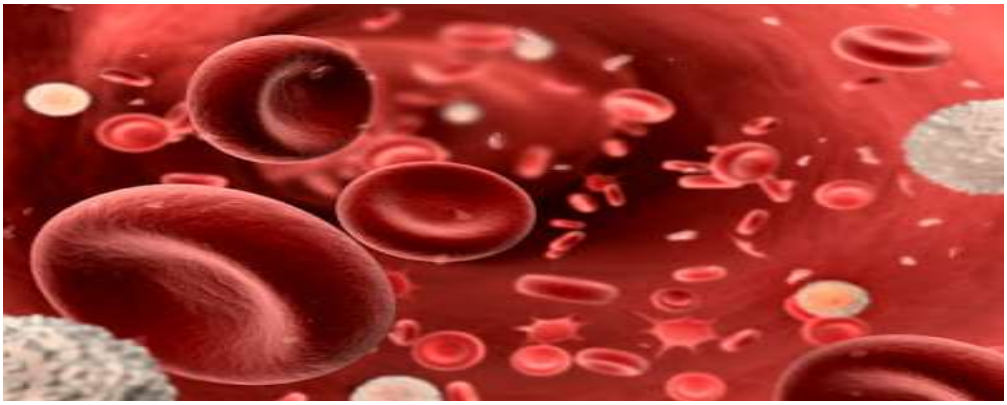

Figure 2.5: Platelets Formation

Source: Chaparro, (2015).

2.4 Packed Cell Volume

Packed cell volume (PCV), is the ratio of the proportion of erythrocytes which is expressed as a percentage of the volume of the whole blood per given sample Kusiluka and Kambarage (2016). Determination of PCV value is fundamental in diagnosing the various pathological and metabolic disorders (Grunwaldt *et al*., 2014; Chaparro, 2015). (Figure 2.6) represent packed cell volume for normal, anaemic and polycytaemia. A low packed cell volume value can depict anaemia, haemorrhage, bone marrow failure, leukaemia, malnutrition or specific nutritional deficiency, multiple myeloma and rheumatoid arthritis. Packed cell volume values higher than the reference values could indicate dehydration due

to diarrhoea, erythrosis and polycythermia packed cell volume is influenced by altitude (Tiboh *et al*., 2016), age (Grunwaldt *et al.*, 2014), health status, ambient temperature, and physiological status (excitement, muscular exercise, pregnancy, estrus, parturition, water balance and transportation depicted in (Figure 2.6) (Osaer *et al*., 2013; Chaparro, 2015). Reduced oxygen tension in mountainous regions results to an elevated production and release of a glycoprotein called erythropoietin which stimulates erythropoiesis as a coping or adaptive mechanism to low oxygen level in such an environment (Tiboh *et al*., 2016). Research has shown that PCV of rodents such as mice, rats, hamsters, and gerbils range from thirty-three to fifty-five percentage (Karen, 2012; Chaparro, 2015).

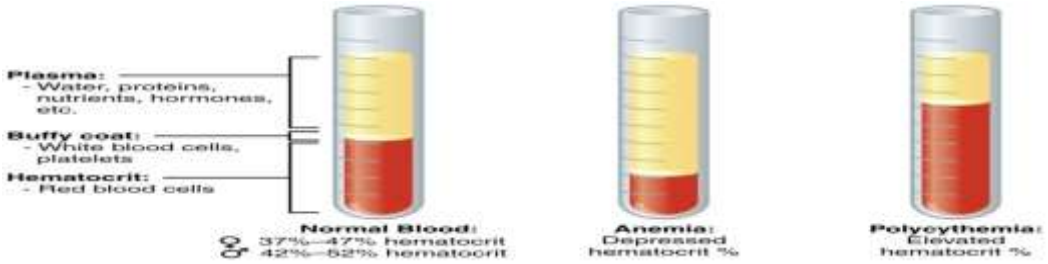

Figure 2.6: Packed Cell Volume

Source: Chaparro, (2015).

2.5 Anaemia Disorder

Anaemia is from Ancient Greek, meaning lack of blood; it is a disorder due to decrease in number of red blood cells (RBCs) or less than the normal quantity of haemoglobin in the blood. However, it can include decreased oxygen-binding ability of each haemoglobin molecule due to deformity or lack in numerical development as in some other types of haemoglobin deficiency (Halterman *et al*., 2016). Haemoglobin which is found inside red blood cells normally carries oxygen from the lungs to the capillaries, anaemia leads to

hypoxia (lack of oxygen) in organs (Chaparro, 2015). Since all human cells depend on oxygen for survival, varying degrees of anaemia can have a wide range of clinical consequences (Halterman *et al*., 2016). Anaemia is the most common disorder of the blood. Several kinds of anaemia are produced by a variety of underlying causes. It can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discernible clinical spectra, etc. The three main classes include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (haemolysis) or deficient red blood cell production (ineffective haematopoiesis) (Chaparro, 2015; Halterman *et al*., 2016).

2.5.1 Iron-Deficiency Anaemia

Iron is essential for the various activities of the human body especially in the haemoglobin synthesis. The following figure shows the distribution and storage of iron (Fe) in the various parts of the human body. Iron deficiency anaemia is a condition in which the body has too little iron in the bloodstream (Muanza *et al*., 2015). This form of anaemia is more common in adolescents and in women before menopause. Blood loss from heavy periods, internal bleeding from the gastrointestinal tract, or donating too much blood can all contribute to this disease. A low level of iron, leading to anaemia, can result from various causes Adam and Fetman (2014). The causes of iron-deficiency anaemia are pregnancy or childhood growth spurts, Heavy menstrual periods, Poor absorption of iron, Bleeding from the gut (intestines), dietary factors (iron poor or restricted diet), medication (aspirin ibuprofen, naproxen and diclofenac), Lack of folic acid and vitamin B12, Bleeding from the kidney, Hookworm infection, Red blood cell problems, Bone marrow problems (Muanza *et al*., 2015).

The symptoms include tiredness, lethargy feeling faint and becoming breathless easily, headaches, irregular heartbeats (palpitations), altered 2taste, sore mouth and ringing in the ears (tinnitus) Adam and Fetman (2014). Anaemia in pregnancy increases the risk of complications in both mother and baby such as low birth weight baby, preterm (premature) delivery and postnatal depression. Low iron reserves in the baby may also lead to anaemia in the new born baby (Muanza *et al*., 2015).

2.5.2 Haemolytic anaemia

Haemolytic anaemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is up. Haemolytic anaemia can affect people of all ages, races and sexes (Wilkins, 2018). Haemolytic anaemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure. Inherited haemolytic anaemias include Sickle cell anaemia, Thalassaemias, hereditary spherocytosis, hereditary elliptocytosis, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Pyruvate kinase deficiency. Acquired haemolytic anaemias include Immune haemolytic anaemia, Autoimmune haemolytic anaemia, Alloimmune haemolytic anaemia, Drug-induced haemolytic anaemia, Mechanical haemolytic anaemias, Paroxysmal nocturnal haemoglobinuria, certain infections and substances can also damage red blood cells and lead to haemolytic anaemia Adam and Fetman (2014).

The most common symptom of anaemia is fatigue. A low red blood cell count can also cause shortness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums and nail beds, as well as chest pain. Symptoms of haemolytic anaemia include Jaundice, Pain in the upper abdomen, Leg ulcers and pain, a severe reaction to a blood transfusion Adam and Fetman (2014). Treatments for haemolytic anaemia include blood

transfusions, medicines, plasmapheresis, surgery, blood and marrow stem cell transplants and lifestyle changes Noshad and Anjum (2018).

2.6 Signs and Symptoms of Anaemia

The general symptoms include:

(i) Tiredness and lethargy.

(ii) Inhibit physical exercise and result in reduced mental performance.

(iii) reduce oxygen carrying capacity of the blood lead to reduced tissue oxygenation and wide spread organ dysfunction.

(iv) Rapid blood loss e.g.; haemorrhage, shock with collapse, and dyspnoea and tachycardia.

(v) Reduce the amount of haemoglobin when the haemoglobin falls below 7 or 8 g/dL there is almost always compensatory increase in cardiac output.

(vi) Increase respiratory rate Acomb and Holden (2017).

2.7 Iron Absorption

Intestinal iron absorption depends on three conditions: the iron content of the diet, the bioavailability of the dietary iron, and the capacity of the mucosal cells to absorb the iron (Miret, 2013; Chaparro, 2015). There are two kinds of dietary iron: heme and non-heme or inorganic iron. Iron-replete persons will absorb proportionally less of any amount of non-heme iron consumed than will those who are iron-deficient. This type of selective absorption is the main mechanism by which iron is regulated in the human body Beard and Osman (2016). The recommended dietary intake of iron for adults is around 13-18 mg per day, out of which only 1 mg is absorbed. Even in iron deficiency, absorption is only

increased to approximately 2-4 mg/day, and in iron overload, it is reduced to 0.5 mg/day (Miret, 2013).

2.7.1 Non-haeme iron absorption

Most dietary iron occurs in the non-heme form, which is present in foods as either the reduced ferrous (Fe^{2+}) or the oxidized ferric (Fe^{3+}) form. Non-haeme iron is found in both plants and animal sources; in plants, it is present in three major forms: as metalloproteins (plant ferritin), as soluble iron in the sap of xylem, phloem and plant vacuoles, and as nonfunctional iron complexed with plant structural or storage components, primarily in the form of phytates. A large amount of dietary non-heme iron is present as contaminant ferric oxides and hydroxides (Chaparro, 2015). In animal-derived foods, iron can be found in meat products as ferritin and hemosiderin; in egg yolk, it is bound to the phosphoproteinphosphovitin, and in milk it is bound to lactoferrin or associated with fat globule membranes and low molecular weight compounds (such as citrate) (Miret, 2013; Chaparro, 2015).

Upon entering the stomach, non-haeme iron is acted upon by gastric juices containing pepsin and hydrochloric acid, which reduce ferric to ferrous iron, making it more bioavailable. Iron is better absorbed in the upper small intestine, mainly in the duodenum, where the low pH enhances its solubility (Miret, 2013). Under normal physiological conditions (i.e. normal pH and presence of oxygen), ferrous iron is quickly oxidized to ferric iron and precipitates as ferric oxyhydroxides; precipitation tends to occur in the luminal contents of the gastrointestinal tract as the pH increases. However, the slightly acidic microclimate in the duodenal surface (pH 6-6.5) helps maintain significant levels of iron in the ferrous form, as does cell surface reductase activity. This microclimate also

provides a proton gradient directed toward the cell interior, creating an additional driving force for iron uptake into the enterocyte (ORiordan *et al.*, 2015).

Non-haeme iron is thought to be taken across the brush-border membrane of the enterocyte after being reduced from ferric to ferrous iron by an apical or brush border ferric reductase called duodenal cytochrome b (DCYTB). Once iron is reduced by ascorbic acid or other reducing agents, ferrous iron can be transported by the divalent metal ion transporter (DMT1), which transports only ferrous iron (Meredith *et al.,* 2019). Iron deficiency and hypoxia stimulate duodenal expression of DMT1, DCTYB and ferroportin (an iron exporter), leading to increased iron absorption (Chaparro, 2015).

2.7.2 Haeme iron absorption

Haemoglobin and myoglobin from animal foods are the main protein sources for heme iron. Heme iron has a high intrinsic bioavailability and is soluble in an alkali environment (Chaparro, 2015). (Figure 2.7), represent the transport of haeme through the duodenal enterocyte. Haeme is released from haemoglobin during digestion in the small intestine and is thought to then bind to a specific receptor on the enterocyte, after which it is internalized via endocytosis (Miret, 2013). Absorbed haeme is acted upon by heme oxygenase 1 (HOX1) in the enterocyte to release iron to the soluble cytoplasmic pool (figure 2.7) (Chaparro, 2015; Beard, 2016). Release of iron from haeme by HOX1appears to be the rate-limiting step in haeme-iron absorption (Miret, 2013).

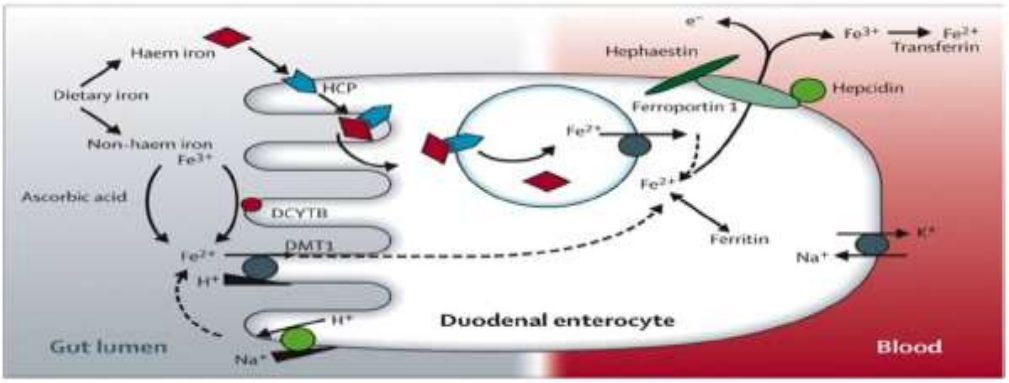

Figure 2.7: Transport of Haeme and Non-Haeme Iron through Enterocyte

Source: Chaparro, (2015).

2.8 Aluminum Chloride ($AlCl_3$)

Aluminum, (Al), is ubiquitous in the environment. Physically it is amber to light pale yellow, almost clear liquid and it is stable at normal temperature and pressure. However, little is known about possible effect of Al as trace element in animals and human in normal condition (Dlugaszek *et al* 2014 and Buraimoh *et al.*, 2016).

The principle mechanisms of absorption of Al is poorly understood. After ingestion, the systemic transfer of aluminum is small but it is greatly affected by the ingestion of certain dietary agents like citrate, that complex with the metal in the intestinal lumen or transiently alters the permeability of the mucosa. The small bowel and colon absorb aluminum passively and paracellularly but stomach dose not (Whitehead *et al*., 2014).

Studies on workers exposed to Al dust in industrial environments demonstrate similar effects (Akila *et al*., 2019). Many researchers have found elevated Al levels to be associated with a decline in visual memory, attention, concentration, frontal lobe function and lower vocabulary scores in haemodialysis patients Schulz and Glaser (2012). Salts of aluminium may bind to DNA, RNA; inhibit such enzymes as hexokinase, acid and alkaline

phosphates, phosphodiesterase and phosphooxydase Scholl and Hediger (2014). Aluminium exposure can cause impairments in glucose utilization, agonist-stimulated inositol phosphate accumulation; free radical mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation Scholl and Hediger (2014). Aluminium induces changes in haemato-biochemical parameters, increases lipid peroxidation and decreases activities of the antioxidant enzymes in plasma and tissues of male rabbits (Rosen, 2016). Al causes deterioration in sperm quality, enhancement of free radicals and alterations in antioxidant enzymes in both invivo and invitro (Newairy *et al*., 2019). The mechanism of aluminium induced toxicity is that it potentiates the activity of Fe^{2+} and Fe^{3+} ions to cause oxidative damage (Rifai, 2013).

2.9 Toxicity of Aluminum Chloride

Recently it is clear that when Al is mobilized from soil by acid rain, it poses hazard to all exposed organs (Buraimoh *et al.,* 2016). The ionic form of Al has no serious biological role, but when accumulate in the body it can induce several clinical disorders such as neurotoxicity, hepatotoxicity, bone marrow diseases and anemia. It also has direct effect on hematopoiesis and its high levels in serum of hem dialysis patients were associated with impaired erythropoiesis and iron (Fe) deficiency anemia. Furthermore, Aluminum is known to disrupt cellular functions by perturbing Fe homeostasis (Osman *et al.,* 2014). Aluminum chloride also increases protein carbonyl group concentration. The long-term aluminum intoxication of rats and mice besides other harmful effects causes an increase in oxidative stress and accumulated in bone and liver (Kowalczyk *et al.,* 2013 and Dlugaszek *et al.,* 2014).

2.10 Aluminium Induced Anaemia

There is no clearcut mechanism as to how aluminium induces anaemia. Anaemia may result from a decreased haeme synthesis, decreased globulin synthesis and increased hemolysis (Rang *et al.,* 2017). Aluminium chloride may have an effect on the metabolism of iron by influencing the absorption of iron through the intestine.

Studies have shown that aluminium has an affinity for transferrin. Aluminium may hinder the transport of iron in the serum and displaces iron from binding to transferrin. Transferrin plays the essential role of iron uptake and transport but is also the major serum binding protein (Rajangam *et al.,* 2017). Exposure to aluminium favors its binding to serum transferrin to form a complex called aluminium-transferrin which interacts with the same receptors as iron-transferrin thereby making serum transferrin unavailable for iron to bind. An exposure to a high dose of aluminium can change iron metabolism in different animal species Preeti and Shalini (2014). Aluminum may cause anaemia through decreased heme synthesis, decreased globulin synthesis and increased hemolysis. Patients with anaemia from aluminum toxicity often have increased reticulocyte counts, decreased haemoglobin concentration, decreased hematocrite value, decreased mean corpuscular volume and decreased mean corpuscular haemoglobin Hernberg and Nikkanen (2020). These toxic effects of aluminum on RBCs have been reported to generate reactive oxygen species and induce oxidative stress, which results in the oxidative deterioration of cellular lipids (through lipid peroxidation), proteins and DNA (Chinoy *et al*., 2018). These toxic effects of aluminum appear to result from free radical generation.

Recent research shows that aluminum may induce changes in the activity of a number of antioxidative enzymes (xanthine oxidase, glutathione peroxidase, superoxide dismutase).

Several studies by (Chinoy *et al*., 2018) have implicated lipid peroxidation as one of the molecular mechanisms underlying aluminum toxicity in vitro and in vivo. Recent report suggests that aluminum can induce morphological and functional alterations in erythroid cells by a direct action on circulating erythrocytes, suggesting membrane alterations due to lipid peroxidation mechanisms (Al-Hashem *et al*., 2018). In line with this, aluminum decreases erythrocytic membrane fluidity. However, a causal link between haematological changes and lipid peroxidation after exposure to aluminum is lacking in literature (Forstner, 2016). Studies suggest that chronic exposure to relatively high doses of aluminum can change iron metabolism in different animal species. However, the findings are not always in the same direction. In fact, some studies have indicated a reduction in iron in serum of rats exposed to high levels of aluminum, whereas other investigators have found no alterations or an increase in iron stores after exposure to aluminum. Consequently, data concerning the mechanism of Aluminum toxicity on haematological system and iron metabolism after administration of aluminum are contradictory and seem to depend on the conditions of toxicity, which refer mainly to different doses and different routes of administration (Al-Hashem *et al.*, 2018)

2.11 Mechanisms of Aluminum-Induced Anaemia

The mechanism by which aluminum causes anaemia is unknown. The absence of reticulocytosis and the occurrence of microcytosis followed by a fall in haemoglobin and haematocrit suggests that decreased red cell production is responsible. The anaemia of lead toxicity is characterized by the inhibition of delta aminolevulinic acid dehydratase activity (Garnica, 2015; Meredith 2019). Since aluminum is known to alter the activity of many enzymes (Harison *et al.*, 2012; Marquis *et al.*, 2014) and the toxic effects of chronic oral

lead and chronic oral aluminum exposure that aluminum-induced anaemia is secondary to interference with enzymes involved in haeme biosynthesis. Inhibition of delta aminolevulinic acid dehydratase activity, the second and rate-limiting enzyme in haeme biosynthesis, is now widely accepted as an accurate measure of human lead exposure (Wingfield, 2012; Garnica, 2015 and Hernberg, 2020). Although the effect of aluminum on delta aminolevulinic acid dehydratase activity has been studied, the results are difficult to interpret. Delta aminolevulinic acid dehydratase activity is significantly decreased in hemodialysis patients, but there is no relationship between decreased enzyme activity and plasma aluminum concentration (Meredith *et al.*, 2019). In vitro studies shows that aluminum inhibits delta amino levulinic acid dehydratase activity, while in vivo studies by the same investigators demonstrate activation of the enzyme by aluminum (Abdullah, 2019). Other in vitro studies show enhanced delta amino levulinic acid dehydratase activity with 2 mmol/L aluminum, but inhibition with four mmollL aluminum. (Garnica, 2015). Delta amino levulinic acid dehydratase activity is activated by zinc and aluminum in vivo, but zinc-mediated activation is inhibited by increasing aluminum concentrations in vitro (Abdullah, 2019). In addition, lead and aluminum additively inhibit delta amino levulinic acid dehydratase activity, but the addition of zinc leads to reactivation of the enzyme (Abdullah, 2019). The specific effects of aluminum on heme biosynthesis in haemodialysis patients are unknown. In addition, the exact role of the interaction of aluminum with other metals in this process awaits clarification. However, the hypothesis that aluminum alone or through its interaction with other metals could profoundly affect haeme biosynthesis remains attractive.

2.12 Ferrous Sulphate

Iron is an important constituent of haemoglobin as each molecule of it contains four atoms of iron amounting to 1.1 mg of iron per milliliter of red blood cells (Okuda *et al*., 2018). An adult man requires on an average 14 μg/kg/ day. This is equivalent to about 1 mg per day however, a menstruating woman requires about 30 μg/kg/ day, which is about 1.4 mg/per day (Okuda *et al*., 2018). Pregnant women in the last two trimesters require nearly 80 μg/kg/ day which is about 5-6 mg per day.

Anaemic patients are prescribed iron preparations to overcome their iron deficiencies and associated medical problems. It is well established that orally administered ferrous sulphate is the treatment of choice for iron deficiency and also the most economical. Absorption of ferrous salts is three times the absorption of the corresponding ferric salts (Okochi *et al*., 2017). It is also observed that sulphate, succinate, gluconate, fumarate and other ferrous salts are absorbed in the body to approximately the same extent. Ferrous sulphate hydrate contains 20 % elemental iron, dried ferrous sulphate has 32 % iron, ferrous fumarate 33 % and ferrous gluconate 12 % iron (Okochi *et al*., 2018). Polysaccharide-iron complex is another preparation being used in the treatment of iron deficiency anaemia (Okigbo *et al*., 2018). The amount of iron, rather than the mass of the total salt in the tablets/capsules, is important. Cereals are the most important source of iron in the diets of a large majority of the population in Nigeria and other developing countries (Ogbonnia *et al*., 2019). Other important sources of iron are legumes and green vegetables. Meat, fish and eggs are important sources of iron in all advanced countries. Milk is a poor source of iron (Oduro *et al*., 2018). Generally, it is recommended that the average dose of iron for the treatment of iron deficiency anaemia is about 200 mg of iron per day (2-3 mg/kg for infants and children), given in three equal doses of 65 mg Ndong and Katsumata (2017). When the

objective is the prevention of iron deficiency in pregnant women, doses of 15-30 mg of iron per day are adequate to meet the 3-6 mg daily requirement of the last two trimesters (Newairy *et al*., 2019). As stated by Yakubu and Musa (2012), a total dose of about 100 mg per day may be used, in selected cases depending upon the degree of anaemia. Intolerance to iron preparations occurs in about 25 % of individuals. Side effects such as heartburn, nausea, upper gastric discomfort, constipation and diarrhoea may occur and this aspect should be considered in the treatment plan (Njukwe *et al*., 2014). Treatment of anaemia requires 1 to 2 months therapy with antianaemia drugs. The duration of treatment is governed by the rate of recovery of haemoglobin and the desire to create iron stores (Nordeide *et al*., 2016). The former depends on the severity of the anemia. With a daily rate of repair of 2 g of haemoglobin per liter of whole blood, the red cell mass usually is reconstituted within 1 to 2 months (Noga *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials Used

3.1.1 Plant materials

Fresh leaves of *Carica papaya* (pawpaw) were collected from biological garden of Federal University of Technology, Minna, Niger State in November 2018. While the seeds were obtained from fruit sellers in Bosso Local Government Minna, Niger State in November 2018. Taxonomic identification of the plants was done by Mr Dangana, M. C. of Biological Science Department, Federal University of Technology Minna, Nigeria. Both the leaves and seeds were cleaned, washed and air-dried at ambient temperature and pulverized using electronic blending machine.

3.1.2 Reagents and chemicals

Aluminum chlorides, hydrochloric acid, sodium hydroxide, iron (iii) chloride, concentrated ammonium hydroxide, methanol, n-hexane and sulphuric acid used in the present research were of analytical grade and were products of Sigma Chemical Co., USA.

3.1.3 Equipment and apparatus

Test tubes, beakers, conical flasks, round bottom flasks, measuring cylinders, masking tape, volumetric flasks, separating funnels, pipette, whatman filter paper (No. 1), cuvette, water bath, Systex haematologic analyser, electronic weighing balance, foil paper and electronic blending machine.

3.1.4 Experimental animals

A total of seventy (70) rats were obtained from the Animal breeding unit of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. These animals weighed 125 g to 160 g, the animals were housed in plastic cages and given commercial diet (rat pellet) and water *ad-libitum* and were maintained under standard laboratory conditions. This study was conducted in confined environment; the temperature was maintained at 22 ` ±3 ^{0}C. The relative humidity was maintained at 50 % ± 5 %, the light & dark cycle (12 hrs dark & 12 hrs light). The diet and the quantity of drinking water were standardized and maintained continuously (Jigam *et al.,* 2009).

3.2 Chemical Methods

3.2.1 Plant preparation and extraction

This was carried out according to the method described by Kabiru *et al.* (2012). The collected fresh leaves and seeds of *C. papaya Linn* were destalked, washed with clean water, air-dried at room temperature and blended using Electric blending machine. Both the pulverized dried leaves and seeds of *Carica papaya* were extracted with Methanol.

Fifty grams (50 g) of the powdered sample of *C.papaya* Leaves and seeds was soaked in 400 mL of methanol and refluxed for 2 hours in a distillation flask mounted on a heating mantle according to the method described Kabiru *et al.* (2012). The extract was filtered using a cheese cloth and the filtrate evaporated using rotary evaporator and concentrated using a water bath. The crude extract was weighed and stored in a refrigerator until required for use.

$$\%\ yield = \frac{Weight\ (g)\ of\ extract}{Weight\ (g)\ of\ pulverized\ sample} \times 100 \qquad (3.1)$$

3.2.2 Qualitative phytochemical analysis

Qualitative phytochemical components of *C. papaya* leaves and seeds extracts were determined according to the standard method of (American association of analytical chemist, 1999).

3.2.2.1 *Test for alkaloids*

Presence of Alkaloids in the sample was determined according to the method described by Harborne (1948). In a test tube, (0.2 g) of the sample was boiled with 5 mL of 2 % HCl on a steam bath. The mixture was filtered and 1 mL portion of the filtrate was treated with 2 drops of Dragendorff's reagent: A red precipitate indicated the presence of alkaloids.

3.2.2.2 *Test for flavonoids*

Presence of Flavonoids in the sample was determined according to the method described by Chang *et al*. (2002), in a test tube, (0.2 g) of the sample was heated with 10 mL ethyl acetate in boiling water for 3 minutes. The mixture was filtered off and the filtrate was used for Ammonium test: A quantity, 4 mL of the filtrate was shaken with 1ml of dilute ammonium solution. The layers were allowed to separate. A yellow precipitate observed at the ammonium layer indicated the presence of flavonoids.

3.2.2.3 *Test for saponins*

Presence of saponins in the sample was determined by the method described by Oloyede (2005), in a test tube, (0.1 g) of the sample was boiled with 5 mL of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for Frothing test: A

quantity, 1 mL of the filtrate was diluted with 4 mL of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

3.2.2.4 *Test for tannins*

The presence of tannins in the sample was determined by the method described by Harborne (1973). In a test tube, (2 g) of the sample was boiled with 5 mL of 45 % ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with Ferric chloride solution: a quantity, 1 mL of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicated the presence of tannins

3.2.2.5 *Test for phenolic compounds*

The presence of phenol in the sample was determined by the method reported by Singleton *et al*. (1999). The, extract (50 mg) is dissolved in 5 mL of distilled water. To this few drops of neutral 5 % ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

3.2.3 Quantitative Phytochemical Analysis

Quantitative phytochemical components of *C. papaya* leaves and seeds extracts were determined according to the method described by AOAC (1999).

3.2.3.1 *Alkaloid content determination*

The determination of alkaloid content was carried out using the method of Harborne (1973). A portion (0.5 g) of the sample was weighed into a 50 mL beaker and 20 mL of 10 % acetic acid in ethanol was added, covered and allowed to stand for 2 hrs. This was

filtered and the extract was concentrated on a water bath to one - quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract to obtain the precipitate which was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. A weighed amount of the crude alkaloid was dissolved in 96 % ethanol 20 % H_2SO_4 so that the concentration of alkaloid was between 0.2 and 3.0 mM/L (Molecular weight of solanine is 868.04 g/mol). The alkaloid solution (1 mL) was mixed without special cooling with 5 mL 60 % H_2SO_4 and after 5 min, 5 mL of 0.5 % solution of formaldehyde in 60 % H_2SO_4 was added. This was left to stand for 180 min and then the absorbance was measured at 570 nm. The actual amount of alkaloid in the crude isolate was determined by reference to absorbance measurements on pure alkaloid solutions treated in the same way with formaldehyde in 1 M H_2SO_4.

3.2.3.2 *Determination of flavonoid content*

The method described by Chang *et al*. (2002), was used to estimate total flavonoid contents of the extract solution based on the formation of a complex, flavonoid-aluminium. To 0.5 mL of extract solution was added 0.5 mL of 2 % $AlCl_3$-ethanol solution. After one hour of incubation at room temperature, the absorbance was measured at 420 nm using UV-Vis spectrophotometer. All determinations were done in triplicate and values were calculated from calibration curve obtained from quercetin.

3.2.3.3 *Determination of tannins*

The method of association of official analytical chemists, (1984) was used for the determination of the tannin content of *Carica papaya* leaf and seed extract. A quantity, 0.2

g of finely ground sample was measured into 50 mL beaker. About 20 mL of 50 % methanol was added and covered with paraffin and placed in a water bath at 77-80 ^{0}C for 1 hour and stirred with a glass rod to prevent bumping. The extract was filtered using a double layer of Whatman No. 1 filter paper into a 50 mL volumetric flask, then, 20 mL distilled water, 2.5 mL Folin-Denis reagent and 10 mL of 17 % Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water and allowed to stand for 20 mins, when a bluish-green colouration developed. Standard tannic acid solutions of range 0-10 ppm were treated similarly as 1 mL of sample above. The absorbances of the tannic acid standard solutions as well as samples were read after colour development at 760 nm. The tannin content was calculated using the formula:

3.2.3.4 *Determination of phenols*

The total phenolic content of *Carica papaya* extract was determined using the method of Singleton *et al*. (1999). An aliquot of the extract (0.5 mL) was mixed with 2.5 mL of 10 % Folin-Ciocalteau reagent and 2 mL of Na_2CO_3 (75 % w/v). The resulting mixture was vortexed for 15 seconds and incubated at 40 ^{o}C for 30 mins for colour development. The absorbance of the sample was measured spectrophotometrically at 765 nm. Total phenolic content was expressed as mg/g tannic acid equivalent from the calibration curve.

3.2.3.5 *Determination of saponins*

The spectrophotometric method of Oloyede, (2005) was used for the estimation of saponins in the plant sample. A portion (0.1 g) of ground sample was weighed into a 25 mL beaker and 10 mL of ethanol was added. The mixture was vortexed on a mechanical shaker for 5 hrs to ensure uniform mixing. After, it was filtered through a Whatman No. 1 filter paper

into a 100 mL beaker and 20 mL of 40 % solution of magnesium carbonate was added. The mixture obtained with magnesium carbonate was again filtered to obtain a clear, colourless solution. Then, 1 mL of the colourless solution was pipetted into a 50 mL volumetric flask and 2 mL of 5 % $FeCl_3$ solution was added and made up to mark with distilled water and was allowed to stand. Standard saponin (0-10 ppm) was prepared from saponins stock solution. The standard solutions were treated similarly with 2 mL of 5 % $FeCl_3$. The absorbance of the sample as well as standard saponin solution was read after colour development on a spectrophotometer at a wavelength of 380 nm.

$$\% \, Saponin = \frac{Absorbance \; of \; sample \; x \; Gradient \; Factor \; x \; Dillution \; Factor}{Weight \; of \; sample} x \; 100 \qquad (3.2)$$

3.2.4 Extraction of total flavonoid

Extraction of total flavonoids was carried out according to the method described by Jouad *et al*. (2001). The powdered samples of *C. papaya* seed and leaf (350 g) were extracted with methanol in a soxhlet extractor. After concentration under reduced pressure using rotary evaporator, 41 g methanol extract was obtained. The methanol extract was dissolved in 400 mL of distilled water and extracted with n-butanol saturated with distilled water. After evaporation under reduced pressure using rotary evaporator, the butanol extract was subjected to column chromatography on silica gel eluted with n-hexane and methanol.

$$\% \, Yield = \frac{Weight \; of \; the \; flavonoid}{Weight \; of \; the \; ground \; C. \; papaya} x \; 100 \qquad (3.3)$$

3.2.5 Extraction of total akaloid

The extraction of the alkaloid was done by the continuous extraction method using the Soxhlet apparatus as described by Gonzalez *et al*. (2014). Three hundred grams (300 g) of ground *C. papaya* leaf and seed was weighed and packed in a cheese cloth bag which

served as an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with sufficient amount of 95 % ethanol. This was made alkaline with sufficient quantity of ammonia and mixed thoroughly. The sample in the thimble was macerated overnight and then placed in the Soxhlet extractor on the next day. Sufficient amount of 95 % ethanol was placed in the solvent flask. The sample was extracted for about 3 – 4 hours. The ethanol extract was filtered and was concentrated in a Soxhlet distilling apparatus at 60 ^{o}C. The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia and placed in a separatory funnel. Measured quantities of chloroform were added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The combined chloroform extract was concentrated in Soxhlet distilling apparatus at 60 ^{o}C and evaporated in water bath maintained at that temperature until semi-dry. The residue was weighed and percentage yield was calculated using the formula:

$$\%\, Yield = \frac{Weight\ of\ the\ alkaloidal\ \ residue}{Weight\ of\ the\ ground\ C.\ papaya} x\ 100 \qquad (3.4)$$

3.2.6 Acute toxicity test of crude extract of *Carica papaya*

Acute toxicity test was carried out using the method of Lorke, (1983). This method has two phases which are phases 1 and 2 respectively.

Phase 1

This phase comprise nine animals. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality will occur (Lorke, 1983).

Phase 2

This phase involved the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality (Lorke, 1983).

Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = highest dose that gave no mortality

D_{100} = lowest dose that produced mortality

3.2.7 Determination of the effect of crude methanol extracts and alkaloids and flavonoids fractions of *Carica papaya* leaves and seeds on bodyweight and haematological parameters of $AlCl_3$-induced wistar rats

The effect of crude extracts and alkaloid and flavonoid fractions of *C. papaya* were carried out using Aluminium chloride-induced anaemia models.

3.2.7.1 *Stage one: grouping of animals*

Experiment one determined the protective potential of methanol extract of *C. papaya* leaves and seeds in aluminium chloride induced anaemic rats. Thirty-five (35) adult Wistar rats were used in this study and it was conducted for fourteen (14) days according to the method described by Osman *et al.* (2012). Prior to this study, the rats were acclimatized for two weeks and following acclimatization, the rats were designed into nine (9) groups, each containing three (3) rats. Group one, two and three were administered with distilled water (1 mL), $AlCl_3$ (0.5 mg/kg) and ferrous sulphate (3 mg/kg) respectively, while groups four, five, six and seven, eight and nine were induced with $AlCl_3$ (0.5 mg/kg) for ten minutes prior treatment with ferrous sulphate (2.86 mg/kg), 100 mg/kg, 300 mg/kg and 500 mg/kg extract of *C. papaya* leaf and seed respectively for 14 days.

3.2.7.2 *Stage two: Grouping of animals*

Experiment two also determined the protective potential of methanol extract of *C. papaya* leaves in aluminium chloride induced anaemic rats treated with alkaloid and flavonoid fractions of the leaves and seeds of *C. papaya*. Another set of thirty-five (35) adult albino rats were used in this study and it was also conducted for fourteen (14) days. Prior to this study, the rats were also acclimatized for two weeks and following acclimatization, the rats were designed into eleven (11) groups, each containing three (3) rats. Group one, two and three were administered with distilled water (1 mL), $AlCl_3$ (0.5 mg/kg) and ferrous sulphate (3 mg/kg) respectively, while group four and five were treated with (75, 150 mg/kg) alkaloid leaves extract. Similarly, group six, and seven were treated with (75, 150 mg/kg) flavonoid leaves extract. Group eight and nine were also treated with (75, 150 mg/kg)

flavonoid seeds extract, while group ten and eleven were treated with (75, 150 mg/kg) alkaloid seed extract for fourteen (14) days.

All treatments were administered orally 10 minutes after the administration of 0.5mg/kg $AlCl_3$. All treatment was administered orally once daily for 14 days. PCV was recorded during the experimentation on 3 days interval for two weeks

3.2.7.3 *Determination of body weight*

The body weights of experimental rats were determined using Ultra V-bile P055 electronic compact scale every three days interval.

3.2.7.4 *Blood sample collection*

On day 15 of the experimental period, the animals were sacrificed according to the method described by Yusuf *et al.* (2018). Rats were placed under diethyl ether anesthesia and bout 3 mL of blood sample was collected by cardiac puncture into a well labeled EDTA sample bottles and were analyzed immediately for hematological parameters.

3.2.7.5 *Determination of haematological parameters*

The hematological components including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet count (PLC) and differential count (granulocyte count, lymphocytes, eosinophils, monocytes and neutrophils) were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (2002).

3.2.8 Data analysis

Data generated were analyzed using Statistical Package for Social Science (SPSS). All data were reported as Mean ± SEM and comparison between groups was done with One Way ANOVA (analysis of variance) and values with $p<0.05$ were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Plant extract and phytochemicals yields of *Carica papaya*

The results of percentage yield of *C. papaya* leaves and seeds extracts are pressented in (Table 4.1). The percentage yields of *C. papaya* leaves extract (17.51 %) was significantly ($p<0.05$) higher than the percentage yield of crude seeds extract (11.59 %). The flavonoid yield from *C. papaya* leaves (7.05±0.65 %) and seeds (7.96±0.29 %) were significantly ($p<0.05$) higher than the alkaloid yield of leaves (2.65±0.03 %) and seeds (2.97±0.04 %) respectively (Table 4.2).

Plant Samples	**% Yield**
C. Papaya crude (seeds) extract	11.59

Table 4.1: Percentage Yield of Crude Methanol Extract of Leaves and Seeds of *Carica papaya*

C. Papaya crude (leaves) extract	17.51*

*= significantly higher as compared to crude (seeds) extract

Table 4.2: Percentage Yields of Alkaloid and Flavonoid Fractions of *Carica papaya*

Fractions	% Yields
Alkaloids (seeds) extract	2.97±0.04[a]
Flavonoids (seeds) extract	7.96±0.29[b]
Alkaloids (leaves) extract	2.65±0.03[a]
Flavonoids (leaves) extract	7.05±0.65[b]

Data are presented in mean ± standard error of two paired determinations. Data followed by different superscript alphabet along the same column were significantly different $p<0.05$.

4.1.2 Quantitative phytochemical compositions of *Carica papaya*

The secondary metabolites detected in the leaves and seeds of *C. papaya* in different concentrations included phenols, flavonoids, tannins, alkaloids and saponins respectively. The result of quantitative phytochemical composition of crude extracts of *C. papaya* leaves and seeds are shown in (Table 4.3). Saponins had the highest concentrations (1249.83±13.05 and 723.65±0.39 mg/g) for the leaves and seeds respectively, followed by phenols (261.34±1.07 and 171.45±0.91 mg/g) and alkaloids (67.75±1.06 and 35.42±0.50 mg/g) and tannins (45.25±0.46 and 40.67±0.50 mg/g) and flavonoids (34.89±0.66 and 25.80±0.99 mg/g) respectively.

Table 4.3: Quantitative Phytochemical Composition of Crude Seeds and Leaves Extracts of *Carica papaya*

Parameters	Seeds (mg/g)	Leaves (mg/g)

Phenols	171.45±0.91[b]	261.34±1.07[a]
Flavonoids	25.80±0.99[b]	34.89±0.66[a]
Tannins	40.67±0.50[b]	45.25±0.46[a]
Alkaloids	35.42±0.50[b]	67.75±1.06[a]
Saponins	723.65±0.39[b]	1249.83±13.05[a]

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabet are significantly different across a row at $p<0.05$.

4.1.3 Acute oral toxicity of crude methanol extracts of leaves and seeds of *Carica papaya*

The result of acute oral toxicity of crude methanol extract of seeds and leaves of *C. papaya* is presented in (Tables 4.4 and 4.5). The LD_{50} of the methanol seeds and leaves extract of *C. papaya* in rats were > 5000 mg/kg and safe doses of the methanol seeds and leaves extract of *C. papaya* in rats were 2900 and 1600 mg/kg bw respectively. No death was recorded in any of the animals at all doses of extracts tested. However, animals administered 2900mg/kg and 5000 mg/kg bw of the *C. papaya* seeds and leaves extract showed some behavioral changes including; Rubbing of mouth on the surface of the cage, hyperactivity/restlessness but no death was recorded.

Table 4.4: Safe Dose of Methanol Seeds Extract of *Carica papaya*

First phase	Dose (mg/kg)	Number of Rats	Number of Death after 24 hours	% Mortality	Sign of toxicity
PHASE 1					
Group 1	10	3	0	0	Nil
Group 2	100	3	0	0	Nil
Group 3	1000	3	0	0	Nil
	PHASE 2				
Group 1	1600	3	0	0	Nil
Group 2	2900	3	0	0	Rubbing of the mouth on the surface of the cage
Group 3	5000	3	0	0	Hyperactivity/restlessness

LD_{50} is greater than 5000mg/kg body weight

Table 4.5: Safe Dose of Methanol Leaves Extract of *Carica papaya*

First phase	Dose (mg/kg)	Number of Rats	Number of Death after 24 hours	% Mortality	Sign of toxicity
PHASE 1					
Group 1	10	3	0	0	Nil
Group 2	100	3	0	0	Nil
Group 3	1000	3	0	0	Nil
	PHASE 2				
Group 1	1600	3	0	0	Nil
Group 2	2900	3	0	0	Nil
Group 3	5000	3	0	0	Hyperactivity/restlessness

LD_{50} is greater than 5000mg/kg body weight

4.1.4 Effect of crude methanol extracts of *Carica papaya* leaves and seeds on body weight of $AlCl_3$-induced anaemic rats

The result of weight changes in rats treated with methanol leaves and seeds extract of *C. papaya* in $AlCl_3$ induced anaemic rats are presented in (Table 4.6). Exposure to $AlCl_3$ resulted in significant ($P<0.05$) decrease in weight of negative control group. The normal control group (rats administered only distilled water) showed significantly increase in body weight (209.75±37.25) than the untreated group (71.00±11.00). Similarly, the extract treated groups showed significant ($P<0.05$) increase in body weight (185.00±5.50) when compared with group administered only $AlCl_3$ (71.00±11.00).

Table 4.6: Effect of Methanol Crude Leaves and Seeds Extracts of *Carica papaya* on Body Weight of $AlCl_3$-induced Anaemic Rats

Group	Dosage (mg/kg bw)	Day 0	Day 3	Day 7	Day10	Day14
Normal Control		191.50±10.50^{ab}	196.50±23.50^{a}	199.25±36.45^{b}	203.00±39.00^{bc}	209.75±37.25^{b}
Negative Control	0.5 mg/kg bw	107.00±33.00^{b}	102.00±30.00^{b}	94.05±11.35^{ab}	81.50±9.50^{c}	71.00±11.00^{ab}
Positive standard	3 mg/kg bw	133.50±19.50^{a}	137.75±12.75^{c}	142.00±17.00^{a}	148.00±26.00^{ab}	152.50±11.50^{a}
Crude*C. papaya* leaves	100 mg/kg bw	163.25±2.75^{ab}	154.75±0.25^{ab}	162.50±1.50^{ab}	171.50±6.50^{ac}	185.00±5.50^{ab}
Crude*C. papaya* leaves	300 mg/kg bw	196.25±23.75^{ab}	180.50±30.20^{ac}	201.50±22.50^{ab}	190.00±37.00^{abc}	206.75±39.75^{ab}
Crude*C. papaya* leaves	500 mg/kg bw	161.50±10.50^{ab}	168.50±9.50^{abc}	175.75±7.25^{ab}	169.50±6.50^{ac}	177.80±4.20^{ab}
Crude *C. papaya* seeds	100 mg/kg bw	202.50±10.50^{ab}	209.50±14.50^{a}	222.00±19.00^{ab}	226.00±33.00^{abcd}	230.25±12.75^{ab}
Crude *C. papaya* seeds	300 mg/kg bw	156.25±0.25^{ab}	173.50±10.50^{ac}	186.350±3.50^{ab}	186.00±12.00^{abc}	191.50±15.00^{ab}
Crude *C. papaya* seeds	500 mg/kg bw	134.00±38.00^{a}	143.00±36.00^{c}	151.35±34.35^{a}	151.50±37.50^{ab}	168.25±28.25^{a}

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p<0.05$).

4.1.5 Effect of crude methanol extracts of *Carica papaya* leaves and seeds on packed cell volume, haemoglobin and red blood cells of aluminum chloride induced anaemic rats

The effect of leaves and seeds extracts of *C. papaya* on Packed Cell Volume (PCV), Haemoglobin (Hgb) and Red Blood Cells (RBC) in $AlCl_3$-induced anaemic rats is presented in (Figure 4.1). At the end of the treatment period, the negative control group (rats that were not treated) showed significantly ($P<0.05$) lower PCV (26.50±3.50 %), Hgb (8.55±1.25 g/dL) and RBC (5.05±1.25 10^{12}/L) counts when compared to the normal control PCV (43.00±2.00 %), Hgb (13.65±0.65 g/dL), RBC (7.15±0.15 10^{12}/L) and extract treated group PCV (47.00±0.50 and 40.00±0,00 %), Hgb (14.35±0.45 and 13.65±0.15 g/dL) and RBC (10.88±0.50 and 10.66±0.85 10^{12}/L). The groups treated with leaves and seeds extract (test groups) showed dose dependent significant ($P<0.05$) increase in RBC (10.88±0.50 and 10.66±0.85 10^{12}/L) when compared with the normal control group (7.15±0.15 10^{12}/L). However, PCV and Hgb in extract treated groups at all concentrations tested were not significantly different ($P>0.05$) when compared with normal control group. A significant ($p<0.05$) increase in PCV, Hgb and RBC counts was observed in the extract treated groups when compared with the negative control group.

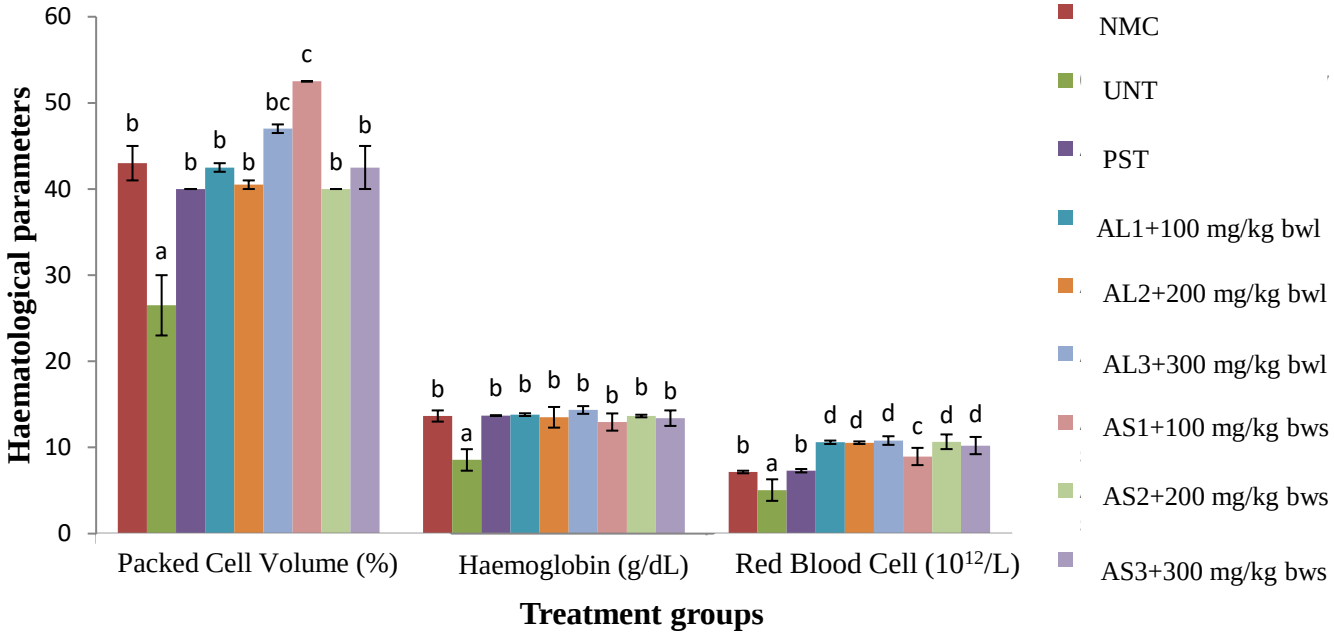

Figure 4.1: Effect of Leaves and Seeds Extracts of *C. papaya* on Packed Cell Volume (PCV), Haemoglobin (Hgb) and Red Blood Cells (RBC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats recieved 0.5 mg/kg bw $AlCl_3$ and were not treated; PST;Positive standard group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* leaves extract; AL2;Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* seeds extract;ACL2; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* seeds extract;ACL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* seeds extract.

4.1.6 Effect of crude methanol extracts of *Carica papaya* leaves and seeds on haematological parameters of aluminium chloride–induced anaemic rats

The result of leaves and seeds extracts *C. papaya* on white blood cell (WBC), lymphocytes (L), neutrophils (N) and red blood cell wide counts (RDWC) in $AlCl_3$ induced anaemic rats is presented in (Figure 4.2). The WBC (2.50±0.60 10^9/L) and lymphocytes (16.50±0.50 %) showed significant ($P<0.05$) decrease in the negative control group when compared to the normal control WBC (6.45±0.35 10^9/L) lymphocytes (51.50±1.50 %) and extract treated group WBC (9.40±1.00 and 6.50±0.10 10^9/L) and lymphocytes (61.50±1.30 and 59.00±0.10 %). However, WBC, RDWC and lymphocytes in extract treated groups at all concentrations tested were not significantly ($P>0.05$) different when compared with normal control group. Neutrophils showed significant ($P<0.05$) increase in the extract treated group (30.25±99.1 %) than the normal (13.00±3.00 %) control group.

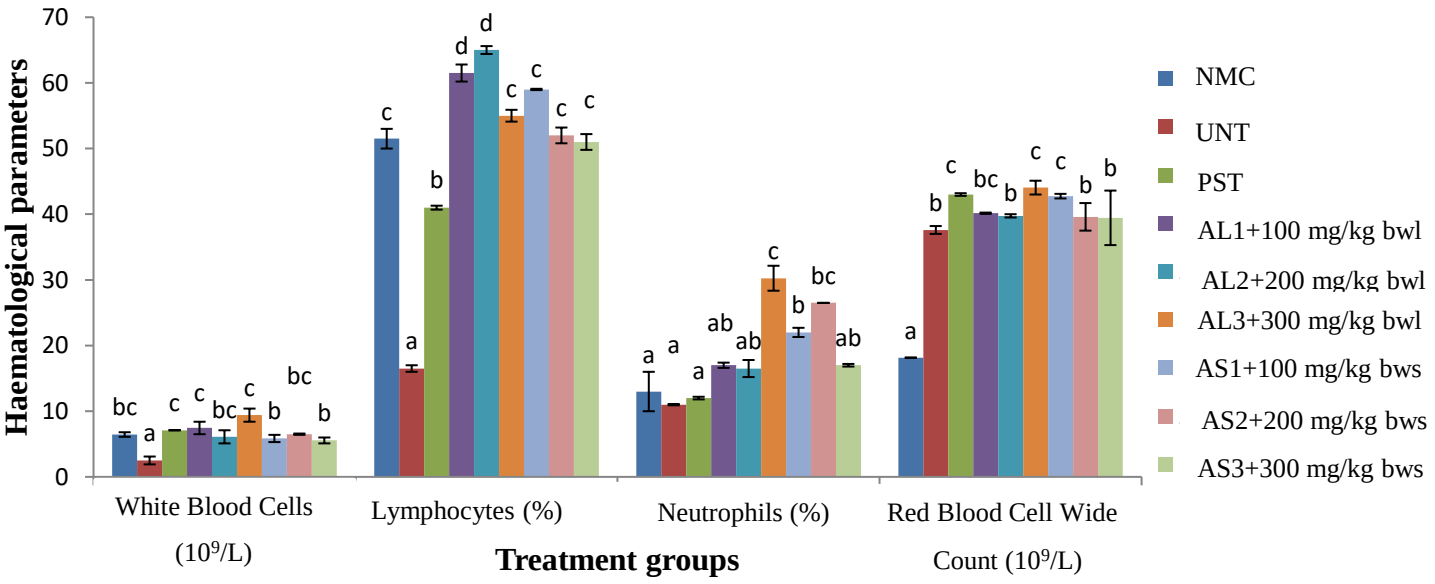

Figure 4.2: Effect of Leaves and Seeds Extracts *C. papaya* on White Blood Cell (WBC), Lymphocytes (L), Neutrophils (N) and Red Blood Cell Wide Counts (RDWC) in $AlCl_3$- induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats recieved 0.5 mg/kg bw $AlCl_3$ and were not treated; PST;Positive standard group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* leaves extract; AL2;Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* seeds extract;ACL2; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* seeds extract;ACL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* seeds extract.

4.1.7 Effect of crude methanol extracts of *Carica papaya* leaves and seeds on red blood cells indices of aluminum chloride induced anaemic rats

The result of leaves and seeds extracts of *C. papaya* on mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin Counts (MCHC) in $AlCl_3$-induced anaemic rats are presented in (Figure 4.3). The MCH (18.00±0.00 and 16.50±1.50 pg) and MCHC (34.50±1.50 and 32.50±2.50 g/dL) in all experimental groups were not significantly ($p>0.05$) different when compared with $AlCl_3$ MCH (16.00±1.00 pg) and MCHC (31.00±1.00 g/dL) (untreated rats) and normal control MCH (19.00±0.0 pg) and MCHC (38.50±0.50 g/dL) group. There was significant ($p<0.05$) increase in the group of rats treated with a dose of 300mg/kg body weight when compared with normal and extract treated group (test groups). However, there was no significant ($p>0.05$) different in MCV (58.00±6.00 and 51.00±0.00 fL) between the extract treated group (test groups) and normal control (47.00±1.00 fL) group.

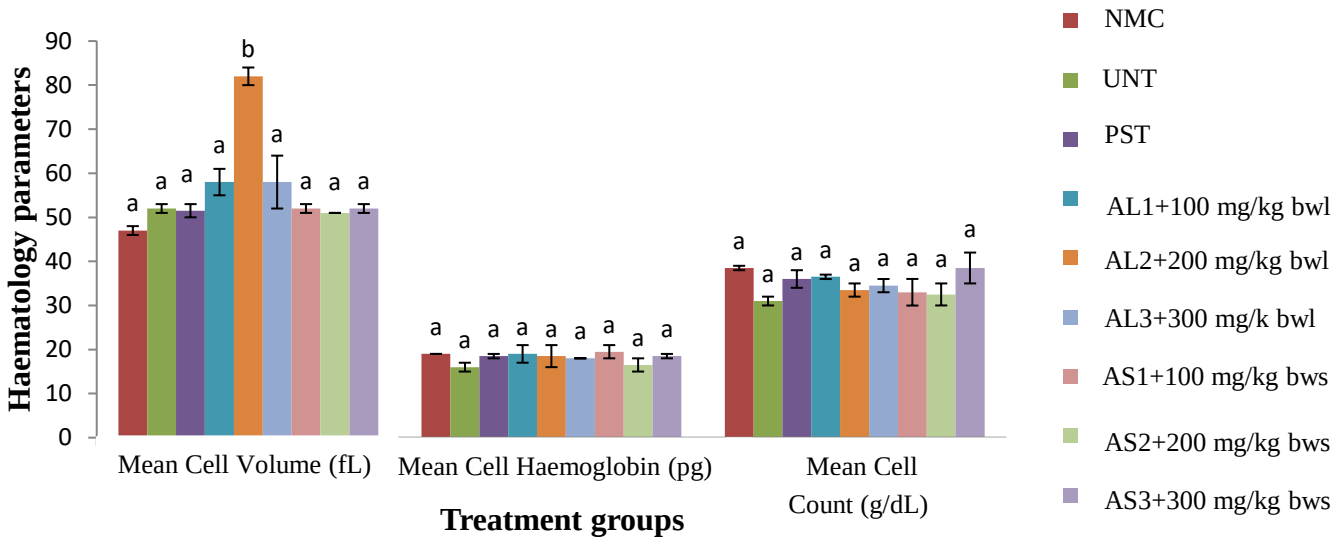

Figure 4.3: Effect of Leaves and Seeds Extracts of *C. papaya* on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats recieved 0.5 mg/kg bw $AlCl_3$ and were not treated; PST;Positive standard group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* leaves extract; AL2;Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* seeds extract;ACL2; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* seeds extract;ACL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* seeds extract.

4.1.8 Effect of crude methanol extracts of *Carica papaya* leaves and seeds on platelets count of aluminum chloride-induced anaemic rats

The effect of leaves and seeds extracts of *C. papaya* on Platelet count in $AlCl_3$-induced anaemic rats is presented in (Figure 4.4). The Platelet count showed insignificant ($p>0.05$) decrease in $AlCl_3$ administered rats (434.00 ± 30.00 10^9/L) (untreated group) when compared with extract treated groups (423.50 ± 40.00 and 556.50 ± 85.50 10^9/L). However, there was significant ($p<0.05$) difference in $AlCl_3$ induced anaemic rats (434.00 ± 30.00 10^9/L) when compared with normal control (738.00 ± 53.00 10^9/L) group, the extract treated group showed significant increase when compared with untreated group.

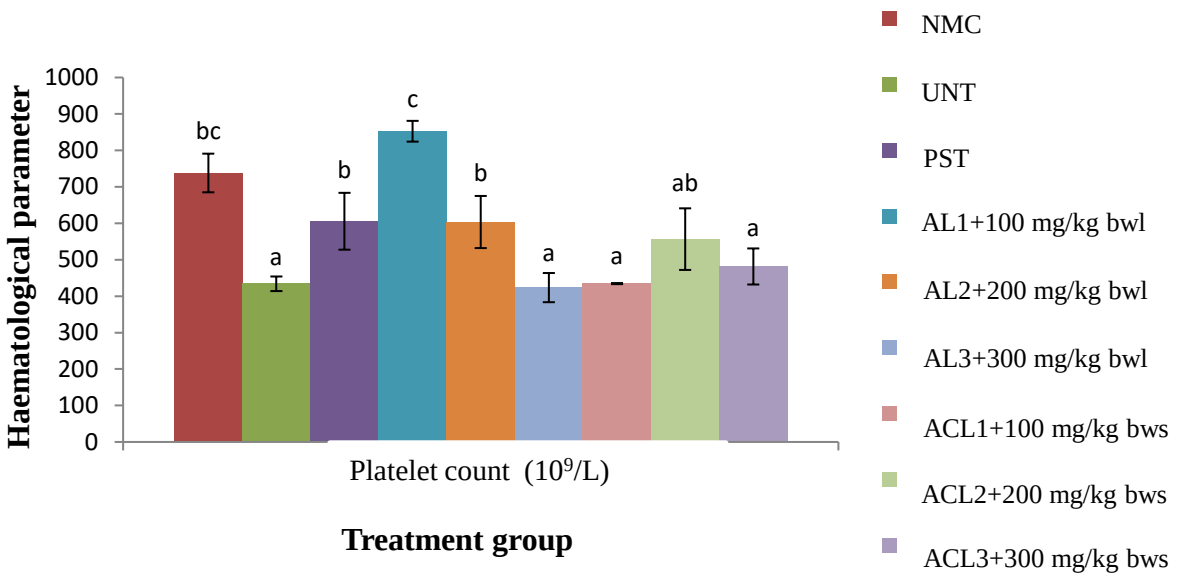

Figure 4.4: Effect of Leaves and Seeds Extracts of *C. papaya* on Platelet Count (PLC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$)

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats recieved 0.5 mg/kg bw $AlCl_3$ and were not treated; PST;Positive standard group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* leaves extract; AL2;Group of rats recieved 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 100 mg/kg bw *C. papaya* seeds extract;ACL2; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 200 mg/kg bw *C. papaya* seeds extract;ACL3; Group of rats received 0.5 mg/kg bw $AlCl_3$ and treated with 300 mg/kg bw *C. papaya* seeds extract.

4.1.9 Effect of alkaloid and flavonoid fractions of *Carica papaya* seeds on change in body weights of rats

The effect of crude methanol leaves and seeds extracts on weight changes in Aluminium chloride-induced anaemic rats treated with alkaloid and flavonoid fractions of *C. papaya* are presenred in (Table 4.8). Aluminium chloride ($AlCl_3$) administration resulted in significantly ($p<0.05$) lower bodyweight in the negative control group (87.00±7.81). The normal control group showed significantly ($p<0.05$) higher body weight (119.33±3.18) throughout the study period. Similarly, the extract treated groups significantly ($p<0.05$) increase in bodyweight (114.00±16.16) during the experimental period.

Table 4.8: Effect of Alkaloid and Flavonoid Fractions of *C. Papaya* on Bodyweight Changes in $AlCl_3$-induced Anaemic Rats

Group	Day 0	Day 3	Day 7	Day10	Day14	Weight Change
Normal Control 1mL Distilled Water	105.67 ± 5.78^{ab}	109.33 ± 6.64^{ab}	115.00 ± 4.93^{abc}	117.67 ± 3.48^{abc}	119.33 ± 3.18^{abc}	14
$AlCl_3$ 0.5mg/kgbw only	99.66 ± 34.48^{ac}	96.67 ± 9.24^{ab}	93.33 ± 9.52^{ab}	92.00 ± 10.12^{a}	87.00 ± 7.81^{a}	-12
Positive 3mg/kgbw $FeSO_4$	116.33 ± 2.91^{abc}	120.00 ± 2.08^{abc}	123.33 ± 2.40^{abc}	128.00 ± 2.65^{abc}	129.33 ± 1.86^{abc}	13
G4:$AlCl_3$+75mg/kgbw Alkaloids seed	95.33 ± 16.15^{ac}	104.67 ± 19.38^{ab}	108.00 ± 19.86^{ab}	112.00 ± 16.46^{ab}	114.00 ± 16.16^{ab}	19
G4:$AlCl_3$+150mg/kgbw Alkaloids seed	98.66 ± 10.68^{ac}	110.67 ± 14.89^{ab}	106.67 ± 12.45^{ab}	108.67 ± 16.46^{ab}	109.33 ± 11.72^{ab}	11
G6:$AlCl_3$+75mg/kgbw Flavonoids seed	81.00 ± 7.21^{a}	87.67 ± 5.78^{ab}	90.67 ± 6.33^{a}	95.33 ± 6.57^{a}	98.00 ± 6.43^{b}	17
G6:$AlCl_3$+150mg/kgbw Flavonoids seed	72.66 ± 4.67^{c}	76.67 ± 4.91^{a}	81.33 ± 4.25^{a}	87.67 ± 6.01^{a}	89.33 ± 5.93^{a}	17

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p<0.05$).

4.1.10 Effect of alkaloid and flavonoid fractions of *Carica papaya* seeds on packed cell volume, haemoglobin and white blood cells of $AlCl_3$-induced anaemic rats

The result of Alkaloid and Flavonoid fractions of *C. papaya* on packed cell volume (PCV), haemoglobin (Hgb) and red blood cells (RBC) in $AlCl_3$-induced anaemic rats are presented in (Figure 4.5). The PCV (26.50±3.50 %), Hgb (8.55±1.25 g/dL) and RBC (5.05±1.25 10^{12}/L) count were not significantly ($P>0.05$) different in negative control group (rats that received only $AlCl_3$) when compared to the PCV (23.67±3.28 %), Hgb (5.17±1.09 g/dL) and RBC (4.10±1.09 10^{12}/L) of the group treated with flavonoid fraction of *C. papaya* seeds. However, PCV (40.00±2.00 %), Hgb (13.35±0.65 g/dL) and RBC (7.05±0.15 10^{12}/L) count were significantly ($p<0.05$) higher in normal control when compared with $AlCl_3$ induced untreated rats and group treated with flavonoid fraction of *C. papaya.*

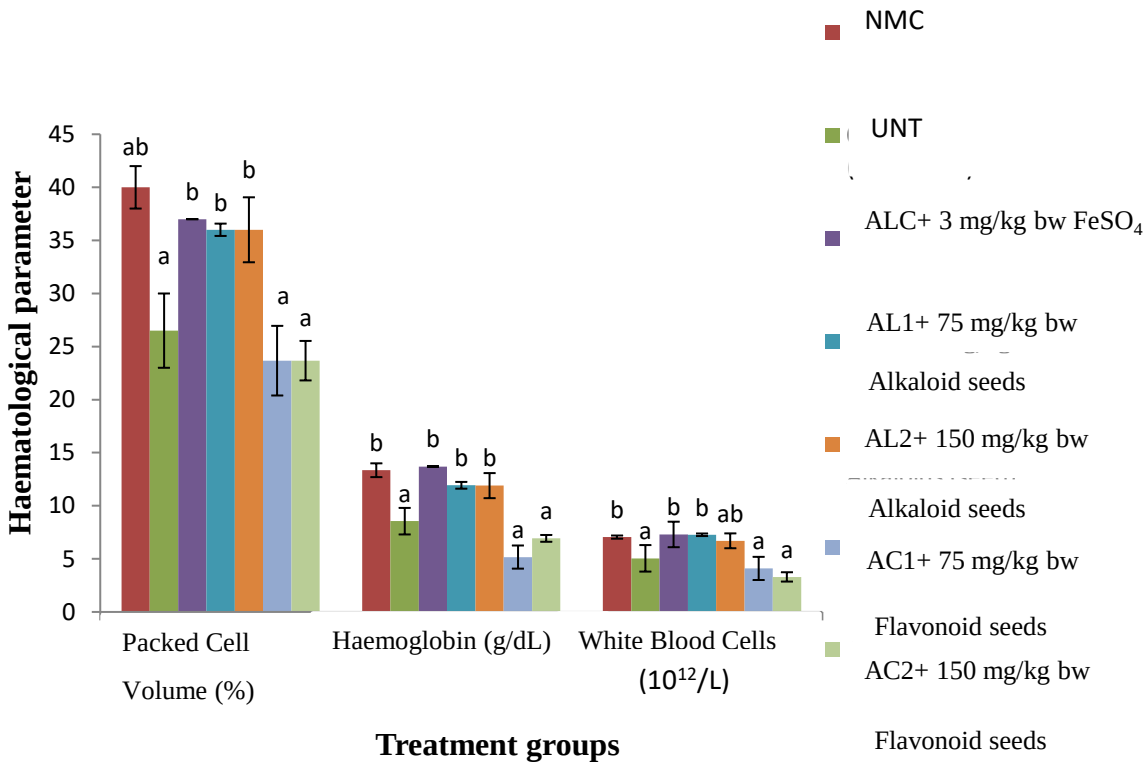

Figure 4.5: Effect of Alkaloid and Flavonoid of *C. papaya* Seeds Extract on Red Blood Cells (RBC), Haemoglobin (Hgb) and White Blood Cells (WBC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;
NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid seeds extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid seeds extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid seeds extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid seeds extract.

4.1.11 Effect of alkaloid and flavonoid fractions of *Carica papaya* seeds on red blood cells indices of aluminium chlorid-induce anaemic rats

The result of alkaloid and flavonoid fractions of *C. papaya* seeds extracts on mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin counts (MCHC) in $AlCl_3$-induced anaemic rats are presented in (Figure 4.6). MCH (18.33±0.33 pg) were not significantly ($p<0.05$) different in all experimental group when compared with normal control (19.00±0.00 pg). Rats that dosed $AlCl_3$ only (untreated group) reveal significant ($p<0.05$) decrease in MCHC (27.05±2.90 g/dL) when compared with normal control (38.50±0.50 g/dL). However, the MCH (18.33±0.33 pg) had no significant ($p>0.05$) different in all experimental groups when compared with normal control group (19.00±0.00 pg). MCV (84.00±4.00 fL) was significantly higher in group treated with alkaloid and flavonoid fractions of *C. papaya* seed when compared with $AlCl_3$ administered rats (52.00±1.00 fL) and normal control group (47.00±1.00 fL).

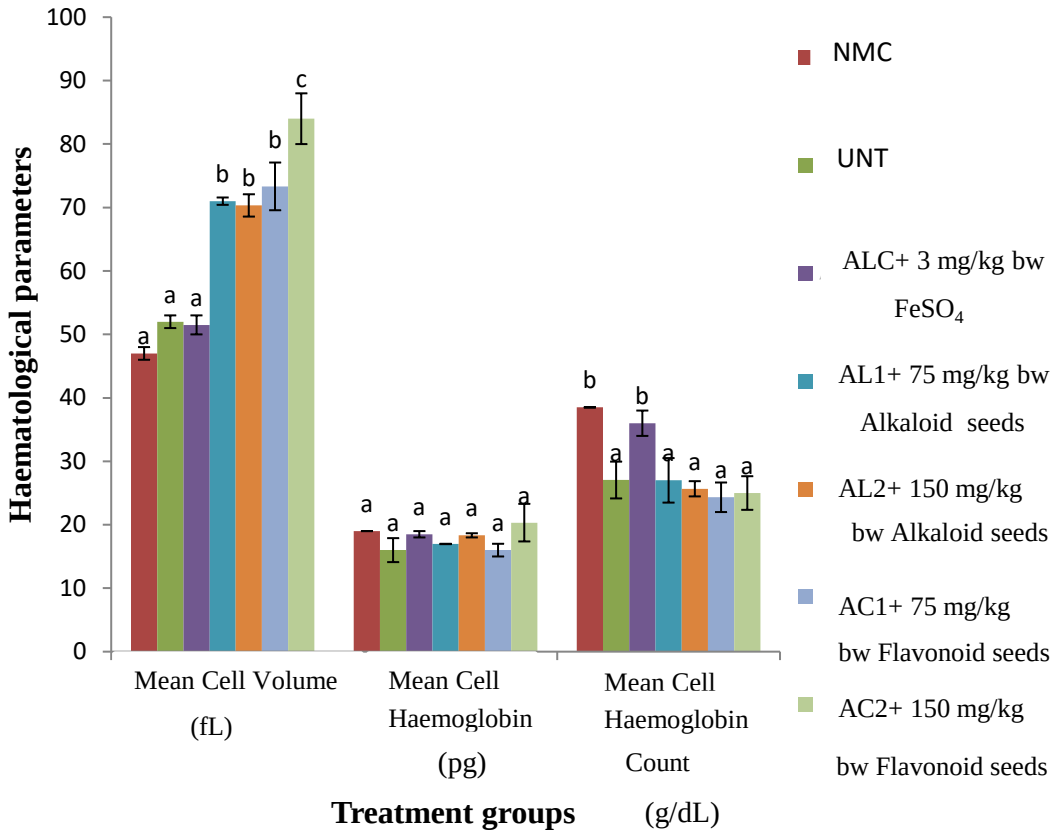

Figure 4.6: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Seeds Extracts on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid seeds extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid seeds extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid seeds extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid seeds extract.

4.1.12 Effect of alkaloid and flavonoid fractions of *Carica papaya* seeds on haematological parameters of aluminium chloride-induced anaemic rats

The effect of alkaloid and flavonoid fraction of *C. papaya* seed extracts on white blood cell (WBC), neutrophils (N), lymphocytes (L) and red blood cell wide count (RDWC) in $AlCl_3$ induced anaemic rats are presented in (Figure 4.7). WBC (8.50±0.31 10^9/L), neutrophils (21.00±1.00 %), lymphocytes (67.00±1.15 %) and RDWC (65.06±2.44 10^9/L) were significantly ($p<0.05$) higher in group treated with alkaloid fraction of *C. papaya* seed when compared with normal control WBC (6.45±0.35 10^9/L), neutrophils (13.00±3.00 %), lymphocytes (51.50±1.50 %) and RCDWC (18.15±0.00 10^9/L) and the untreated group WBC (2.50±0.60 10^9/L), neutrophils (11.00±0.10 %), lymphocytes (16.50±0.50 %) and RCDWC (37.60±0.60 10^9/L). WBC reveal insignificant ($p>0.05$) difference in group treated with flavonoids fraction of *C. papaya* seed when compared with normal control and induced untreated group. Neutrophils (26.66±1.20 %), Lymphocytes (46.33±1.11 %) and RDWC (58.23±5.46 10^9/L) reveal significant ($p<0.05$) difference in some group treated with flavonoids fraction of *C. papaya* seeds when compared with $AlCl_3$ induced rats neutrophils (11.00±0.10 %), lymphocytes (16.50±0.50 %) and RCDWC (37.60±0.60 10^9/L) and normal control group neutrophils (13.00±3.00 %), lymphocytes (51.50±1.50 %), RDWC (18.15±0.00 10^9/L).

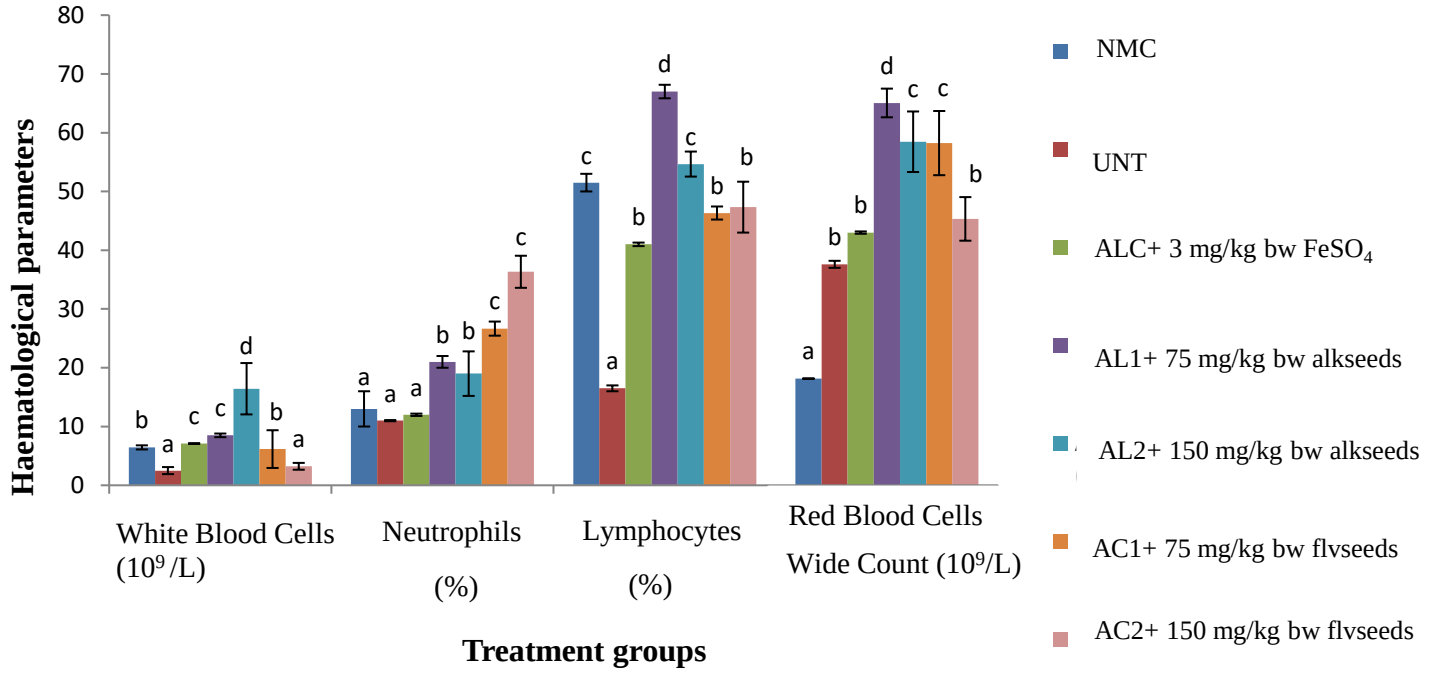

Figure 4.7: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Seed Extracts on White Blood Cell (WBC), Neutrophils (N), Lymphocytes (L) and Red Blood Cell Wide Count (RDWC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid seeds extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid seeds

extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid seeds extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid seeds extract.

4.1.13 Effect of alkaloid and flavonoid fractions of *Carica papaya* seeds platelets count of aluminium chloride-induce anaemic rats

The result of alkaloid and flavonoid fractions of *C. papaya* seeds extracts on Platelet count in $AlCl_3$-induced anaemic rats is presented in (Figure 4.8). The $AlCl_3$-induced untreated group exhibited insignificant ($p>0.05$) different in platelet count ($434.00\pm30.00\ 10^9$/L) when compared with 150mg/kg body weight ($441.00\pm10.69\ 10^9$/L) flavonoids fraction of *C. papaya* seeds. However, $AlCl_3$ only administered rats reveal significant ($p<0.05$) decrease in platelet count ($434.00\pm30.00\ 10^9$/L) when compared to normal control ($738.00\pm53.00\ 10^9$/L) and extract treated group ($608.33\pm69.34\ 10^9$/L). Platelet count was significantly ($p<0.05$) higher in normal control when compared with all experimental groups.

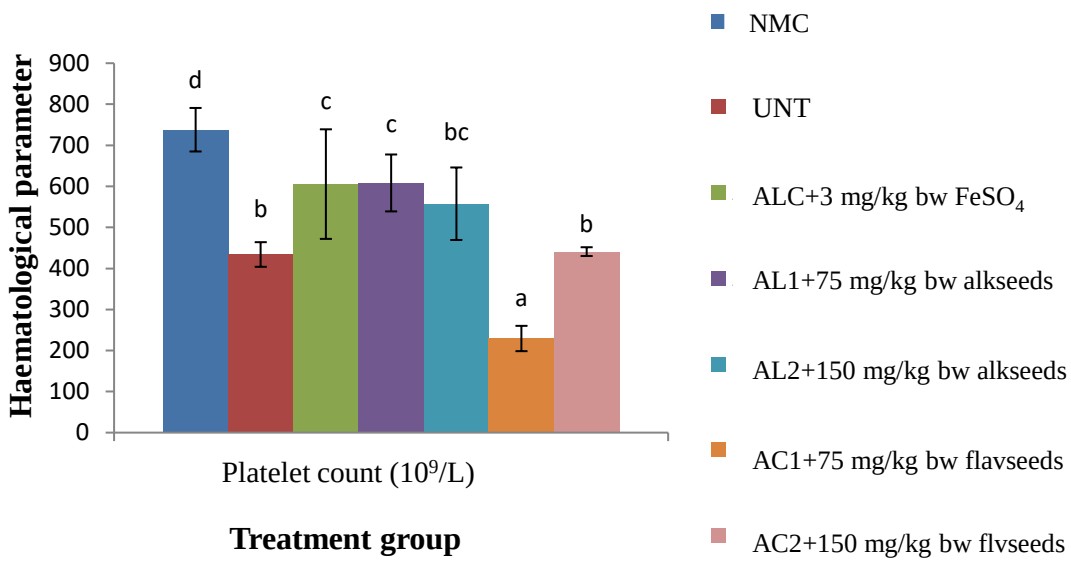

Figure 4.8: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Seeds Extracts on Platelet Count (PLC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid seeds extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid seeds extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid seeds extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid seeds extract.

4.1.14 Effect of alkaloid and flavonoid fractions of *Carica papaya* leaves on changes bodyweight of $AlCl_3$-induced rats

The result of bodyweight changes in rats treated with alkaloid and flavonoid fractions of *C. papaya* leaves in $AlCl_3$-induced anaemic rats are presented in (Table 4.10). $AlCl_3$ induced untreated group showed significant ($P<0.05$) decrease in weight (87.00±7.81) compared with normal control (119.33±3.18) and extract treated groups (129.33±1.86). The normal control group (119.33±3.18) and the extract group (129.33±1.86) showed significant ($P<0.05$) increase in body weight when compared with the $AlCl_3$ only administered rats (87.00±7.81).

Table 4.10: Effect of Alkaloid and Flavonoid Fractions of *C. Papaya* Leaves on Bodyweight Changes in $AlCl_3$-induced Anaemic Rats.

Group	Day 0	Day 3	Day 7	Day10	Day14	Weight Change
Normal Control 1ml Distilled Water	105.67 ± 5.78^{ab}	109.33 ± 6.64^{ab}	115.00 ± 4.93^{abc}	117.67 ± 3.48^{abc}	119.33 ± 3.18^{abc}	14
$AlCl_3$ 0.5mg/kgbw only	99.66 ± 34.48^{ab}	96.67 ± 9.24^{ab}	93.33 ± 9.52^{ab}	92.00 ± 10.12^{a}	87.00 ± 7.81^{a}	-12
Positive 3mg/kgbw $FeSO_4$	116.33 ± 2.91^{ab}	120.00 ± 2.08^{abc}	123.33 ± 2.40^{abc}	128.00 ± 2.65^{abc}	129.33 ± 1.86^{abc}	13
G4:$AlCl_3$+75mg/kgbw Alkaloids seed	95.33 ± 16.15^{ac}	104.67 ± 19.38^{ab}	108.00 ± 19.86^{ab}	112.00 ± 16.46^{ab}	114.00 ± 16.16^{ab}	19
G4:$AlCl_3$+150mg/kgbw Alkaloids seed	98.66 ± 10.68^{ac}	110.67 ± 14.89^{ab}	106.67 ± 12.45^{ab}	108.67 ± 16.46^{ab}	109.33 ± 11.72^{ab}	11
G6:$AlCl_3$+75mg/kgbw Flavonoids seed	81.00 ± 7.21^{a}	87.67 ± 5.78^{ab}	90.67 ± 6.33^{a}	95.33 ± 6.57^{a}	98.00 ± 6.43^{b}	17
G6:$AlCl_3$+150mg/kgbw Flavonoids seed	72.66 ± 4.67^{c}	76.67 ± 4.91^{a}	81.33 ± 4.25^{a}	87.67 ± 6.01^{a}	89.33 ± 5.93^{a}	17

Values are presented in Mean ± Standard Error of three replicate determinations. Values with different superscript alphabets on the same colunm are significantly different ($p<0.05$).

4.1.15 Effect of alkaloid and flavonoid fractions of *Carica papaya* leaves on packed cell volume, haemoglobin and red blood cell of rats

The result of alkaloid and flavonoid fractions of *C. papaya* leaves on packed cell volume (PCV), haemoglobin (Hgb) and red blood cells (RBC) in $AlCl_3$-induced anaemic rats are presented in (Figure 4.9). The PCV (40.00±2.00 %), Hgb (13.35±0.65 g/dL) and RBC (7.05±0.15 10^{12}/L) count were significantly ($p<0.05$) higher in normal control group when compared with $AlCl_3$-induced and untreated group PCV (26.50±3.50 %), Hgb (8.55±1.25 g/dL) and RBC (5.05±1.25 10^{12}/L). However, The PCV, Hgb and RBC in normal control group were not gnificantly ($p>0.05$) difference when compared with those treated with alkaloid fraction of *C. papaya* leaf and 150 mg/kg body weight flavonoid fraction of *C. papaya* leaves. The PCV, Hgb and RBC count in $AlCl_3$ induced and untreated rats PCV (26.50±3.50 %), Hgb (8.55±1.25 g/dL) and RBC (5.05±1.25 10^{12}/L) were not significantly ($p>0.05$) different from those treated with flavonoid fraction of *C. papaya* leaves (29.66±2.40 %), Hgb (9.53±0.93 g/dL) and RBC (5.26±0.73 10^{12}/L).

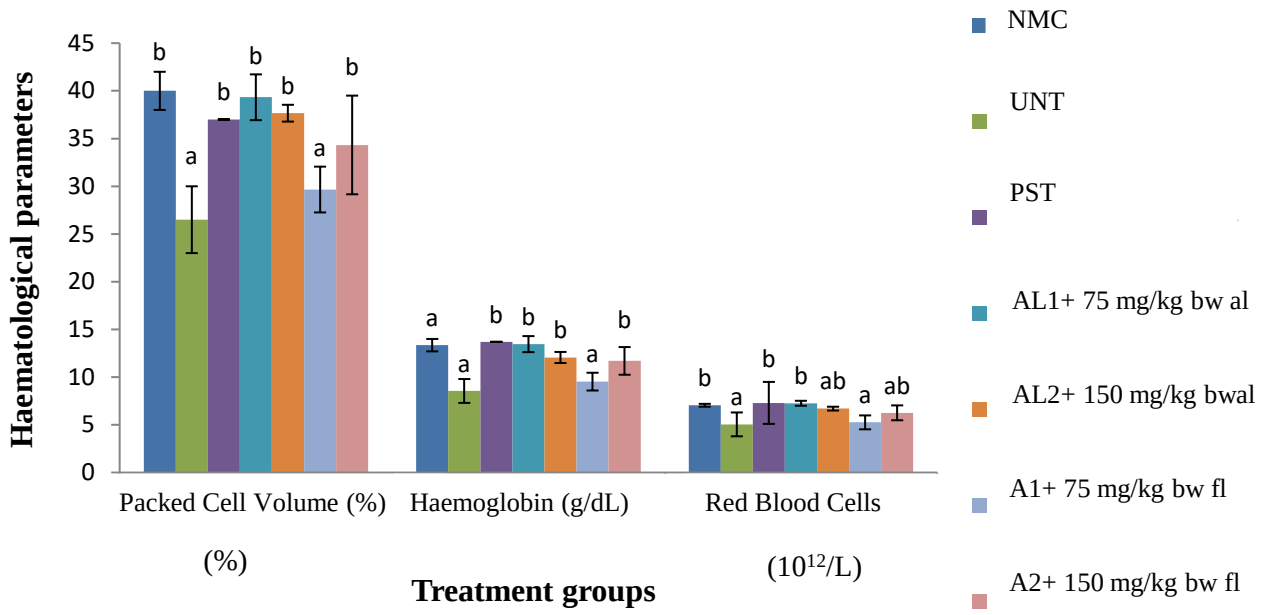

Figure 4.9: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves on Packed Cell Volume (PCV), Haemoglobin (Hgb) and Red Blood Cells (RBC) in $AlCl_3$-induced Anaemic Rats

Values are presented in Mean ± Standard Error of three replicate determinations.Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid leaves extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid leaves extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid leaves extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid leaves extract.

4.1.16 Effect of alkaloid and flavonoid fractions of *Carica papaya* leaves on haematological parameters of aluminium chloride-induce anaemic rats

The result alkaloid and flavonoid fractions of *C. papaya* leaves on white blood cell (WBC), lymphocytes (L), neutrophils (N) and red blood cell wide count (RDWC) in $AlCl_3$-induced anaemic rats are presented in (Figure 4.10). WBC (13.06±1.15 and 10.73±3.72 10^9/L), lymphocytes (68.33±3.28 and 57.00±1.53 %), neutrophils (20.33±2.85 and 60.33±17.17 %) and RDWC (51.93±0.62 and 46.00±0.58 10^9/L) were significantly ($p<0.05$) higher in rats treated with alkaloid and flavonoid leaves fractions at all concentration tested when compared with $AlCl_3$ untreated rats WBC (2.50±0.60 10^9/L), lymphocytes (16.50±0.50 %), neutrophils (11.00±0.10 %) and RDWC (37.60±0.60 10^9/L) and normal control group WBC (6.45±0.35 10^9/L), lymphocytes (51.50±1.50 %), neutrophils (13.00±3.00 %) and RDWC (18.15±0.00 10^9/L). However, rats that dosed $AlCl_3$ only in showed significant ($p<0.05$) difference in WBC when compared to normal and extract treated groups. The rats that dosed $AlCl_3$ only in lymphocytes and neutrophils were significantly ($p<0.05$) difference when compared to extract treated groups and normal control. However, the rats that dosed $AlCl_3$ only showed that there was no significant ($p>0.05$) difference in RDWC when compared with extract treated groups, the RDWC were significantly ($p<0.05$) lower when compared with the normal control group.

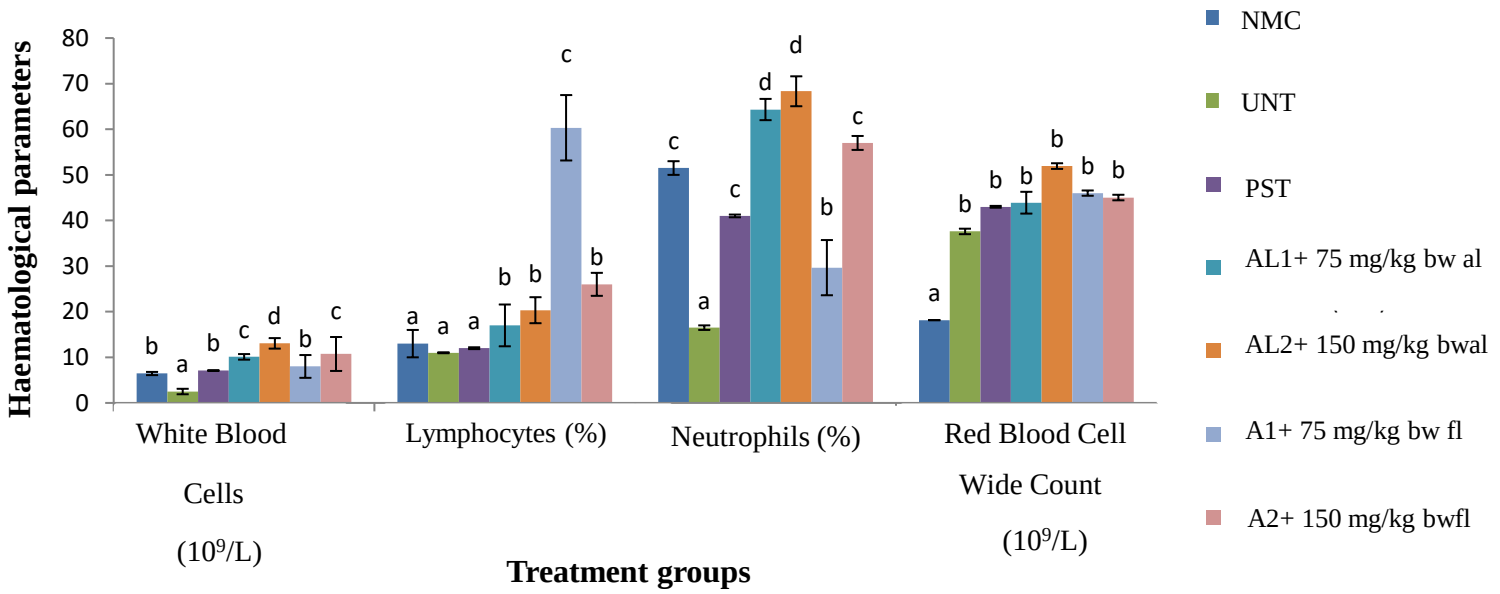

Figure 4.10: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves on White Blood Cell (WBC), Lymphocytes (L), Neutrophils (N) and Red Blood Cell Wide Count (RDWC) in $AlCl_3$-induced Anaemic Rats

Values are presented in mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid leaves extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid leaves extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid leaves extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid leaves extract.

4.1.17 Effect of alkaloid and flavonoid fractions of *Carica papaya* leaves on red blood cells indices of aluminium chloride-induce anaemic rats

The result of alkaloid and flavonoid fractions of *C. papaya* leaves extracts on mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin counts (MCHC) in $AlCl_3$ induced anaemic rats are presented in (Figure 4.11). MCV (67.33±2.73 and 71.00±1.53 fL) was significantly ($p<0.05$) higher in rats treated with alkaloid and flavonoid fractions of *C. papaya* leaves when compared with normal control (47.00±1.00 fL) and $AlCl_3$ induced untreated groups (52.00±1.00 fL). MCH (19.33±0.88 and 20.66±1.76 pg) were not significantly ($p>0.05$) different in all extract treated groups when compared with normal control group (19.00±0.00 pg). However, MCHC (38.50±0.50 g/dL) was significantly ($p<0.05$) higher in normal control group when compared with all extract treated groups (31.33±2.33 and 29.33±1.76 g/dL) and $AlCl_3$ induced untreated group (31.00±1.00 g/dL).

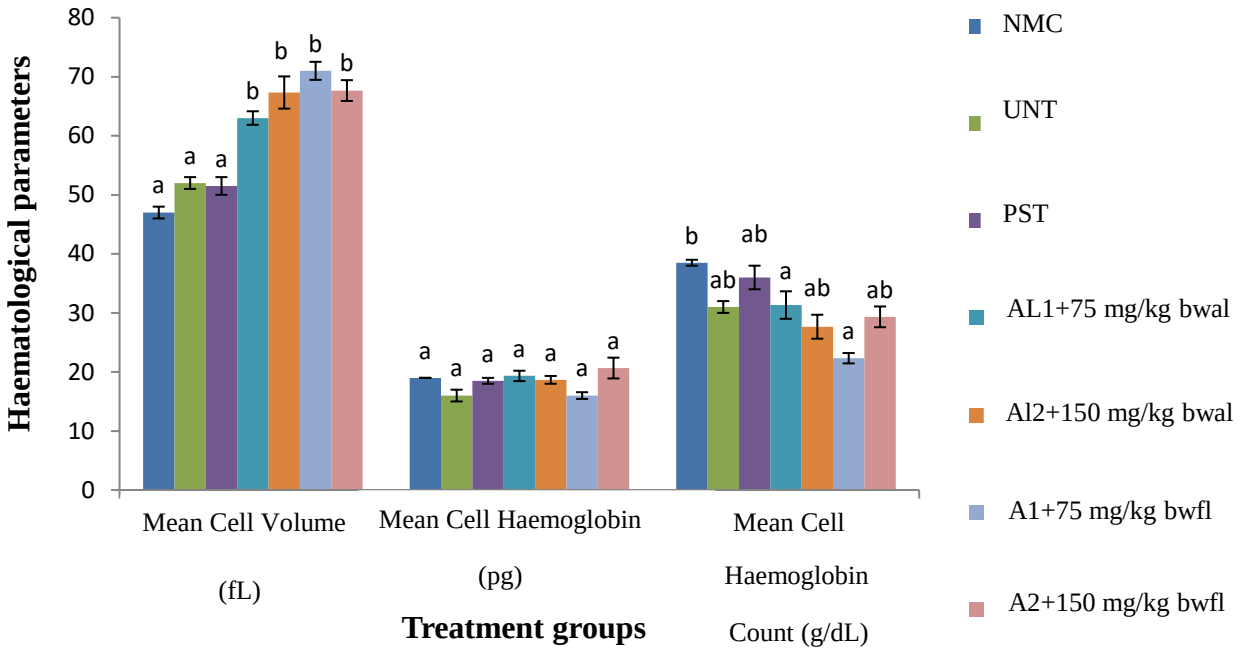

Figure 4.11: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves Extracts on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in $AlCl_3$-induced Anaemic Rats

Values are presented as mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$)

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid leaves extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid leaves extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid leaves extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid leaves extract.

4.1.18 Effect of alkaloid and flavonoid fractions of *Carica papaya* leaves on platelets count of aluminium chloride-induce anaemic rats

The result of alkaloid and flavonoid fractions of *C. papaya* leaves on Platelet count in $AlCl_3$-induced anaemic rats is presented in (Figure 4.12). Platelet count (738.00±53.00 10^9/L) was significantly ($p<0.05$) higher in normal control group when compared with extract treated groups (619.33±119.90 and 439.00±74.50 10^9/L) and $AlCl_3$ induced untreated group (434.00±30.00 10^9/L). However, Rats that dosed $AlCl_3$ only reveal significant ($p<0.05$) decrease in platelet count (434.00±30.00 10^9/L) when compared with (619.33±119.90 10^9/L) group treated with 150mg/kg body weight alkaloids fraction of *C. papaya.*

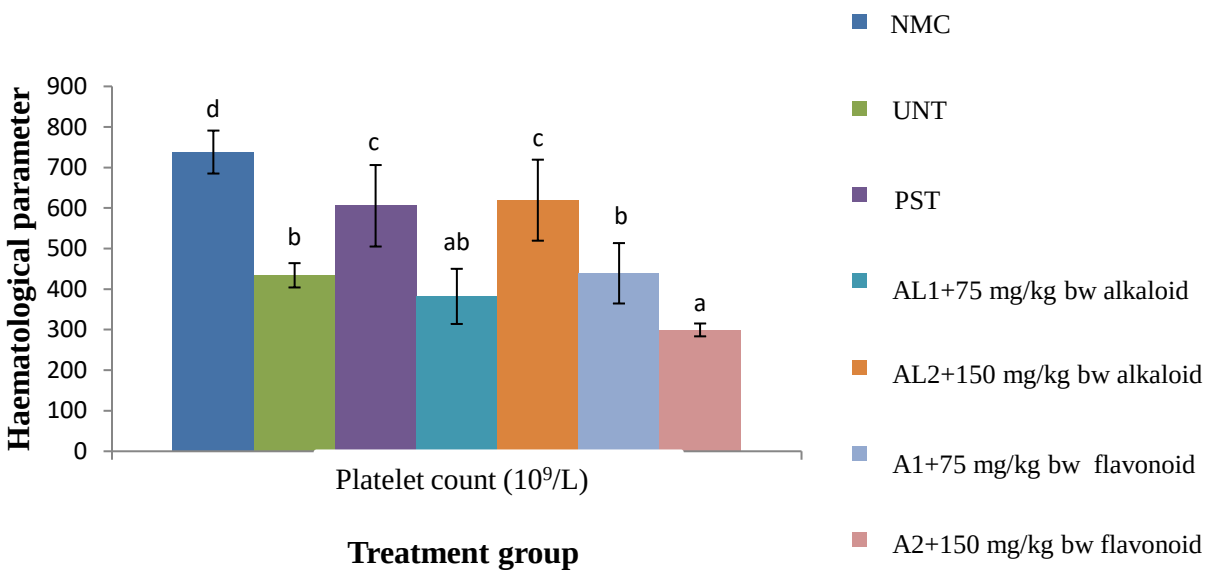

Figure 4.12: Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves on Platelet Count (PLC) in $AlCl_3$-induced Anaemic Rats

Values are presented in mean ± Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p<0.05$)

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT;Group of rats that received 0.5 mg/kg bw $AlCl_3$ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 3 mg/kg bw $FeSO_4$; AL1; Group of rats that recived 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw alkaloid leaves extract; AL2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw alkaloid leaves extract; AC1;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 75 mg/kg bw flavonoid leaves extract; AC2;Group of rats that recieved 0.5 mg/kg bw $AlCl_3$ and treated with 150 mg/kg bw flavonoid leaves extract.

4.2 Discussion

Medicinal plants contain substances that can be used for therapeutic purposes or contains precursors for the synthesis of useful drugs (Ogamba *et al*., 2018). It was reported that crude material of medicinal plant contains significant quantity of phytochemicals with several medicinal properties; these phytochemicals have also been reported to exert therapeutic effect and maintain good health (Ogamba *et al*., 2018).

The results of the present study showed that *Carica papaya* extracts contain alkaloids, flavonoids, saponins, phenols and tannins in the leaves and seed of *C. papaya*. The same observations were reported by Ayoola *et al.* (2010) and (Doughari, 2017) which found out that the leaves of papaya contained saponins, phenols, cardiac glycosides and alkaloids. This study also attest to the report of Akhila *et al.* (2015) which found phyto compounds like alkaloids, phenolics, flavonoids and also amino acids in *C. papaya* leaves. This study also matched the results obtained by previous studies of (Ikeyi *et al.*, 2013; Marshall *et al.*, 2015 and Bhushan and Mrina 2016) which reported that *C. papaya* leaves contained anthraquinones, phenols, glycoside amino-acid, terpenoid, reducing sugar, saponin, tannin, alkaloids and flavonoids. These phytochemicals could be responsible for the medicinal potentials and dietary values of *C. papaya.* On the other hand, the findings of the present study agree with the findings of Umar *et al*. (2018) in which the seed material of *C. papaya* was found to contain alkaloids, saponins, flavonoids, tannins and reducing-sugar.

As reported by Odebiyi and Sofowora (2008) and Swarna and Ravindhran (2013), alkaloids are nitrogen-containing naturally occurring bioactive compounds, commonly known to have antimicrobial properties due to their ability to intercalate with DNA of

microorganisms (Kasolo *et al*., 2017). The intercalating effect of alkaloids on DNA has led to the development of anti-microbial drugs. They exhibit their bioactive activity even at very low concentrations, and many are widely used as drugs such as cocaine, morphine, atropine, colchicines, quinine, and strychnine. Alkaloid has been used as stimulant for central nervous system, anaesthetic in ophthalmology, pain relievers, anti-puretic agents, among other uses (Heikens *et al*., 2015).

As reported by Odebiyi and Sofowora (2008), flavonoids are essential biological antioxidant having the ability to mediate against the deleterious effects of reactive oxygen species such as singlet oxygen, superoxides, peroxyl radicals, hydroxyl radicals and peroxynitriles. Low antioxidants to reactive oxygen species ratio may result in oxidative stress in cells, leading to cellular damage. Oxidative stress has been linked to various inflammatory diseases (Palozza, 2018). The flavonoid content of *c. papaya* in this study could mean that it could be used in the prevention and management of diseases resulting from oxidative stress such as cardiovascular diseases, atherosclerosis, chronic ulcer, arthritis, ischemic injury and neuro-degenerative diseases Burlon and Ingold (2014). Several studies have also shown that flavonoids such as genistein and procyanidin B5 can protect Low-density Lipoproteins (LDL) from being oxidized Donald and Cristobal (2016). The oxidation of LDL has been recognized to play an important role in the onset of atherosclerosis which lead to immune system cells macrophages recognition and engulfing of oxidized LDL, a process that leads to the formation of atherosclerotic plagues in the arterial wall.

As reported by Odebiyi and Sofowora (2008), the high phenol contents of *C. papaya* leaves extract may be relevant in treatment of ailments emerging from microbial contamination,

since phenols and phenolic compounds have been extensively used as disinfectant and remain the standard for other bactericides (Akinyeye *et al*., 2014). Phenols are also known to have scavenging activity due to their hydroxyl group (Okwu, 2014).

As reported by Gabriel and Donald (2017), the variation observed in their results when compared to the result obtained from the present study may be due to environmental factors such as climate, soil type and harvesting period. Saponins are glycosides with distinctive foaming characteristics (Judd *et al*., 2019). They are natural detergents found in many plants (Ajali, 2014). Saponins have both antibacterial and antifungal properties and are used extensively in cosmetics, such as lipsticks and shampoo. The high saponins content observed in the present study may be responsible for the foaming characteristics of the extract. Saponins have been reported to have cholesterol lowering potential. Studies have shown its role in killing cancer cells, bone health and stimulation of the immune system (Price *et al*., 2017; Oakenful and Sidhu 2019). Saponins inhibit Na^+ efflux by the blockage of the entrance of Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+-Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} in flux through this anti porter helps to strengthen the contractions of heart muscle Schneider and Woliling (2014).

As reported by Akindahunsi and Salawu (2015), tannins have been reported to be effective in curbing hemorrhages as well as joint swellings. While tannins are said to be haemostatic, they are also beneficial when applied on mucosal coating in mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches (Akinyeye *et al*., 2014). When applied internally, tannins affect the walls of the stomach and other digestive parts. They sour the mucus secretions and contract or squeeze

the membranes in such a manner that secretions from the cells are restricted. Tannins have been proven to be harmful to both man and farm animals and some are species specific Odebiyi and Sofowora (2008). Tannins have been demonstrated to possess anti nutritional effects, following their ability to reduce palatability and digestibility of feedstuff Odebiyi and Sofowora (2008).

One major and overriding criterion in the selection of herbal medicine for use in health services is safety. Plant extracts should not only be efficacious but safe for consumption. Safety of long-term use or consumption of medicinal plants is becoming important as most of this preparation will be for general health and will be used for long term duration. In order to elucidate such information, a proper toxicological evaluation is carried out in various experimental animal models to predict toxicity and to select a safe dose for human use. Subsequent to that, the safety data on human subject should be conducted through the various phases of clinical trial.

Crude extract of *C. papaya* leaves and seed showed high safety margin since no animal died within 24 hours after treatment with the extracts. This implies that *C. papaya* are not toxic to the animals at the dose tested. The major signs of toxicity noticed within 24 hours included rubbing of the mouth on the surface of the cage and hyperactivity. This finding agrees with the findings of Sunil *et al*. (2012) which reported that *C. papaya* did not show any toxic effects in rats when treated with 5000 mg/kg body weight extract (Halim *et al.*, 2011; Sunil *et al*., 2012).

According to (Lorke's, 1983), any substance that does not result to mortality at 5000 mg/kg body weight is safe and non-lethal. The implication of this is that *C. papaya* seeds and

leaves extract should be non-toxic. According to Clarke and Clarke (2007), any compound or drug with the oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe. This finding of this study agrees with the study of Ismail *et al.* (2014) which reported that *C. papaya* leaf extract for 13 weeks at a dose up to fourteen times the levels employed in traditional medicine practice did not cause any significant toxic effect. Findings from this study also agree with Halim *et al.* (2011). Which showed the acute toxicity study of *C. papaya* leaves juice in rats showed dehydration as demonstrated by an increase in red cell mass, However, LD_{50} has not been considered as a biological constant because many variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the $LD_{50\ value}$ obtained. Hence, there are considerable uncertainties in extrapolating the $LD_{50\ value}$ obtained for a species to other species (Zbinden and Roversi 2013; Devaki *et al.*, 2017)

The administration of the leaves and seeds extracts of *C. papaya* ameliorated the effect of aluminium induced toxicity as shown in. The increases observed in the body weight gain of treated rats in comparison with anemic rats could be due to the presence of amino acids, vitamins and minerals particularly iron found in the leaf and seed extract of *C. papaya* (Bhushan, 2016). The gain in weight after administration of the extract as observed in this study is in agreement with the findings of Opasich *et al*. (2015). This gain in weight can be explained by the high content of some bioactive phytochemicals in *C. papaya*. The leaf and seed extracts of *C. papaya* are rich in flavonoids and saponins. Flavonoids are essential biological antioxidant having the ability to mediate against the deleterious effects of reactive oxygen species such as singlet oxygen, superoxides, peroxyl radicals, hydroxyl radicals and peroxynitriles (Oloyede, 2009), while saponins have the ability to stimulate

immune response against any form of toxicant (Price *et al*., 2017, Oakenful and Sidhu, 2019). It has been reported by Shackley and Sohi (2019), that flavonoids can exhibit anti-oxidant activity in aluminium induced oxidative stress.

The group of rats administered with $AlCl_3$ without treatment had significant ($p<0.05$) loss in weight when compared to the normal control and extract treated group. This observation is in agreement with the findings of Al-hashem *et al*. (2018) which found out that Alminium-chloride caused impaired erythrocytes deformities and oxidative damage from free radicals. This significant loss in weight could also be linked to aluminium induced oxidative stress which may result in free radical mediated cytotoxicity and reduced cholinergic function Shackley and Sohi (2019). Salts of aluminium may inhibit enzymes such as hexokinase, acid and alkaline phosphatase, phosphodiesterase and phosphooxydase and consequently results in tissue wasting Scholl and Hediger (2014). The decrease of the body weight of rats is an indication of anaemia, this may be due to lack of appetite in anemic rats. This observation is in agreement with the finding of (Aurelie *et al.*, 2015). The reduction in the body weight of anemic rats has been reportedly linked with the decrease in dissacharidases (Sucrase and lactase enzymes that catalyzed the last stage of carbohydrate digestion) activities in anemic rats (Viera *et al.*, 2015).

Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status (Muhammad *et al.*, 2018). Assessment of haematological parameters can be used to determine the toxic effects of xenobiotics including plant extracts on the blood constituents of an animal (Berinyuy *et al.*, 2015). Such analysis is relevant to risk evaluation because changes in the haematological system are highly predictive for human toxicity, when data are translated from animal

studies (Shatoor, 2015). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu *et al.,* 2012).

Several mechanisms have been proposed for the aluminum-induced anemia. But the precise mechanism of aluminum-induced anemia is unknown. The proposed mechanism appears to involve inhibition of heme synthesis, either by inhibition of enzyme activity, or interference with iron incorporation or utilization (Kaiser and Shwartz 2015; Ganchev *et al.,* 2016).

The administration of $AlCl_3$ in rats without treatment resulted in significantly lower haematological parameters. The significant decrease in RBC, hemoglobin concentration and PCV in anemic group could be due to toxicity caused by $AlCl_3$ which may involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization (Ganchev *et al.,* 2016). Some of these abnormalities might also be due to destruction of matured red blood cells leading to the low Hb counts accompanied by the fall in the RBC and PCV Muhammad and Oloyede (2013). However, aluminum-induced haematolotgical alterations in MCV, MCH and MCHC. The significant ($P<0.05$) decrease in MCV and MCH indicated that the rats established microcytic hypochromic anemia. This study is similar to previous reports on the same blood parameters in rats (Zaman *et al.,* 2013; Chmielnicka *et al.,* 2014; Savage *et al.,* 2018).

The reduction observed in RBC count, Hgb and PCV in the groups of rats administered with $AlCl_3$ may be as a result of the ability of aluminium to promote oxidation of Fe^{2+} to Fe^{3+}. Aluminium ion (Al^{3+}) may replace Ferric ion (Fe^{3+}) during haemoglobin formation Omoregie and Osagie (2017). The alterations in RBC, Hgb and PCV might be as a result of destruction of matured red blood cells leading to the lowering of Hgb concentration and

PCV because Hgb and PCV are directly linked to RBC (Rang *et al.*, 2017). Aluminium has also been reported to inhibit ferrochelatase; a hemesynthetase, the mitochondrial enzyme involved in the final step in the heme biosynthetic pathway (Rang *et al.*, 2017). This also supports the work of Ameh and Alafi (2018), in which $AlCl_3$ decreased Hgb, RBC and PCV levels. The features of aluminium induced anaemia are consistent with a defect in hemoglobin synthesis. This may be due to the inhibition of erythrocyte ∂-aminolevulinic acid dehydratase by aluminium ion Preeti and Shalini (2014). The catalytic activity of ∂-aminolevulinic acid dehydratase has been shown to be considerably reduced in patients on hemodialysis and in anaemic patients Preeti and Shalini (2014). Omoregie and Osagie (2017) reported that aluminum exerts an inhibitory action in uroporphyrinogen decarboxylase, another step in the heme biosynthetic pathway. Aluminium is reported to increase lipid peroxidation and decreases the activities of antioxidant enzymes resulting in free radical generation (Newairy *et al*., 2019). This also agrees with an earlier report observed by Fairbark (1967), that xenobiotics can cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane is oxidized which inflicts injury to the erythrocyte membrane.

However, the administration of the leaf and seed extract of *C. papaya* improved the effect of aluminium induced. However, in the extract treated group, there was a significant ($p<0.05$) increase in the haematological parameters when compared to the anemic group. The significantly higher RBC count, PCV and haemoglobin concentration in treated groups compared to non-treated group as observed in this study may be due to the phyto constituents present in *C. papaya* leaf and seed extract which are well known haemopoietic factors that have indirect influence on blood production (Beltowski *et al*., 2015).

Haematopoietin are hormone that stimulate the released of a proptein (erythropoietin) into the kidney which is the humoral regulator of RBC production (Beltowski *et al.*, 2015). Haematopoietin increase the number of erythropoietin committed stem cells in the bone marrow that are converted to RBCs and then to matured erythrocytes (Ganong, 2012). *C. papaya* was reported to rank among the first fruits for vitamin C, vitamin A, riboflavin, foliate, calcium, thiamine, iron, niacin, potassium and fibre and may probably be responsible for *C. papaya* conferring erythropoietic properties on the treated rats Ayoola and Adeyeye (2010). *C. papaya* has also been reported to confer protective effect on haematological components during CCl_4 intoxication (Sule *et al.*, 2016).

Saponins are also known to inhibits platelet aggregation and thrombosis. Saponin containing herbs have been successfully used in the management of liver inflammation, as tonic sedative formula and to promote and vitalize blood circulation (Wang *et al.*, 2014; Singh *et al.*, 2016).

Since saponins are membrane active agents that's lyse RBCs and other wall, it is possible that the RBCs were initially lysed by this plant; the cells overcome this inhibition by producing glycosidic enzyme which cleaves some of the terminal's sugars from the saponin, thereby detoxifying it (Pathirana *et al.*, 2017). This detoxification of saponins enhanced the proper utilization of the iron contained in the extract of *C. Papaya* to synthesize haemoglobin (haeme) for new RBCs thus leading to an improved Hb, PCV and RBC.

Earlier report made by Oladunmoye and Osho (2017), revealed that the higher values of these haemological indices in rats treated with *C. papaya* may be due to the inability of $AlCl_3$ to cause haemolysis resulting from the anti-inflammatory potentials inherent in the

herbs. Findings from this study is also in agreement with the earlier reports by Oladunmoye and Osho (2017) which showed that Hb and PCV levels increased significantly in rats orogastrically dosed with Salmonellela typhi and Staphyloccus aureus and treated with ethanolic leaf extract from *C. papaya.*

The significant ($p<0.05$) increase values of lymphocytes in rats treated with *C. papaya* when compared with rats administered with $AlCl_3$ only may go a long way to suggests that this plant may have influenced the defense mechanism of the test rats. So, the continuous exposure of the body systems of animals to *C. papaya* may cause lymphocytosis, which may then account for the use of this plant for medicinal purposes (Keenwe *et al.,* 2016).

The significant decrease in white blood cell count of the negative control group (rats administered $AlCl_3$ only without treatment) might have resulted from deactivation of the immune system or decreases in normal cell-mediated immune response (El-Demerdash, 2014). This may imply a reduction in the ability of the rats to resist infection. There was an increase in the levels of WBC in the extract treated group which suggest that the extract possess some potentials that are capable of boosting the immune system in the rats (Ganong, 2010).

The result of this study also showed a decrease in the platelet count of the anemic group when compared with the normal control group. This suggests that the process of clot-formation (blood coagulation) will be delayed resulting in excessive loss of blood in the case of injury Guyton and Hall (2012).

However, there was a significant increase ($p<0.05$) in platelet count in extracts treated groups when compared with the normal and anaemic group, thus this shows that the extract has a stimulating effect on platelet production.

According to Osman *et al.* (2012), patients with anemia caused by aluminum toxicity often have increased reticulocytes counts, decreased mean corpuscular volume, and mean corpuscular hemoglobin concentration and these disagreed with results of the index obtained in this study. The findings with MCV, MCH and MCHC in this study disagree with previous studies who reported that MCV, MCH and MCHC increases in pathological conditions like liver cirrhosis and hemolytic anemia Sembulingam and Sembulingam (2016; Murakami *et al.*, 2018). The effect of oral administration of *C. papaya* leaf extract irrespective of the dose has the tendency to increase blood parameters such as WBC, RBC, Hb, PCV etc. as well as alleviate blood disorders. Literature has shown medicinal plants with anti-anemic properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain. This is in agreement with the findings of this study. Summing these facts, it is plausible for the alkaloids and flavonoids constituents of *C. papaya* extract to be responsible for the observed biological effects.

The group of rats administered with $AlCl_3$ without treatment with the extract lost weight compared to normal control group (rats that were not pretreated and not induced) that gained weight as shown in. The weight loss which was significant could arise from the deleterious effect of the reactive oxides induced by administration of aluminium chloride Shackley and Sohi (2019).

The significant gain in weight in the experimental rats treated with alkaloids and flavonoids of *C. papaya* seed and leaves as compared to anaemic group may mean that the extract had some protective effects on the animals. This weight gain could be linked to the high concentration of flavonoids and alkaloids in the extract. It has been reported that flavonoids have antioxidant activity against aluminium induced oxidative stress (Arika *et al*., 2015).

The experimental rats administered with $AlCl_3$ without treatment with the alkaloids and flavonoids fractions of *C. papaya* leaf had significantly lower haematological parameters. This is in agreement with the findings of Olorunnisola *et al*. (2013) which reported that some of the abnormalities exhibited by rats following administration of $AlCl_3$ might be due to destruction of matured red blood cells by aluminium resulting in lower RBC, haemoglobin and PCV. This fact is also supported by the findings of Muhammad and Oloyede (2013), and (Ogbonnia *et al*., 2019) that haeme biosynthesis is impaired in aluminium induced anaemia. The reduction in RBC count and PCV may also be as a result of the hemolytic action of aluminium resulting from the replacement of Fe^{3+} by Al^{3+} during haemoglobin formation Omoregie and Osagie (2012).

Treatment with alkaloids and flavonoids fractions of *C. papaya* leaf prevented the occurrence of anaemia in the experimental groups as significantly higher RBC count, haemoglobin and PCV were obtained as compared to the non-treated group ($AlCl_3$ only administered rats). This finding is in agreement with the findings of Ezekwe *et al*. (2013), which reported that oral treatment with alkaloids and flavonoids fractions of *C. papaya* showed a tremendious organ protective potential against the incidence of the deleterious effect of anaemia following the exposure of experimental rats to $AlCl_3$. The higher haematological parameter values in the treated groups may be due to the ability of the

phytochemical constituents of *C. papaya* extract. Phytochemicals such as flavonoids and alkaloids have antioxidants properties that have direct influence on blood formation with the ability to inhibit free radical induced blood cell damages (Beltowski *et al*., 2015). Findings from this study showed that alkaloids and flavonoids leaf fractions of *C. papaya* restored the normal values of haematological indices in the anemic rats. Thus indicating that both phytochemicals are responsible for the haematopoietic properties of *C. papaya* leaf.

Results from this present study also indicated that flavonoid fraction of *C. papaya* seed administered to anemic rats at doses levels of 75 and 150 mg/kg bw had no positive effect on haematopoietic system of anemic rats. Findings from this study however suggest that flavonoids fractions of *C. papaya* seed are not a major haematopoietic stimulatory bioactive metabolite in *C. papaya* seed. However, treatment of $AlCl_3$ induced rats with 75 and 150 mg/kg alkaloid fraction of *C. papaya* seed significantly improved the hematological status of the animals to a level comparable to the normal rats. This shows that alkaloid is the major haematopoietic stimulatory bioactive metabolite in *C. papaya* seed. The higher haematological parameter values in the groups treated with 75 and 150 mg/kg alkaloid fractions of *C. papaya* seed may be due to the ability of the phytochemical constituent of *C. papaya* extract. Phytochemicals such as flavonoids and alkaloids have antioxidant properties that have direct influence on blood formation with the ability to inhibit free radical induced blood cell damages (Beltowski *et al*., 2015). Findings from this study showed that alkaloids seed fractions of *C. papaya* restored the normal values of haematological indices in the anemic rats, indicating that only alkaloids fraction of *C. papaya* seed are responsible for the haematopoietic properties of *C. papaya*.

The significant decrease in white blood cell count of the negative control group (rats administered $AlCl_3$ only without treatment) might have resulted from deactivation of the immune system or decreases in normal cell-mediated immune response (El-Demerdash, 2014). This may imply a reduction in the ability of the rats to resist infection. There was an increase in the levels of WBC in the extract treated group which suggest that the extract possess some potentials that are capable of boosting the immune system in rats (Ganong, 2010).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study revealed that the methanol extracts of *Carica papaya* leaves and seeds are rich in phyto-compounds which includes saponins (1249.83±13.05 and 723.65±0.39 mg/g) followed by phenols (261.34±1.07 and 171.45±0.91 mg/g) and alkaloids (67.75±1.06 and 35.42±0.50 mg/g) and tannins (45.25±0.46 and 40.67±0.50 mg/g) and flavonoids (34.89±0.66 and 25.80±0.99 mg/g). This makes it promising in phyto-therapy and could contribute to it uses in the protective and management of diseases resulting from oxidative stress such as anaemia.

The crude methanol extracts of *C. papaya* leaves and seeds had shown the ability to protect and restore the integrity of haemoglobin (14.35±0.45 and 13.40±0.90 g/dL), packed cell volume (47.00±0.50 and 40.00±0.00 %), and red blood cell count (10.88±0.50 and 10.66±0.85 10^{12}/L), that were compromised by $AlCl_3$-exposure including the haemoglobin (8.55±1.25 g/dL), packed cell volume (26.50±3.50 %) and red blood cell count (5.05±1.25 10^{12}/L). The alkaloid and flavonoid fractions of *C. papaya* leaves and seeds significantly improved red blood cell count (8.76±0.26 and 7.26±0.78 10^{12}/L), haemoglobin (13.46±0.84 and 10.93±0.93 g/dL), and packed cell volume (41.33±2.40 and 35.44±1.55 %) compared to the non-treated group having red blood cell count (5.05±1.25 10^{12}/L), haemoglobin (8.55±1.25 g/dL), and packed cell volume (26.50±3.50 %). Therefore, it could be speculated that the crude methanol extracts of *C. papaya* leaves and seeds, and alkaloid and flavonoid fractions of *C. papaya* leaves may contribute to the observed haemato-protective and hematinic potentials.

5.2 Recommendations

Based on the present report, the following studies are recommended to be undertaken:

i. The mode of action by which *C. Papaya* is able prevent the $AlCl_3$– toxicity should be elucidated.

ii. Further study should be carried out on *C. papaya* to investigate the heamoglobin regenerating potential of this plant in iron deficiency anaemia.

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APPENDICES

APPENDIX A

Lethal Dose Determination of Crude Methanol Extracts of *Carica Papaya*

(i) Lethal Dose Determination of Methanol Extract of *C. Papaya* Seeds in Albino Rats for 24 Hourrs (First Phase).

First phase	Dose (mg/kg)	Number of Rats	Number of Death after 24 hours	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0

LD_{50} is greater than 5000mg/kg body weight.

(ii) Lethal Dose Determination of Methanol Extract of *C. Papaya* Leaves in Albino Rats for 24 Hourrs (Second Phase).

	Dose (mg/kg)	Number of Rats	Number of Death after 48 hours	% Mortality
Group 1	1600	3	0	0
Group 2	2900	3	0	0
Group 3	5000	3	0	0

LD_{50} is greater than 5000mg/kg body weight.

APPENDIX B

Effects of Administration of Methanol Extracts of Leaves and Seeds of *Carica Papaya* on Hematological Parameters in $AlCl_3$ Induced Anaemic Rats

GROUPS PARAMETERS	HCT (%)	HGB (g/dl)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^9/L)	WBC (10^9/L)	Neutrophils (%)	Lymphocytes (%)	RDWC (10^9/L)
G1: Normal 1ml DW	43.00±2.00^{ab}	13.65±0.65^{b}	7.15±0.15^{a}	47.00±1.00^{a}	19.00±0.00^{a}	38.50±0.50^{b}	738.00±53.00^{bc}	6.45±0.35^{bc}	13.00±3.00^{ab}	51.50±1.50^{c}	18.15±0.00^{a}
G2:0.5mg/kgbw $AlCl_3$ only (Untreated)	26.50±3.50^{a}	8.55±1.25^{a}	5.05±1.25^{a}	52.00±1.00^{a}	16.00±1.00^{a}	31.00±1.00^{ab}	434.00±30.00^{ab}	2.50±0.60^{a}	11.00±0.10^{a}	16.50±0.50^{a}	37.60±0.60^{b}
G3:$AlCl_3$+3mg/kgw $FeSO_4$	40.00±0.00^{b}	13.70±0.00^{b}	7.30±0.20^{a}	51.50±1.50^{a}	18.50±0.50^{a}	36.00±2.00^{ab}	605.50±183.50^{abc}	7.10±0.00^{bc}	12.00±0.20^{a}	41.00±0.30^{b}	43.00±0.20^{ab}
G4:$AlCl_3$+100mg/kgbw (*c.papaya*.l)	42.50±0.50^{b}	13.81±0.16^{b}	10.60±0.20^{b}	58.00±3.00^{a}	49.00±29.00^{a}	36.50±0.50^{ab}	852.50±28.50^{c}	7.45±0.95^{bc}	17.00±0.40^{ab}	61.50±1.30^{e}	40.15±0.10^{ab}
G5:$AlCl_3$+300mg/kgbw (*c.papaya*.l)	40.50±0.50^{b}	13.50±1.20^{b}	10.55±0.15^{b}	82.00±20.0^{b}	48.50±29.50^{a}	33.50±1.50^{ab}	603.50±71.50^{a}	6.10±1.00^{abc}	16.50±1.30^{ab}	65.00±0.60^{f}	39.75±0.25^{ab}
G6:$AlCl_3$+500mg/kgbw (*c.papaya*.l)	47.00±0.50^{cd}	14.35±0.45^{b}	10.80±0.50^{b}	58.00±6.00^{a}	18.00±0.00^{a}	34.50±1.50^{ab}	423.50±40.00^{ab}	9.40±1.00^{c}	30.25±99.10^{b}	55.00±0.90^{d}	44.05±1.05^{c}
G7:$AlCl_3$+100mg/kgbw (*c.papaya*.s)	49.50±0.00^{d}	12.95±1.00^{b}	8.95±1.00^{b}	52.00±1.00^{a}	19.50±1.50^{a}	33.00±3.00^{ab}	384.50±1.50^{ab}	5.85±2.55^{abc}	22.00±0.70^{a}	59.00±0.10^{e}	42.75±0.35^{ab}
G8:$AlCl_3$+300mg/kgbw (*c.papaya*.s)	40.00±0.00^{b}	13.65±0.15^{b}	10.66±0.85^{b}	51.00±0.00^{a}	16.50±1.50^{a}	32.50±2.50^{ab}	556.50±284.50^{abc}	6.50±0.10^{abc}	26.50±0.00^{ab}	52.0±1.20^{cd}	39.60±2.10^{ab}
G9:$AlCl_3$+500mg/kgbw (*c.papaya*.s)	42.50±2.50^{ab}	13.40±0.90^{b}	10.22±1.00^{b}	52.00±1.00^{a}	18.50±0.50^{a}	38.50±3.50^{b}	481.50±49.50^{ab}	5.55±0.45^{ab}	17.00±0.20^{ab}	51.00±1.20^{c}	39.45±4.15^{ab}

APPENDIX C

Effects of administration of methanol extracts of Leaves and seeds of *Carica papaya* on immunological parameters in $AlCl_3$ induced anaemic rats

GROUPS PARMETERS	WBC (10^9/L)	Neutrophils (%)	Lymphocytes (%)	RDWC (10^9/L)
G1: Normal 1ml DW	6.45 ± 0.35^{bc}	13.00 ± 3.00^{ab}	51.50 ± 1.50^{c}	18.15 ± 0.00^{a}
G2:$AlCl_3$0.5mg/kgbw $AlCl_3$	2.50 ± 0.60^{a}	11.00 ± 0.10^{a}	16.50 ± 0.50^{a}	37.60 ± 0.60^{b}
G3: $AlCl_3$+3mg/kgw $FeSO_4$	7.10 ± 0.00^{bc}	12.00 ± 0.20^{a}	41.00 ± 0.30^{b}	43.00 ± 0.20^{ab}
G4:$AlCl_3$+100mg/kgbw (*c.papaya*.l)	7.45 ± 0.95^{bc}	17.00 ± 0.40^{ab}	61.50 ± 1.30^{e}	40.15 ± 0.10^{ab}
G5:$AlCl_3$+300mg/kgbw (*c.papaya*.l)	6.10 ± 1.00^{abc}	16.50 ± 1.30^{ab}	65.00 ± 0.60^{f}	39.75 ± 0.25^{ab}
G6:$AlCl_3$+500mg/kgbw (*c.papaya*.l)	9.40 ± 1.00^{c}	30.25 ± 99.10^{b}	55.00 ± 0.90^{d}	44.05 ± 1.05^{c}
G7:$AlCl_3$+100mg/kgbw (*c.papaya*.s)	5.85 ± 2.55^{abc}	22.00 ± 0.70^{a}	59.00 ± 0.10^{e}	42.75 ± 0.35^{ab}
G8:$AlCl_3$+300mg/kgbw (*c.papaya*.s)	6.50 ± 0.10^{abc}	26.50 ± 0.00^{ab}	52.0 ± 1.20^{cd}	39.60 ± 2.10^{ab}
G9:$AlCl_3$+500mg/kgbw (*c.papaya*.s)	5.55 ± 0.45^{ab}	17.00 ± 0.20^{ab}	51.00 ± 1.20^{c}	39.45 ± 4.15^{ab}

Data are Mean ±SEM of triplicate determination. Data followed by different superscript alphabet along the same column were significantly different

APPENDIX D

Effects of Alkaloid and Flavonoid Fraction of *Carica Papaya* Seed on Hematological Parameters in Aluminum Chloride-Induced Anaemic Rats.

	PCV (%)	HGB (g/dl)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^9/L)	WBC (10^9/L)	Neutrophils (%)	Lymphocytes (%)	RDWC (10^9/L)
Normal 1ml DW	40.00±2.00^{ab}	13.35±0.65^{b}	7.05±0.15^{b}	47.00±1.00^{a}	19.00±0.00^{a}	38.50±0.50^{b}	738.00±53.00^{d}	6.45±0.35^{b}	13.00±3.00^{a}	51.50±1.50^{c}	18.15±0.00^{a}
0.5mg/kgbw $AlCl_3$ only (Untreated)	26.50±3.50^{a}	8.55±1.25^{a}	5.05±1.25^{a}	52.00±1.00^{a}	16.00±1.89^{a}	27.05±2.90^{a}	434.00±30.00^{b}	2.50±0.60^{a}	11.00±0.10^{a}	16.50±0.50^{a}	37.60±0.60^{b}
$AlCl_3$+3mg/kgw $FeSO_4$	37.00±0.00^{b}	13.70±0.00^{b}	7.30±1.20^{b}	51.50±1.50^{a}	18.50±0.50^{a}	36.00±2.00^{b}	605.50±183.50^{c}	7.10±0.00^{c}	12.00±0.20^{a}	41.00±0.30^{b}	43.00±0.20^{b}
$AlCl_3$+75mg/kgbw Alkaloids seed	36.00±0.58^{b}	11.93±0.32^{b}	7.27±0.12^{b}	71.00±0.58^{b}	16.97±0.03^{a}	27.00±3.51^{a}	608.33±69.34^{c}	8.50±0.31^{c}	21.00±1.00^{b}	67.00±1.15^{d}	65.06±2.44^{d}
$AlCl_3$+150mg/kgbw Alkaloids seed	36.00±3.06^{b}	11.90±1.18^{b}	6.70±0.70^{ab}	70.33±1.76^{b}	18.33±0.33^{a}	25.67±1.20^{a}	557.67±88.34^{bc}	16.43±4.37^{d}	19.00±3.79^{b}	54.66±2.13^{c}	58.46±5.15^{c}
$AlCl_3$+75mg/kgbw Flavonoids seed	23.67±3.28^{a}	5.17±1.09^{a}	4.10±1.09^{a}	73.33±3.76^{b}	16.00±1.00^{a}	24.33±2.33^{a}	229.33±30.82^{a}	6.16±3.21^{b}	26.66±1.20^{c}	46.33±1.11^{b}	58.23±5.46^{c}
$AlCl_3$+150mg/kgbw Flavonoids seed	23.67±1.86^{a}	6.93±0.32^{a}	3.30±0.44^{a}	84.00±4.00^{c}	20.33±2.96^{a}	25.00±2.65^{a}	441.00±10.69^{b}	3.23±0.58^{a}	36.33±2.73^{c}	47.33±4.33^{b}	415.33±3.71^{b}

Data are Mean ±SEM of triplicate determination. Data followed by different superscript alphabet along the same column were significantly different

APPEENDIX E

Effects of Alkaloid and Flavonoid Fraction of *Carica Papaya* leaves On Hematological Parameters in Aluminum Chloride-Induced Anaemic Rats.

GROUPS PARMETERS	PCV (%)	HGB (g/dl)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^9/L)	WBC (10^9/L)	Neutrophils (%)	Lymphocytes (%)	RDWC (10^9/L)
Normal 1ml DW	40.00±2.00^{b}	13.35±0.65^{b}	7.05±0.15^{b}	47.00±1.00^{a}	19.00±0.00^{a}	38.50±0.50^{b}	738.00±53.00^{d}	6.45±0.35^{b}	13.00±3.00^{a}	51.50±1.50^{c}	18.15±0.00^{a}
5mg/kgbw $AlCl_3$only	26.50±3.50^{a}	8.55±1.25^{a}	5.05±1.25^{a}	52.00±1.00^{a}	16.00±1.00^{a}	31.00±1.00^{a}	434.00±30.00^{b}	2.50±0.60^{a}	11.00±0.10^{a}	16.50±0.50^{a}	37.60±0.60^{b}
$AlCl_3$+3mg/kgw $FeSO_4$	37.00±0.00^{b}	13.70±0.00^{b}	7.30±2.20^{b}	51.50±1.50^{a}	18.50±0.50^{a}	36.00±2.00^{a}	605.50±183.50^{c}	7.10±0.00^{b}	12.00±0.20^{a}	41.00±0.30^{c}	43.00±0.20^{b}
$AlCl_3$+75mg/kgbw Alkaloids leaf	39.33±2.40^{b}	13.46±0.84^{b}	7.26±0.26^{b}	63.00±1.15^{b}	19.33±0.88^{a}	31.33±2.33^{a}	382.00±68.00^{ab}	10.10±0.61^{c}	17.00±4.58^{b}	64.33±2.33^{d}	43.90±2.39^{b}
$AlCl_3$+150mg/kgbw Alkaloids leaf	37.66±0.88^{b}	12.06±0.58^{b}	6.70±0.2^{ab}	67.33±2.73^{b}	18.66±0.66^{a}	27.66±2.03^{a}	619.33±119.90^{c}	13.06±1.15^{d}	20.33±2.85^{b}	68.33±3.28^{d}	51.93±0.62^{b}
$AlCl_3$+75mg/kgbw Flavonoids leaf	29.66±2.40^{a}	9.53±0.93^{a}	5.26±0.73^{a}	71.00±1.53^{b}	16.00±0.57^{a}	22.33±0.88^{a}	439.00±74.50^{b}	8.00±2.50^{b}	60.33±17.17^{c}	29.66±16.05^{b}	46.00±0.58^{b}
$AlCl_3$+150mg/kgbw Flavonoids leaf	34.33±5.17^{b}	11.70±1.45^{b}	6.26±0.78^{a} ^{b}	67.66±1.76^{b}	20.66±1.76^{a}	29.33±1.76^{a} ^{b}	299.33±15.85^{a}	10.73±3.72^{c}	26.00±2.52^{b}	57.00±1.53^{c}	45.03±0.61^{b}

Data are Mean ±SEM of triplicate determination. Data followed by different superscript alphabet along the same column were significantly diferent ($p<0.05$)