EFFECTS OF DICHLOROMETHANE-METHANOLIC LEAF EXTRACTS OF Carissa edulis (Forssk.)Vahl ON HAEMATOLOGICAL AND SERUM LIPID PROFILES IN NORMAL RAT MODELS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

July, 2016
Declaration

I, Obel Jorum Humphrey, duly declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution.

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DEDICATION

This thesis is hereby dedicated to my family for their selfless sacrifices towards my education.
ACKNOWLEDGEMENTS

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Above all I most sincerely thank the Almighty God our father for giving me strength, good health and sound mind to accomplish this project. From Him is all knowledge, understanding, power and wisdom, glory be to His holy name.

Lastly, to all who contributed to the success of my work mentioned or not, may God bless you in a mighty way.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated Thrombostin Time</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>CD4⁺ T</td>
<td>Cluster of Differentiation Thymus Cells</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate.</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte Colony Stimulating Factor</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HCT</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>HMG CoA</td>
<td>β-Hydroxy β-Methylglutaryl Coenzyme A</td>
</tr>
<tr>
<td>HSCT</td>
<td>Hematopoietic Stem Cell Transplantation</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpes Simplex Virus</td>
</tr>
<tr>
<td>IDL</td>
<td>Intermediate Density Lipoprotein</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>ITP</td>
<td>Idiopathic Thrombocytopenic Purpura</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Carpsular Haemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean Carpsular Haemoglobin Concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Carpsular Volume</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean Platelet Volume</td>
</tr>
<tr>
<td>NK-C</td>
<td>Natural Killer Cells</td>
</tr>
<tr>
<td>PAMPs</td>
<td>Pathogenic Associated Molecular Patterns</td>
</tr>
<tr>
<td>PCOs</td>
<td>Polycystic Ovarian Syndrome</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed Cell Volume.</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome Proliferator Activated Receptor</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood cell</td>
</tr>
<tr>
<td>RDW</td>
<td>Red cell Distribution Width</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe Combined Immunodeficiency</td>
</tr>
<tr>
<td>SCN</td>
<td>Severe Congenital Neutropenia</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic Lupus Erythematosis</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>TIP-HUS</td>
<td>Thrombocytopenic Idiopathic Purpura-Hemolytic</td>
</tr>
<tr>
<td>TLRs</td>
<td>Toll-Like Receptors</td>
</tr>
<tr>
<td>VEGFA</td>
<td>Vascular Endothelial Growth Factor-A</td>
</tr>
<tr>
<td>VWD</td>
<td>Von Willerbrand Disease</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
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ABSTRACT

Assessment of hematological parameters and serum lipid profiles can be used to explain blood related functions of a plant extract. The blood can act as pathological reflector and also as an indicator of the physiological state of an animal. Hematological and lipid related disorders are increasingly on the rise while conventional management of these disorders are not easily accessible. This has prompted an increased use of medicinal plants which are readily available in the management of blood disorders. *Carissa edulis* (Forssk.) Vahl (Apocynaceae) like other terrestrial plants, has ethnopharmacological relevance and has been exploited by the local people in search for remedies for various diseases including those of the blood. Although *C. edulis* is widely used in managing blood related disorders in traditional system of medicine, no scientific research have been undertaken to evaluate its effects on the hematological and serum lipid profiles. This study therefore was designed to investigate the effects of Dichloromethane-Methanolic leaf extract of of *C. edulis* on hematological parameters and serum lipid profiles. The plant leaves were collected from Siakago-Mbeere North Sub-County, Embu County, Kenya. The samples were prepared and extraction of the active compounds carried out using organic solvents; dichloromethane and methanol in the ratio of 1:1. Experimental rats were divided into four groups each consisting of five normal rats. The groups received oral doses of 50, 75 and 100 mg/kgbw of the extract while one group was used as control and did not receive any dosage. Blood samples were drawn from the rats at intervals of seven days then the hematological and serum lipid profiles were analysed using an auto-analyser. Screening for plants phytochemicals was conducted using the standard recommended procedures. The results of this study showed that DCM-MeOH leaf extract of of *C. edulis* induced general increase in the levels of red blood cells, Hemoglobin and related parameter profiles across the 50, 75 and 100 mg/kgbw dose levels (p<0.05). The total and differential white blood cell counts also increased significantly at all the dose levels during the study period (p<0.05). Platelets and the related parameter levels also significantly increased at all dose levels during this study period (p<0.05). The triglycerides, total cholesterol and low density lipoprotein cholesterol levels, however, decreased significantly at all the dose levels (p>0.05), while high density lipoprotein cholesterol levels increased significantly during this study. Qualitative phytochemical screening confirmed the presence of various phytochemicals which included alkaloid, flavonoids, tannins, phenols, terpenes and traces of steroids which have the ability to protect the erythrocytes from oxidative damage as well as possess erythropoietin stimulatory, immune-stimulatory and thrombopoietic stimulatory activities. The phytochemicals also have the ability to alter lipid metabolism. It was therefore concluded that the plant extract, subject to various stipulated assays, moderation and approvals, may be useful in the management of hematological and lipid related disorders.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Haematology refers to the study of the number and morphology of the cellular elements of blood particularly; the red blood cells (erythrocytes), white blood cells (leucocytes) and the platelets (thrombocytes) in addition to the use of these results in the diagnosis and monitoring of diseases (Ovuru and Ekweozor, 2004; Merck, 2012). Therefore, laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body (Waugh et al., 2001; Ogunbajo et al., 2013).

Erythrocytes have three main functions; to distribute oxygen to the periphery from the lungs through the pulmonary capillaries, remove carbon dioxide from the tissues back to the lungs through the systemic capillaries and to ensure that the acidic and basic values of the body are normal (Jagger et al., 2001; Ellsworth, 2004; Sprague and Ellsworth, 2012).

White blood cells (WBCs), also called leukocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All leukocytes are produced and derived from a multipotent 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., 1997; LaFleur-Brooks et al., 2008).

Platelets are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries (Laki, 1972). Platelets have no cell nucleus, they are fragments of cytoplasm which are derived from the megakaryocytes of the
bone marrow and then enter the circulation (Machlus et al., 2014). These unactivated platelets are biconvex discoid structures (Jain, 1975). They are 2–3 µm in greatest diameter (Paulus, 1975).

Blood lipids are lipids in the blood, either free or bound to other molecules. They are mostly transported in a protein capsule, and the density of the lipids and type of protein determines the fate of the particle and its influence on metabolism. The concentration of blood lipids depends on intake and excretion from the intestine, and uptake and secretion from cells. Blood lipids are mainly fatty acids and cholesterol (Criqui et al., 1993; Ramzi et al., 1994; Price and Schrier, 2008).

Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazzi et al., 2004). Blood disorders that affect red blood cells include: Anemia which is characterized by low number of red blood cells (Cook et al., 2003; Richard et al., 2008). Neutropenia is the disorder that is characterized by decreased levels of neutrophils (Lee et al., 2006; Kenneth et al., 2010). Most platelet disorders are due to an insufficient number of platelets, a condition known as thrombocytopenia. The classification of these disorders can be divided into two, congenital and acquired disorders (Heinz, 2008). Hyperlipidemia is characterized by abnormally elevated levels of any or all lipids and lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels). Lipids (fat-soluble molecules) are transported in a protein capsule. The size of that capsule, or lipoprotein, determines its density. People with high LDL cholesterol and
high triglycerides are at an increased risk of developing heart disease (Chait et al., 1990; Maton et al., 1993).

Conventional means of treatment against anemia includes use of iron pills to boost haemoglobin synthesis, blood transfusion, kidney injections of a synthetic hormone such as epogen to stimulate the production of blood cells (Richard et al., 2008). Neutropenia may be treated with cytokines, granulocyte colony—stimulating factor or granulocyte-macrophage colony—stimulating factor (Newman and Akhtari, 2011). Lipid disorders management include use of drugs such as Statins to treat high cholesterol, cholesterol absorption inhibitors, Bile-acid-binding resins and supplements such as Omega-3 fatty acids are polyunsaturated fats that work to increase the heart’s health while Niacin is used to increase the level of HDL production (Waugh et al., 2001; Mora et al., 2013).

Most conventional ways of managing anemia, neutropenia, thrombocytopenia and hyperlipidemias may have undesired side effects (Keidan, 1989). Blood transfusion limits its usefulness because of risk of infection and formation of antibodies (Lonn et al., 2006; Shander et al., 2007). Iron supplement used in anemic conditions often lead to diarrhoea, epigastric abdominal discomfort (Toblli et al., 2007; Newman and Akhtari, 2011). Epogen used to treat anemia resulting from chronic kidney disease can lead to high blood pressure, crippling cluster mignraine, joint pain and clotting at the infection sites. Skin rash, flu-like symptoms, allergic reactions, seizures, thrombotic events are among other possible side effects (Drueke et al., 2006; Neunert et al., 2011).
Many medicinal plants have been used traditionally to manage hematological and serum lipid disorders. *Bulbine natalensis* stem extracts have been widely used by communities in eastern and northern parts of South Africa to boost immune system and consequently treat immune related disorders such as neutropenia. Leaves of *Sorghum bicolor* and bark of *Magnifera indica*, are used by local communities in South Western Nigeria to treat anemia (Majolagbe et al., 2013; Onyenekwe et al., 2013). *Ficus benghalensis* has been used by ayurvedic practitioners, in India to boost the immune system to fight a number of diseases (Gabhe et al., 2006; Pandit et al., 2011). Unripe pods and leaves of *Bauhinia purpurea*, leaves of *Cinnamomum tamala* and the roots of *Commiphora mukkul* herbs have been used by herbalists in India to manage lifestyle diseases such as obesity, heart conditions and hyperlipidemia (Oben et al., 2006).

*Carissa edulis* (Forssk.)Vahl belongs to the family Apocynaceae. Its leaves bark and roots are often used to manage various disorders. The plant is native in the East African, West Africa and south African countries Like other terrestrial plants, *Carissa edulis* has ethnopharmacological relevance and has also been exploited by the local people in the search for remedies for various ailments to increase vitality and manage hematological disorders (Tolo et al., 2006). Its effects in these activities has not been scientifically studied or validated. It was against this background that this study was undertaken to test the hematological claimed effect of the plant extracts in normal rats.

1.2 Problem statement

Haematological and lipid related disorders are on the rise with the ever increasing negative lifestyles and increased tendency of the present human population being exposed to harmful chemicals in the environment, poor eating habits and sedentary lifestyles, while conventional
methods used to treat haematological disorders and hyperlipidemias are not easily accessible and their use have various adverse effects (Keidan, 1989). As a result, many people turn to medicinal plants for treatment because professional care is not immediately available.

Even though *C. edulis* has been widely used in traditional medicine to treat various disorders such as headache, chest complications, rheumatism, syphilis among others (Achenbach *et al.*, 1985). The effects of this plant extracts on hematological parameters and serum lipid profiles are still unconfirmed.

**1.3 Justification**

In the view of the aforementioned setbacks, there is need to develop agents that are effective, cheaply available and with arguably reduced side effects as the alternative medical intervention. Unlike conventional drugs which are based on a single active ingredient targeting just one haematological component, the plant derived agents comprise of a mixture of therapeutically active phytochemicals that act together to restore a normal physiological state. There is therefore urgent need to develop agents that are effective, cheaply available and with minimal side effects as alternative means of treatment. It is, therefore, important to try and establish if the extracts of *C. edulis* exert their activities through alteration of haematological parameters and serum lipid profiles. This study was designed to assess the effects of DCM-MeOH leaf extracts of *C. edulis* on the levels of haematological and serum lipid profiles in normal rat models.
1.4 Research questions

i. What are the effects of DCM-MeOH leaf extract of *Carissa edulis* on the levels of haematological parameters of normal laboratory rats?

ii. What are the effects of DCM-MeOH leaf extract of *Carissa edulis* on the levels of serum lipid profiles of normal laboratory rats?

iii. What is the phytochemical composition in the DCM-MeOH leaf extract of *Carissa edulis*?

1.5 Objectives

1.5.1 General objective

To determine the effects of DCM-MeOH leaf extracts of *Carissa edulis* on haematological and serum lipid profiles in laboratory rats.

1.5.2 Specific objectives

i. To determine the effects of DCM-MeOH leaf extracts of *C. edulis* on haematological parameters in normal rats.

ii. To determine the effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats.

iii. To determine the qualitative phytochemical composition of DCM-MeOH leaf extracts of *C. edulis*. 
2.1 Role of red blood cells and hemoglobin in health

2.1.1 Erythrocytes

Erythrocytes are the most common type of blood cells and are the vertebrate organism's principal means of delivering oxygen (O$_2$) to the body tissues via blood flow through the circulatory system. RBCs take up oxygen in the lungs and release it into tissues while squeezing through the body's capillaries and are also involved in the elimination of carbon dioxide and many molecules from circulation, including products of immunological reactions. They possess adhesion molecules that bind complement fragments and are therefore important for capturing and delivering such complexes to the reticuloendothelial system for elimination from circulation (Horakova, 2004).

Some erythrocyte antigens are also known to bind inflammatory mediators with potential to affect innate immune responses (Hadley and Peiper, 1997). More recently, however, erythrocytes have been identified to reversibly bind, transport and release nitric oxide (NO) within the cardiovascular system. Nitric oxide modulates both the function of the vascular wall and that of blood cells, such as platelets and leukocytes (Loscalzo, 2001).

2.1.2 Hemoglobin

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of most vertebrates as well as the tissues of some invertebrates (Sidell et al., 2006). Hemoglobin in the blood carries oxygen from the respiratory organs to the rest of the body. There, it releases the
oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism.

In mammals, the protein makes up about 96% of the red blood cells’ dry content, and around 35% of the total content (including water). Hemoglobin has an oxygen-binding capacity of 1.34 mL O₂ per gram, which increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules (Weed et al., 1963).

Hemoglobin is involved in the transport of other gases: It carries some of the body’s respiratory carbon dioxide as carbaminohemoglobin, in which CO₂ is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen (Epstein et al., 1998). Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism. The human hemoglobin molecules are a set of very closely related proteins formed by symmetric pairing of a dimer of polypeptide chains, the α- and β-globins, into a tetrameric structural and functional unit. The α2β2 molecule forms the major adult hemoglobin (Alan, 2008).
2.2 Role of white blood cells in health

2.2.1 White blood cells

Leucocytes (white blood cells) are the main cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All leukocytes are produced and derived from a multipotent 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., 1997).

They provide either innate or specific adaptive immunity. They are of five different classes; neutrophils, monocytes, lymphocytes, eosinophils and basophils. 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 $11 \times 10^9$/L in adults. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leucopenia (Bruce et al., 2002).

2.2.2 Neutrophils

Neutrophils are the most abundant (40 to 75%) type of white blood cells in most mammals and form an essential part of the innate immune system. They are the first leukocytes to be recruited to an inflammatory site and eliminate pathogens through various mechanisms (Phillipson and Kubes, 2011). They are involved in removal of dead cells and bacteria by their phagocytic activity. They migrate towards the target through a process known as chemotaxis and kill microorganisms intracellularly by formation of oxygen radicals (Basaran et al., 1997).
Neutrophils directly release growth factors, such as vascular endothelial growth factor-A (VEGFA) that have been shown to promote angiogenesis in the cornea (Gong and Koh, 2010).

Apoptotic neutrophils release 'find me' signals that attract monocytes which are recruited to phagocytose apoptotic neutrophils and other dying cells in the tissue. So, by promoting their own removal from the tissue, neutrophils contribute to the resolution of inflammation and promote healing and tissue repair (Soehnlein and Lindbom, 2009).

Though considered to be exclusively pro-inflammatory in nature, there is increasing evidence that some types of neutrophils may exhibit anti-inflammatory or healing characteristics. Moreover, removal of neutrophils from an inflamed tissue often results in more tissue pathology, even in the setting of sterile injury (Fournier and Parkos, 2012).

Overall, it seems that neutrophils affect adaptive immunity by promoting humoral immune response but suppressing cellular immune response. The identification of neutrophils in places such as the lymph node supports their role in adaptive immune regulation (Alberto et al., 2011).

### 2.2.3 Basophils

Basophils are the least common of the granulocytes, representing about 0.01 to 0.3% of circulating white blood cells. They are generally associated with type 2 immune responses and develop in response to allergens and multicellular parasites, such as parasitic worms (helminths) or ticks (Migalovich-Sheikhet, 2012). In addition to their effector functions, basophils and mast
cells can rapidly respond to environmental signals and might function as modulators of immune responses by enhancing, suppressing or polarizing adaptive immunity (Siracusa et al., 2011).

Basophils are thought to contribute to the pathogenesis of allergic contact dermatitis, atopic dermatitis, allergic drug reactions, immediate hypersensitivity reactions including anaphylaxis, asthma, Crohn’s disease, skin and kidney allograft responses and acute and chronic myelogenous leukemia (Mark et al., 2013). They respond to a variety of environmental stimuli such as drugs, venoms and pollens, and their reactivity can be assessed by the basophil activation test. Recent studies in humans and murine systems have shown that basophils perform non-redundant effector functions and significantly contribute to the development and progression of TH2 cytokine-mediated inflammation (Mark et al., 2013).

2.2.4 Eosinophils

Eosinophils are white blood cells and one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood (Yamaguchi et al., 1988).

These cells are 'acid-loving' as shown by their affinity to coal tar dyes: Normally transparent, it is this affinity that causes them to appear brick-red after staining with eosin, a red dye, using the Romanowsky method (Metcalf et al., 1986). The staining is concentrated in small granules within the cellular cytoplasm, which contain many chemical mediators, such as histamines and
proteins such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases (DNase), lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues (Yamaguchi et al., 1988).

Eosinophils synthesize, store, and release stem cell factor and transforming growth factor (Hartman et al., 2001). When added to fibroblasts, they stimulate fibroblast proliferation, collagen synthesis, and lattice contraction mostly by transforming growth factor (Levi-Schaffer et al., 1999). In addition, they are a source for preformed nerve growth factor that is also a mast cell survival and activating factor (Kobayashi et al., 2002). Interestingly, nerve growth factor can act on eosinophils in an autocrine manner by activating them to release eosinophil peroxidase (Smith and Levi-Schaffer, 2000). The latter can activate mast cells to release histamine (Patella et al., 1996), suggesting a role for eosinophil-derived nerve growth factor in mast cell–eosinophil cross talk (Mauch and Krieg, 1990).

Moreover, eosinophils can modulate fibroblast properties by other growth factors such as fibroblast growth factor-2, nerve growth factor and vascular endothelial growth factor (Horiuchi and Weller, 1997). Substantial evidence shows that IL-4 and IL-13, also expressed by eosinophils, promote fibroblast functions by up regulating fibroblast chemokine and matrix protein expression (Richter et al., 2001).
2.2.5 Monocytes

Monocytes are the largest type of all leukocytes. They are part of the innate immune system of vertebrates (Ziegler, 2007). They are amoeboïd in shape, having agranulated cytoplasm. Monocytes have unilobar nuclei, which makes them one of the types of mononuclear leukocytes (containing azurophil granules). The archetypal idea of the nucleus is that it is bean-shaped or kidney-shaped, although the most important distinction is that it is not deeply furcated into lobes, as occurs in polymorphonuclear leukocytes (Sozzani et al., 1996).

Monocytes constitute 2 to 10% of all leukocytes in the human body. They play multiple roles in immune function. Such roles include: replenishing resident macrophages under normal conditions and in response to inflammation signals, monocytes can move quickly to sites of infection in the tissues and differentiate into macrophages and dendritic cells to elicit an immune response. Half of them are stored in the spleen. Monocytes are usually identified in stained smears by their large kidney shaped or notched nucleus (Ziegler, 2007).

Recent advances in immunology research have shown that monocytes are heterogenic and can be divided into three subsets based on specific surface markers and that each subset displays specific functions. Identified monocyte subsets exhibit distinct pathophysiological roles. Classical inflammatory monocytes are equipped with a set of Toll-like receptors (TLRs) and scavenger receptors, recognizing pathogen-associated molecular patterns (PAMPs) and removing microorganisms, lipids, and dying cells via phagocytosis. They produce effector molecules such as cytokines, myeloperoxidase, superoxide, and initiate inflammation (Yasaka et al., 1981).
Inflammatory monocytes selectively traffic to the sites of inflammation, produce inflammatory cytokines and contribute to local and systemic inflammation. They are highly infiltrative and can be differentiated into inflammatory macrophages, which remove PAMPs and cell debris. In steady state, the patrolling anti-inflammatory monocytes patrol the vasculature to monitor PAMPs and become tissue resident macrophages. During inflammation, they differentiate into anti-inflammatory macrophages, which repair damaged tissues (Yasaka et al., 1981).

2.2.6 Lymphocytes

Lymphocytes are the main type of cells found in the lymph, hence the name. They include natural killer cells (NK cells) which function in cell-mediated, cytotoxic innate immunity, T cells which function in cell-mediated, cytotoxic adaptive immunity and B cells which function in cells humoral, antibody-driven adaptive immunity. The T-lymphocytes which are produced in the bone marrow pass through thymus and mature there while B-lymphocytes do not pass through thymus but mature in bone marrow (Mossman and Coffman, 1989; Kendall, 1990; Nestor, 2001).

T-lymphocytes are further classified into T helper (Th) cells that regulate the antibody-forming function of B-lymphocytes and participate in rejection of transplants, T-suppressor cells (T-s) and T-cytotoxic cells (T-c) that are involved in the defences against viral infections. The T-cytotoxic cells are capable of destroying a target cell, that is infected with virus or that expresses some form of foreign antigen and are the major immune effectors of the cellular immune response. Both T- Helper cells and T-suppressor cells down-modulate immune responses (Levine et al., 1998).
The B-lymphocytes are a population of cells that express clonally diverse cell surface immunoglobulin (Ig) receptors recognizing specific antigenic epitopes (Tucker and Thomas, 2015). They mature into antibody-producing plasma cells found in lymph nodes and the spleen. Once the B-cells have created a specific antibody to attack a specific pathogen, their primitive intelligence remembers this information and will know it later should it encounter it (Roitt et al., 1998). In addition to their essential role in humoral immunity, B cells are required for the initiation of T-cell immune responses (Ron et al., 1981). They can also function as polarized cytokine-producing effector cells that influence T-cell differentiation (Harris et al., 2000).

2.3 Role of platelets in health

2.3.1 Platelets

Platelets are produced in the bone marrow from megakaryocytes as cytoplasmic fragments without genomic DNA (Italiano and Shivdasani, 2003). Upon tissue trauma platelets adhere to the extracellular matrix leading to initial tethering and rolling of platelets over the damaged vessel wall, eventually resulting in firm adhesion. Platelet adhesion triggers a signaling cascade mediated by tyrosine kinases and G-protein coupled receptors, which guide full activation of the platelet and concomitant granule release, in turn resulting in recruitment and activation of additional platelets. Platelet adhesion and activation leads to platelet aggregation and the presentation of a procoagulant surface promoting formation of a fibrin-rich hemostatic plug at the injured site (Katleen et al., 2011).
The main function of platelets is to contribute to hemostasis which is 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. The first step is adhesion, whereby platelets attach to substances outside the interrupted endothelium. The second step is activation, whereby the platelets change shape, turn on receptors and secrete chemical messengers. The third step is aggregation, where the platelets connect to each other through receptor bridges. Formation of this platelet plug is associated with activation of the coagulation cascade with resultant fibrin deposition and linking (Yip et al., 2005). These processes may overlap, the spectrum is from a predominantly platelet plug to a predominantly fibrin clot or the more typical mixture. The final result is the formation of clot. In addition, platelets play a role in wound healing and repair as well as activation of inflammatory and immune responses (Weyrich and Zimmerman, 2004).

2.4 Role of serum lipids in health

2.4.1 Triglycerides

A triglyceride is a triester derived from glycerol and three fatty acids (Nelson et al., 2000). As a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so. Triglycerides are the main constituents of vegetable oil and animal fats (Nelson et al., 2000). They are also the major component of human skin oils (Lampe et al., 1983).

In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke (Drummond et al., 2014). However, the relative negative impact of raised levels of triglycerides compared to that of LDL:
HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level.

2.4.2 Total cholesterol

Cholesterol, from the ancient Greek *chole*- (bile) and *stereos* (solid) followed by the chemical suffix *-ol* for an alcohol, is an organic molecule. It is a sterol or modified steroid (Hanukoglu, 1992). It is a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of all animal cell membranes that is required to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to function normally without a cell wall (like plants and bacteria) to protect membrane integrity/cell-viability, thus are able to change shape and move about. In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D (Hanukoglu, 1992). Cholesterol is the principal sterol synthesized by animals. All kinds of cells in animals can produce it. In vertebrates the hepatic cells typically produce greater amounts than other cells.

Most ingested cholesterol is esterified, and esterified cholesterol is poorly absorbed. The body also compensates for any absorption of additional cholesterol by reducing cholesterol synthesis (Lecerf *et al.*, 2011). For these reasons, seven to ten hours after ingestion, cholesterol will show little, if any, effect on total body cholesterol content or concentrations of cholesterol in the blood. However, during the first seven hours after ingestion of cholesterol, the levels significantly increase (Dubois *et al.*, 1994). Cholesterol is recycled; the liver excretes it in a non-esterified form (via bile) into the digestive tract. Typically, about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the bloodstream.
Plants make cholesterol in very small amounts (Behrman, 2005). Plants manufacture phytosterols which can compete with cholesterol for reabsorption in the intestinal tract, thus potentially reducing cholesterol reabsorption (John et al., 2007). When intestinal lining cells absorb phytosterols, in place of cholesterol, they usually excrete the phytosterol molecules back into the gastrointestinal tract, an important protective mechanism.

According to the lipid hypothesis, since cholesterol (like all fat molecules) is transported around the body (in the water outside cells) inside lipoprotein particles, elevated cholesterol concentrations (hypercholesterolemia) — potentially offers a lower cost way to detect elevated concentrations of LDL particles; possibly even low concentrations of functional HDL particles — both variations strongly associated with cardiovascular disease because LDL particles promote atheroma development in arteries (John et al., 2007).

2.4.3 High density lipoproteins

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells (Ruidavets et al., 2002). They are typically composed of 80-100 proteins/particle (organized by one, two or three ApoA; more as the particles enlarge picking up and carrying more fat molecules) and transporting none to hundreds fat molecules. Unlike the larger lipoprotein particles which deliver fat molecules to cells, HDL particles remove fat molecules from cells which want to export fat molecules. The fats carried include cholesterol, phospholipids, and triglycerides.
Men tend to have noticeably lower HDL levels, with smaller size and lower cholesterol content, than women. Men also have an increased incidence of atherosclerotic heart disease. Alcohol consumption tends to raise HDL levels and moderate alcohol consumption is associated with lower cardiovascular and all-causes of mortality (Ruidavets, 2002). Recent studies confirm the fact that HDL has a buffering role in balancing the effects of the hypercoagulable state in type 2 diabetics and decreases the high risk of cardiovascular complications in these patients. Also, the results obtained in this study revealed that there was a significant negative correlation between HDL and activated partial thromboplastin time (APTT) (Mard et al., 2012).

Epidemiological studies have shown that high concentrations of HDL (over 60 mg/dL) have protective value against cardiovascular diseases such as ischemic stroke and myocardial infarction. Low concentrations of HDL (below 40 mg/dL for men, below 50 mg/dL for women) increase the risk for atherosclerotic diseases. Data from the landmark Framingham Heart Study showed that, for a given level of LDL, the risk of heart disease increases 10-fold as the HDL varies from high to low. On the converse, however, for a fixed level of HDL, the risk increases 3-fold as LDL varies from low to high (Rubins et al., 2002).

2.4.4 Low Density Lipoprotein (LDL)

Low density lipoprotein (LDL) is one of the five major groups of lipoproteins. In nutrition, LDL is sometimes referred to as the bad cholesterol. Lipoproteins transfer fats around the body in the water outside cells, can be sampled from blood and allow fats to be taken up by the cells of the body by receptor-mediated endocytosis (Dashti et al., 2011). The LDL particles pose a risk for
cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans.

A complex set of biochemical reactions regulates the oxidation of LDL particles, chiefly stimulated by presence of necrotic cell debris and free radicals in the endothelium (Dashti et al., 2011). Increasing concentrations of LDL particles are strongly associated with increasing amounts of atherosclerosis within the walls of arteries over time, eventually resulting in sudden plaque ruptures and triggering clots within the artery opening, or a narrowing or closing of the opening, leading to cardiovascular disease, stroke, and other vascular disease complications (Dashty et al., 2014).

2.4.5 Very Low Density Lipoprotein (VLDL)

Very low density lipoprotein is a type of lipoprotein made by the liver and is one of the five major groups of lipoproteins that enable fats and cholesterol to move within the water-based solution of the bloodstream. The VLDL is assembled in the liver from triglycerides, cholesterol, and apolipoproteins. It is converted in the bloodstream to low-density lipoprotein (LDL). The VLDL particles have a diameter of 30-80 nm and transports endogenous products. It transports endogenous triglycerides, phospholipids, cholesterol, and cholesteryl esters. It functions as the body's internal transport mechanism for lipids. In addition it serves for long-range transport of hydrophobic intercellular messengers (Rubins et al., 2002).

Nascent VLDL released from the liver contains apolipoprotein B100, apolipoprotein C1, apolipoprotein E, cholesterol, cholesteryl esters, and triglycerides. As it circulates in blood, it
picks up apolipoprotein C-II and additional apoE donated from high-density lipoprotein (HDL). At this point, nascent VLDL becomes a mature VLDL. Once in circulation, VLDL will come in contact with lipoprotein lipase (LPL) in the capillary beds in the body (adipose, cardiac, and skeletal muscle) (Dashti et al., 2011).

The LPL will remove triglycerides from VLDL for storage or energy production. Consequently, VLDL now meets back up with HDL where apoC-II is transferred back to HDL (but keeps apoE). The HDL also transfers cholesteryl esters to the VLDL in exchange for phospholipids and triglycerides via cholesterylester transfer protein (CETP). As more and more triglycerides are removed from the VLDL because of the action of LPL and CETP enzymes, the composition of the molecule changes and it becomes intermediate-density lipoprotein (IDL) (Mard et al., 2012).

2.4.6 Chylomicrons

Chylomicrons are lipoprotein particles that consist of triglycerides (85–92%), phospholipids (6–12%), cholesterol (1–3%), and proteins (1–2%). They transport dietary lipids from the intestines to other locations in the body. Chylomicrons are one of the five major groups of lipoproteins that enable fats and cholesterol to move within the water-based solution of the bloodstream.

Chylomicrons transport lipids absorbed from the intestine to adipose, cardiac, and skeletal muscle tissue, where their triglyceride components are hydrolyzed by the activity of lipoprotein lipase and the released free fatty acids are absorbed by the tissue. When a large portion of the triacylglycerol core has been hydrolyzed, chylomicron remnants are formed and are taken up by the liver, hereby transferring dietary fat also to the liver (Rubins et al., 2002).
Chylomicrons are formed in the endoplasmic reticulum in the absorptive cells of small intestines. To be specific, the mucosal cells within the villi of the duodenum. Newly formed chylomicrons are secreted through the baso-lateral membrane into the lymphatic system. Chylomicrons are released from the lymph to the blood systems, and supply the tissue with fat absorbed from the diet (Dashti et al., 2011).

2.5 Hematological disorders

2.5.1 Anemia

Anemia is usually defined as a decrease in the amount of red blood cells (RBCs) or hemoglobin in the blood. It can also be defined as a lowered ability of the blood to carry oxygen and is a widespread public health problem with major consequences for human health as well as social and economic development (Lippincott et al., 2006). It is a hematological disorder characterized by decreased hemoglobin (Hb), red blood cells (RBCs), and packed cell volume (PCV) or hematocrit in peripheral blood compared to that of normal for age, sex and pregnancy state of the subject (Aguwa, 1986; Oma, 1991).

Anemia is the most common disorder of the blood, affecting about a quarter of population globally while iron deficiency anemia affects nearly 1 billion. In the year 2013, anemia due to iron deficiency resulted in about 183,000 deaths – down from 213,000 deaths in 1990. It is more common in females than males among children, during pregnancy and in the elderly. Anemia increases costs of medical care and lowers a person's productivity through a decreased ability to
work. The name is derived from ancient Greek, *anaimia*, meaning lack of blood (Flaxman *et al.*, 2012).

### 2.5.1.1 Causes of anemia

Anemia is present whenever there is a decrease in the normal amount of circulating hemoglobin. This reduction in hemoglobin may result from blood loss, as in common iron deficiency anemia; from increased destruction of red blood cells, as in the hemolytic anemias; from decreased production of red cells, as in pernicious and folic acid deficiency anemias (Cook *et al.*, 2003). The causes in pre-school children are iron and folate deficiency, parasitamia and inherited disorders of erythrocytes such as glucose-6-phosphate dehydrogenase deficiency. Hemolytic anemia may be caused by parasites, bacterial and viral infection and chemicals that cause erythrocyte destruction (Ramzi *et al.*, 1994).

Iron deficiency anemia is the most common of all anemias, affecting approximately 30% of the world’s population and accounting for up to 500 million cases worldwide. Iron deficiency anemia may result from chronic blood loss, such as occurs in menstrual or menopausal bleeding, parturition, bleeding hemorrhoids, or a bleeding malignant lesion or ulcer in the gastrointestinal tract. It also may develop in patients from a variety of causes that may decrease the rate of absorption of iron, such as subtotal or complete gastrectomy, or a habit of clay eating, or as part of malabsorption syndromes (Cook *et al.*, 1994).

An inadequate dietary intake of iron also may be responsible, but the diagnosis of iron deficiency caused by dietary insufficiency must be made with extreme caution. The body zealously guards
its iron stores, and it has been estimated that the adult male can go up to a decade without iron intake before an iron deficiency anemia develops. Women normally lose about 50 mL of blood with each menstrual period and are thereby more likely to become anemic with an iron deficient diet. Chronic iron deficiency anemia is one of the typical findings in gastrointestinal malignancy and in certain forms of parasitic infections. The margin of iron balance (intake versus loss) is decreased in infants, growing children, and menstruating women (Provan et al., 1999).

Hemoglobinopathies such as sickle-cell disease, thalassaemias, and glucose-6-phosphate dehydrogenase deficiency are common in many developing countries (Deyde et al., 2002). These disorders are particularly found in malaria endemic areas and have been associated with anemia. Glucose-6-phosphate dehydrogenase is correlated with chronic hemolytic anemia (Thurlow et al., 2005). Many studies suggested that these red cell polymorphisms are a human body adaptation against adverse effects of malaria. Sickle cell anemia for example results from genetic mutation of allele A, allele S or C of the β chain to provide resistance against plasmodium effect (Modiano et al., 2008).

Nutritional factors including micronutrients are directly or indirectly involved in red blood cell metabolism. Vitamin B₆ is required for activation of δ-aminolevulinic acid synthase that is necessary for heme synthesis. Vitamin B₉ (folate) and B₁₂ (cobalamine) deficiencies result in immature erythrocyte leading to macrocytic anemia (Gropper et al., 2005). Vitamin A deficiency has been associated with anemia (Gamble et al., 2004). Moreover, one of the leading causes of anemia is iron deficiency due to inadequate intake or malabsorption of dietary iron (Zimmermann et al., 2005).
2.5.1.2 Signs and symptoms of anemia

Moderate anemia can typically cause signs and symptoms such as pale skin, fatigue, weakness, tiring easily, breathlessness, drop in blood pressure when standing from a sitting or lying position (orthostatic hypotension) – this may happen after acute blood loss, like a heavy period frequent headaches, racing heart or palpitations, becoming irritated easily, concentration difficulties, cracked or reddened tongue, loss of appetite and strange food cravings (Vogelzang et al., 1997; Ludwig and Strasser, 2001). Patients with iron deficiency anemia also note a tendency of the nails to crack and split. Weakness and dyspnea may be present for some time before the development of other clinical signs or symptoms of anemia. Chronic anemia can result in severe organ damage affecting the cardiovascular system, immune system, lungs, kidneys, and the central nervous system (Ludwig and Strasser, 2001).

2.5.1.3 Management of anemia

Patients with mild thalassemia (α trait or β minor) are clinically normal and require no treatment. In other cases, the patient’s survival depends on blood transfusions. Prevention of a hemoglobin concentration decrease to under 10 g/dL improves the chances of normal development and survival into adulthood. This hypertransfusion treatment results in iron overload with hemosiderosis and iron deposition in all body tissues. As a result, patients may develop abnormalities in cardiac, endocrine, and hepatic functions, with cardiac insufficiency, diabetes, pituitary hypofunction, and a possible bleeding tendency due to liver disease (Kreuzer and Rockstroh, 1997). If regular blood transfusions are given to children with thalassemia to maintain the hemoglobin level between 10 and 14 g/dL, the children develop normally, without the marked skeletal changes. Some patients with thalassemia undergo splenectomy in an attempt
to prolong RBC survival. Folic acid supplement also seems to be of some benefit. The iron overload is treated with continuous injections of a chelator, deferiprone, which mobilizes and excretes the excess iron. Hematopoietic stem cell transplantation constitutes a future hope for the treatment of thalassemia (Abrams et al., 2000).

Oral iron supplements (ferrous fumarate, ferrous sulfate, and ferrous gluconate) are desirable as first-line therapy for treating anemia. These iron preparations are essentially equivalent in terms of bioavailability (Navas-Carretero et al., 2007). Epoetin alfa together with iron supplementation is prescribed for patients with mild symptomatic or moderate HIV-related anemia to meet the demand for iron during enhanced RBC production (Abrams et al., 2000). Oxymetholone and other anabolic steroids have been used to treat anemia. These agents can increase production and urinary excretion of erythropoietin in patients with anemia caused by bone marrow failure, and they can stimulate erythropoiesis in patients with deficient RBC production (Anadrol, 2000).

Anemia can be managed by use of medicinal plants. For instance, Justicia secunda (Vahl) a popular plant is utilized in Kinshasa to manage anemia (Moswa et al., 2005). Leaves of Sorghum bicolor, Bark of Magnifera indica, Telfaria occidentalis, Basella alba and leaves of Hibiscus sabdariffa are used by local communities in South Western Nigeria to treat anemia (Majolagbe et al., 2013). Tectona grandis, Amaranthus spinosus and Stylosanthes erecta are used in South-Eastern Côte d'Ivoire against anemia (Mamidoukane et al., 2012). Many herbalists and naturopathic doctors in North America recommend use of Vernonia amygdalina for their patients as treatment for anemia (Kupchan et al., 1969). Seeds of Piper guineense, the fruit of Eugenia caryophyllum, the leaves of Sorghum bicolor and the stems of Pterocapus osun (Craib) have
been used locally among folk groups in Nigeria to prevent painful crises that are associated with sickle cell anemia (Iyamu et al., 2002).

2.5.2 Neutropenia

Neutropenia may occur alone or as part of a generalized suppression of the bone marrow also affecting the erythrocytes and platelets (aplastic anemia). The degree of neutropenia predicts the risk of serious bacterial infections. Neutropenia is defined as an absolute neutrophil count of more than two standard deviations below a normal mean value. The normal absolute number of neutrophils in the peripheral blood is 3,000/mm$^3$ to 6,000/mm$^3$. Mild neutropenia occurs when 1,000/mm$^3$ to 2,000/mm$^3$ neutrophils are present, moderate neutropenia occurs when 500/mm$^3$ to 1,000/mm$^3$ neutrophils are present, and severe neutropenia occurs when fewer than 500/mm$^3$ neutrophils are present in the peripheral blood (Dale et al., 2003).

Neutrophils fight infection by destroying harmful bacteria and fungi (such as yeast) that invade the body. People who have neutropenia are at increased risk for developing serious infections because they do not have enough neutrophils to destroy harmful microorganisms that cause disease. Neutrophils are contained in the bone marrow, either as mitotically active or postmitotic mature cells constitute about 60% of total leukocytes and thus practically, the depletion of leukocytes implies a decrease in the neutrophil count (Beutler et al., 1995).

2.5.2.1 Causes of neutropenia

Neutropenia has a wide range of underlying causes. Decreased production of neutrophils is associated with deficiencies of vitamin B12 and folic acid (Clay et al., 2010). Certain infections
decrease the number of neutrophils in the circulating blood because of increased migration of neutrophils into the tissues, sequestration of neutrophils, or the direct toxic effect of the microorganism and its toxins on the blood marrow (Neudorf et al., 2004). Infections with viruses, particularly hepatitis A and B viruses, parvovirus, human immunodeficiency virus (HIV-1), and Cytomegalovirus, are associated with neutropenia. Overwhelming bacterial infection, particularly septicemia, can be accompanied by neutropenia because the cells are used at rapid rate to overcome the infection. Diseases causing sequestration of neutrophils include systemic lupus erythematosus and Felty’s syndrome (Shastri and Logue, 1993).

The most common cause of neutropenia is a drug reaction. A large number of drugs can cause neutropenia or aplastic anemia. Neutropenia secondary to a drug reaction results from either a toxic (dose-related) or an idiosyncratic phenomenon. Toxic neutropenia occurs predictably in all people who take the offending drugs at sufficient doses for a sufficient time. These drugs interfere with DNA synthesis, protein synthesis, or mitosis (Neudorf et al., 2004). Drugs that cause toxic neutropenia include those used in cancer chemotherapy, benzene, and alcohol. Increasingly, more commonly used drugs, such as analgesics, antibiotics, and anti-histamines, have been identified as potential causes of severe neutropenia or agranulocytosis. Neutropenia secondary to ionizing radiation also results from a direct toxic effect on the division of bone marrow cells (Bain, 2011).

Idiosyncratic reactions are not dose related and occur only in a small percentage of individuals taking the drug. Idiosyncratic drug reactions causing neutropenia are thought to be either an immunologic reaction affecting the bone marrow or an inherited inability to metabolize the drug.
properly. Drugs that have an increased risk of causing idiosyncratic neutropenia include phenothiazides, phenylbutazone, sulfonamides, and chloramphenicol (Stroncek, 1993).

### 2.5.2.2 Signs and symptoms of neutropenia

Along with feelings of general malaise (headache, discomfort, and muscle aches), the most common complication of neutropenia and agranulocytosis is infection. The clinician must be aware that the localizing clinical signs of infection may be few or absent owing to a decreased inflammatory reaction. Swelling and pus will be minimal. The most common sign of infection in neutropenic patients is fever (Neudorf et al., 2004). Other common manifestations include mucosal ulcers, tachycardia, acute pharyngitis, and lymphadenopathy. Common sites of infection include the lungs, urinary tract, skin, rectum, and mouth. Acute bacterial infections are the most common and usually are caused by *Staphylococcus aureus* or gram-negative bacilli, such as *Klebsiella*, *Pseudomonas*, and *Proteus*. The major signs and symptoms of cyclic neutropenia are related to infection occurring during neutropenic episodes. The most common signs are fever, stomatitis, pharyngitis, and skin abscesses (Stroncek, 1993).

### 2.5.2.3 Management of neutropenia

Broad spectrum parenteral antibiotics should be administered in patients with severe neutropenia because organisms that cause infections in these patients usually comes from the gastrointestinal tract or skin and can result in the rapid onset of overwhelming sepsis (Hughes et al., 1997). Neutropenia resulting from chemotherapy or radiation may, in specific instances, be treated with cytokines such as granulocyte colony—stimulating factor or granulocyte-macrophage colony—stimulating factor.
Recombinant human granulocyte colony-stimulating factor is a safe and effective therapeutic modality in management of autoimmune neutropenia associated with Felty's syndrome and systemic lupus erythematosus, which stimulates neutrophil production (Newman and Akhtari, 2011). Severe congenital neutropenia (SCN) whereby the patient fails to respond to granulocyte colony-stimulating factor treatment, hematopoietic stem cell transplantation (HSCT) can successfully be used in treatment (Kawaguch et al., 2014).

Careful oral hygiene should be practiced to prevent infections of the mucosa and teeth, rectal temperature measurements and rectal examinations should be avoided, administration of stool softeners for constipation and skin infections should be managed by someone with experience in the treatment of infection in neutropenic patients (Freifeld et al., 2011), or can also be managed using fourth-generation cephalosporins or equivalents to combat infection. Fever is treated by administering Cefepime, meropenem, imipenem-cilastatin, or piperacillin-tazobactam empirically as a single agent. Gentamicin or another aminoglycoside should be added if the neutropenic patient’s condition is unstable or the individual appears septic. Vancomycin can be added if infection with methicillin-resistant staphylococcus aureus or a corynebacterium species is suspected (Bow et al., 1996; Kern et al., 1999).

Neutropenia is also managed by use of medicinal plants. For instance, leaves, stems, flowers, roots, seeds, bark and even whole plant of Withania somnifera are used as immune stimulant in patients with low white blood cell counts in Atlantic Ocean to South East Asia and from the Mediterranean region to South Africa (Sitansu and Ajay, 2011). Herbal preparation of Telfaria
ocidentalis has been employed in the treatment of sudden attack of convulsion, malaria and anemia in Nigeria (Gbile, 1986).

2.5.3 Thrombocytopenia

Thrombocytopenia is defined as a platelet count below the normal range for the population (2 standard deviations). In most laboratories, a normal platelet count is between 150,000 to 450,000 per litre. By definition, 5% of the population will have counts outside the “normal” range. No generally accepted definition of mild, moderate or severe thrombocytopenia exists (Cheng et al., 2003). Platelet counts between $100 \times 10^9$ and $150 \times 10^9$ per litre do not necessarily indicate disease if they have been stable for more than 6 months. Furthermore, it is now appreciated that in many non-western countries, the lower threshold of the normal platelet count is lower than $150 \times 10^9$ per litre (Rodeghiero et al., 2009).

2.5.3.1 Causes and classification of thrombocytopenia

Immune thrombocytopenia (ITP) is a relatively common disease in adults. A Danish study noted an incidence rate of 2.68 per 100,000. It is an autoimmune condition caused by antiplatelet antibodies, which result in decreased platelet survival. These antibodies are frequently IgG in nature and directed against platelet antigens GP IIb/IIIa and GP Ib/IX complexes (Amorosi and Ulmann, 1966). The spleen is the major site of platelet destruction. While all ages may be affected, frequently patients are young adult females. Severe thrombocytopenia typically presents without anemia or leukopenia. Autoimmune hemolytic anemia is sometimes seen in association with ITP and this is referred to as Evans syndrome (Frederiksen and Schmidt, 1999).
Thrombotic Thrombocytopenic Purpura—Hemolytic Uremic Syndrome (TTP-HUS) is a relatively uncommon, life-threatening cause of thrombocytopenia. The classic diagnostic pentad of TTP includes microangiopathic hemolytic anemia, thrombocytopenia, renal insufficiency, fever, and mental status changes. However, the pentad is seen in less than 40% of cases (McCrae and Cines, 2000).

Drugs are a common cause of thrombocytopenia and practically any drug can cause thrombocytopenia. When evaluating a patient with thrombocytopenia, a medication history (including over-the-counter medications) should be carefully elicited and any recently initiated drug should be suspected. Cytotoxic chemotherapy and ethanol cause thrombocytopenia by directly inhibiting megakaryocytes (George et al., 1998). Most other drugs cause thrombocytopenia by immune mechanisms. Heparin is the most common cause of drug-induced thrombocytopenia. The GPIIb/IIIa inhibitors are associated with severe thrombocytopenia in approximately 0.5 to 2% of cases. Unlike other drug-induced thrombocytopenias, GPIIb/IIIa inhibitor-induced thrombocytopenia develops within 24 hours of exposure. This may be related to the presence of preformed “naturally occurring” antibodies against neoepitopes exposed by alteration of the GPIIb/IIIa molecule (Abrams and Cines, 2004).

With the increasing prevalence of cancer, chemotherapy has become a common cause of thrombocytopenia. The history is usually readily available and patients often have cytopenia in other cell lines also. With most chemotherapy agents, nadir blood count occurs 7 to 10 days after chemotherapy and recovery over 2 to 3 weeks. Some agents like nitrosoureas and mitomycin can cause more prolonged myelosuppression. Platelet transfusions are occasionally needed and dose
adjustment of future chemotherapy doses may be necessary. It is important to recognize that certain agents like mitomycin can also cause TTP (Sudhir and Vivek, 2006).

2.5.3.2 Signs and symptoms of thrombocytopenia

Some individuals with thrombocytopenia may experience external bleeding such as nosebleeds and bleeding of the gums. Some women may have longer periods or breakthrough bleeding. Bruising, particularly purpura in the forearms may be caused by spontaneous bleeding under the skin. Petechia may also occur on feet and legs. Painless, round and pinpoint petechiae usually appear and fade and sometimes group to form ecchymoses. Patients may also complain of malaise, fatigue and general weakness with or without loss of blood. Adults may develop large, blood filled bullae in the mouth (Abrams and Cines, 2004).

2.5.3.3 Management of thrombocytopenia

Initial management of Immune thrombocytopenia is undertaken with corticosteroids. Recently, short pulses of dexamethasone have been found to be very effective (Cheng et al., 2003). In case of severe thrombocytopenia or evidence of hemorrhage, a patient should receive immediate attention by undertaking platelet transfusion, intra-venal immunoglobulin infusion can be used when rapid platelet increment is desirable. Other treatment options include administration of danazol, cyclophosphamide, azathioprine, rituximab or autologous transplantation (McMillan, 1997).

In thrombotic thrombocytopenic purpura-hemolytic uremic syndrome patients, emergent plasma exchange is the cornerstone of treatment. It has the ability to both remove an unwanted substance
(inhibitory ADAMTS-13 antibody), replace a deficient substance (ADAMTS-13 enzyme), and allow for larger volume of plasma to be infused (Myriam et al., 2014). In case of thrombocytopenia caused by infection, treatment is supportive and consists of treating the underlying cause and providing supportive blood product transfusions—platelets, cryoprecipitate and fresh frozen plasma as needed are used to replace consumed coagulation factors (Sudhir and Vivek, 2006).

Thrombocytopenia is managed by use of medicinal plants. For instance, Euphorbia hirta has been used for ages by native American communities for its immunomodulatory activity in promoting the development of blood platelets, stopping hemorrhage and preventing further bleeding. It also improves nausea and abdominal cramps (Vijaya et al., 1995).

2.6 Lipid-related disorders

2.6.1 Hyperlipidemia

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is also identified as dyslipidemia, to describe the manifestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, dyslipidemia can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL) (Krauss et al., 2000). Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population, and are regarded as a
modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis (Vijaya et al., 1995).

2.6.1.1 Causes of hyperlipidemia
Hyperlipidemia can be caused by: familial combined hypercholesterolemia; familial hypertriglyceridemia; other disease states such as insulin and non-insulin dependent diabetes mellitus, hypothyroidism, cushing’s syndrome, dysproteinemias, nephrotic syndrome and renal failure, cholestatic disorders and low thyroid; drugs such as anabolic steroids, beta blockers, birth control pills and estrogens, corticosteroids, protease inhibitors, retinoids, thiazide diuretics; Diets like cholesterol intake greater than 300 mg per day; Lifestyle involving habitual excessive alcohol use, lack of exercise, smoking; Risk factors such as advancing age, sex (male), stress and postmenopause (Fredrickson et al., 1965).

2.6.1.2 Classification of hyperlipidemia
Hyperlipidemia, a major, modifiable risk factor for atherosclerosis and cardiovascular disease, including coronary heart disease (CHD) is classified under; Primary hyperlipidemias - are probably genetically based, but the genetic defects are known for only a minority of patients (Chait et al., 1990). Examples include;

i. Primary chylomicronemia: Recessive traits of deficiency of lipoprotein lipase or its cofactor (Yamamura et al., 1979).

ii. Familial hypercholesterolemia: A dominant trait, although levels of LDL tend to increase with normal VLDL (Yamamura et al., 1979).
iii. Familial combined (mixed) hyperlipoproteinemia: Elevated levels of VLDL, LDL (Yamamura et al., 1979).

iv. Familial dysbetalipoproteinemia: Increased LDL with increased TG and cholesterol levels (Yamamura et al., 1979).

v. Familial hypertriglyceridemia: Increased VLDL production with normal or decreased LDL (Yamamura et al., 1979).

vi. Familial mixed hypertriglyceridemia: Serum VLDL and chylomicrons are increased (Yamamura et al., 1979).

Secondary hyperlipidemia results from disease states such as Cushing’s syndrome, diabetes, liver disorders, renal disorders, thyroid disease, obesity, as well as alcohol consumption, estrogen administration and other drug-associated changes in lipid metabolism (Fredrickson et al., 1965).

2.6.1.3 Symptoms of hyperlipidemia

Hyperlipidemia usually does not cause symptoms. Very high levels of lipids or triglycerides can cause yellowish nodules of fat in the skin beneath eyes, elbows and knees, and in tendons (xanthomas). Sometimes pain, swelling of organs such as the liver, spleen or pancreas (pancreatitis) or whitish rings around the eye's iris occur (Chait et al., 1990).

2.6.1.4 Management of hyperlipidemia

2.6.1.4.1 Dietary intervention method

This is the primary treatment strategy, but drug therapy may often be added later to augment treatment. The main component of a healthy diet is a food pattern that is low in saturated fat and dietary cholesterol and provides adequate energy to support growth and maintain an appropriate
weight. Specific dietary recommendations include, decreased intakes of saturated fat - most effective in lowering LDL. Sources include stick margarine and commercial fried food such as French fries (James et al., 2006). Decreased intake of trans-fatty acids which are thought to increase LDL-C levels and appear to lower HDL. Decreased intake of dietary cholesterol leads to LDL reduction. Increased fiber intakes, soluble fiber can contribute to LDL reduction and sources of fiber include oats, fruits and vegetables (Chait et al., 1990). Encourage antioxidant food sources such as carotenoids. Reduce serum homocysteine levels, adequate intakes of folate, vitamins B6 and B12 as well as total fat restriction may keep homocysteine levels low (James et al., 2006).

2.6.1.4.2 Drug therapy method

Currently, there are many classes of medications that may be utilized in the pharmacological management of hyperlipidemia (Fredrickson et al., 1965). This includes HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors (statins). Mechanism of action involve reduction of cholesterol synthesis in liver; inhibiting the rate-limiting step in endogenous cholesterol synthesis; compensatory increase in synthesis of LDL receptors on hepatic and extra hepatic tissues; increase in hepatic uptake of circulating LDL which decreases plasma LDL cholesterol; increase in HDL, decrease in TGs and vasodilatation and decrease in atherosclerosis (Fung et al., 2011).

Fibrates are activators of lipoprotein lipase. It acts by; agonising peroxisome proliferator-activated receptor (PPAR); increasing hydrolysis of VLDL and chylomicrons; decreasing serum TGs; increasing clearance of LDL by liver and increase in HDL and expression of genes
responsible for increased activity of plasma lipoprotein lipase enzyme (Fung et al., 2011). Ezetimibe acts by inhibiting intestinal cholesterol, decrease in concentration of intrahepatic cholesterol; increase in uptake of circulating LDL; decrease in serum LDL cholesterol levels and compensatory increase in LDL receptors.

Nicotinic acid (Niacin) is an inhibitor of lipolysis, it acts as a potent inhibitor of lipolysis in adipose tissues, decreases mobilization of FFAs (major precursor of TGs) to the liver, increases HDL levels, decreases LDL and also decreases endothelial dysfunction and thrombosis (Fung et al., 2011).

The bile acid binding resins (bile acids sequestrants) are preferred in the pediatric age group as they are not systemically absorbed. They act as anion exchange resins, bind bile acids in the intestine forming complex that leads to loss of bile acids in the stools, increase the conversion of cholesterol into bile acids in the liver, compensatory increase in LDL receptors leading to decreased concentration of intrahepatic cholesterol, increase hepatic uptake of circulating LDL and decrease serum LDL cholesterol levels (Sameer et al., 2009).

Alternative methods such as use of plant sterols and stanols also assist in the reduction of LDL-C. Plant sterols reduce cholesterol absorption by competing with cholesterol for space within bile salt micelles in the intestinal lumen. The plant stanols, which are the result of the hydrogenation of sterols, are not absorbed as well as sterols. Ingestion of about 2 g per day of plant sterols or stanols produces LDL-C reduction of six percent to fifteen percent (Sameer et al., 2009).
2.7 Herbal management of hematological and lipid-related disorders

Medicinal phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). The term is generally used to refer to those chemicals that may have biological significance (Wagner, 1990). *Phyllanthus amarus* is a tropical shrub indigenous to the rainforest of Amazon and other tropical areas of the world (Samraj, 2001). The plant has been valued in many countries for its medicinal properties and curative potentials for a variety of ailments such as asthma/bronchial infection, jaundice and hepatitis B and other viral infections (Huang *et al*., 2003). It exhibits inhibitory effect on human immune virus (HIV) and reverse transcriptase activity, hypotensive, hypoglycaemic and hypocholestrolemic effect of *P. amarus* extract on hepatocytes of diabetic rats have also been reported as well as the antioxidative effect of the plant extract on S. *typhi* induced oxidative stress in rats. The *in-vitro* and antimicrobial activity of the plant extract against *Staphylococcus*, *Micrococcus* and *Pasteurella* spp has been reported (Agrawal *et al*., 2004).

*Phyllanthus amarus* effects are due to reversal of bone marrow depression thus improving haematopoeitic activity of the cells and the improvement in erythrocyte membrane integrity through the antioxidant potential of the extract, thereby reducing haemolysis (Naaz *et al*., 2007). Also bacterial infection causes deoxyribonucleic acid disintegration and has been shown to be ameliorated by the bacteriocidal effects of the extract, leading to an increase in protein synthesis and cell proliferation (Rajinder *et al*., 2008). Increase in protein synthesis may as well explain the increase in the level of Hb observed in many studies. Expectedly, increase in RBC count on administration of *P. amarus* extract results in increase in MCV, while increase in Hb results to increase in MCH and MCHC.
*Bridelia micrantha* belongs to the family Euphorbiaceae. It is commonly called coastal Golden-leaf in English and Ogaofia (boss of the bush) in Igbo language (Nigeria) and the plant is native to Africa. Herbalists of western Nigeria use the bark for induction of labour in full-term pregnancy and the leaf decoction for management of diabetes, while in Ghana and Ivory Coast, the leaf decoction is given to expel guinea worm and as purgative, respectively. In Senegal, it is administered in various preparations for the treatment of stomach and intestinal problems, sterility and edema (Lin *et al.*, 2002). The leaf extract has also been reported to have antimicrobial, antiulcer, hypoglycemic and antioxidant activities. This functions are attributed to the fact that extracts of this plant contains some phytochemicals that can stimulate the formation of erythropoietin which is a glycoprotein hormone that stimulates stem cells in the bone marrow to produce red blood cells. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia. The lipid lowering activity of extracts of this plant may also be attributed to its phytochemical constituents (Nemenibo, 2003).

These findings suggest that extracts of this plant may be effective in ameliorating cardiac disease complications seen in diabetes mellitus. This indicates the protective potential of the extract against diabetic hyperlipidemia which may be beneficial in preventing diabetic complications. The extract may have achieved this by blocking cholesterol biosynthesis; binding and reducing the production of bile acids or by increased generation of propionate which has been shown to reduce cholesterol levels. The reduction of the hyperlipidemia by extracts of this plant also may have been presumably mediated by a control of lipid metabolism (Cho *et al.*, 2002).
In Siddha medicine, the herb *Achyranthes aspera* (Tamil name – Nayuruvi) is used for leucorrhoea, haemorrhoids, obesity, oligomenorrhoea and gastric ulcers. The paste prepared from the leaf juice is used for syphilitic ulcers. It is also used for regulating menstruation. The root is used for treating dental caries (Harikumar *et al.*, 2012).

The alcoholic extract of *A. aspera*, at 100 mg/kg dose lowered serum cholesterol (TC), phospholipid (PL), triglyceride (TG) and total lipids (TL) levels by 60, 51, 33 and 53%, respectively in triton induced hyperlipidemic rats. The chronic administration of this drug at the same doses to normal rats for 30 days, lowered serum TC, PL, TG and TL by 56, 62, 68 and 67%, respectively followed by significant reduction in the levels of hepatic lipids. The faecal excretion of cholic acid and deoxycholic acid increased by 24 and 40%, respectively, under the action of this drug. The possible mechanism of action of cholesterol lowering activity of *A. aspera* may be due to rapid excretion of bile acids causing low absorption of cholesterol (Harikumar *et al.*, 2012).

While terpenoids mediate immunological processes by enhancing antibody production and supressing T cell response due to presence of sesquesterpen lactones and helenalin (Hall *et al.*, 1979). On the other hand, lectins bind to lymphocytes and induce their mitosis. Others such as Phenols, quinines and lipids contains molecules such as ubiquinone Q7 which is able to induce secretion of colony stimulating factor (CSF) and increase the number of peripheral granulocytes (Wagner *et al.*, 1985).
Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the reduction of lipid profile is considered to be an important therapeutic approach and efforts have been made to identity the lipid lowering effect of various medicinal plants (Onat et al., 2006). The prevention of oxidation of low density lipoprotein cholesterol by the antioxidant compounds like poly-phenolics and flavonoids is therefore important in the prevention of cardiovascular diseases.

2.8 *Carissa edulis* (Forssk.)Vahl

2.8.1 Description and distribution of *Carissa edulis*

*Carissa edulis* (Forssk.)Vahl belongs to the family Apocynaceae. It was formerly known as *C. pubescence* (Hutchison and Dalziel, 1963). *C. edulis* is a spiny, much branched, small tree, evergreen shrub or scrambler, up to 5 m in height, with a milky sap. The barks are grey, smooth, young branchlets with or without hairs. Its spines are simple, straight, 2-5 cm long, usually single. The Leaves are ovate to ovate-elliptic, opposite, occasionally almost circular, 2.5-6 x 1.8-3 cm, leathery, dark green above, paler green below, with or without short, soft hairs; lateral veins obscure; apex tapering, often with a bristle like tip; base rounded to shallowly lobed; margin entire; petiole 1-4 mm long. Its flowers are white tinged with purple, red or pink, up to 1.8 cm long, about 2 cm in diameter, slender, tubular, with corolla lobes overlapping to the right, sweetly scented, in terminal heads about 4 cm in diameter. The Fruits are ovoid to almost spherical, up to 1.1 cm in diameter, red-black, ripening to purplish black, containing 2-4 flat seeds (Najma, 2002; Van wyk et al., 2007; Raimonda et al., 2009).
The plant is native in the following countries: Australia, Botswana, Cambodia, Cameroon, Eritrea, Ethiopia, Ghana, Guinea, Japan, Kenya, Myanmar, Namibia, Nigeria, Papua, Saudi Arabia, Senegal, South Africa, Sudan, Tanzania, Thailand, Uganda, Vietnam and Yemen (Raimonda et al., 2009).

![Picture of C. edulis plant (commons.wikimedia.org)](image)

**Figure 2.1:** Picture of *C. edulis* plant (commons.wikimedia.org)

### 2.8.2 Cultural uses of *C. edulis*

In Central Kenya, a piece of the root is fixed into a hut roof as snake repellent. Its fruits are sweet and pleasant to eat (Bussmann et al., 2006). Animals especially goats in the dry parts of Embu county browse on the plant, ‘Mukawa’ for feed. The species is a source of excellent firewood (Sidigia et al., 1990). The abundant branching habit and the presence of spines make the plant suitable for planting as a protective hedge (Nedi et al., 2004).
The use of local plant materials including *Engamryaki* as normal dietary additives is common among pastoralist tribes namely; Maasai, Sonjo, Gogo, Kurya and Barbaigs in Tanzania. Fruits are sweet and pleasant to eat; in Ghana, they are normally added to the food of sick people as an appetizer. Vinegar has been made from them by fermentation; in Sudan and Kenya, they are made into a jam. The roots are put into water gourds by the Maasai to impart an agreeable taste and are added to soups and stews for the same reason. Goats and camels in the dry parts of Sudan browse on *C. edulis*. The ripe fruits are utilized in curries, tarts, puddings and chutney. When only slightly under ripe, they are made into jelly (Pakrashi *et al*., 1968).

### 2.8.3 Medicinal importance of *C. edulis*

Like other terrestrial plants, *Carissa edulis* has ethnopharmacological relevance and has also been exploited by the local people in the search for remedies for various ailments (Tolo *et al*., 2006). The plant was much more used in traditional as well as in modern era. The plant parts are used in ethno-medicine for wide variety of illnesses, such as epilepsy (Ya’u *et al*., 2008), headache, chest complaints, gonorrhea, syphilis, rheumatism, obesity, rabies and as well as a diuretic (Nedi *et al*., 2004). Other folkloric uses of *C. edulis* include fever, sickle cell anaemia and hernia (Ibrahim, 1997).

*Carissa edulis* is the best known member of the genus *Carissa* as it has been used as a traditional medicinal plant over thousands of years in the ayurvedic system of medicine as it is practiced on the Indian subcontinent. The root is credited with stomachic, antidiarrhoeal and antianthelmintic properties (Pakrashi *et al*., 1968). The herb has been used for the treatment of breast cancer and
malaria among some communities in Kenya (Nedi et al., 2004; Bussmann et al., 2006; Tolo et al., 2006). The root is used to treat glandular inflammation, lumbago and other pains (Burkill, 1985). Various parts of the plant are used in traditional medicine for the treatment of fever, oedema, toothache, cough, ulcer, sickle cell anaemia and hernia. Preparations of *C. edulis* have been used in the Nigerian traditional medicine for the management of fever, sickle cell disease, pain and inflammation for many years and their efficacy is widely acclaimed among the Hausa communities of northern Nigeria (Ya’u et al., 2013).

The available scientific data supports ethnomedical claims on the following pharmacological actions: *Carissa edulis* is reported to have anticonvulsant activity which supports the ethnomedicinal claims of the plant in the treatment of epilepsy (Ya’u et al., 2008). They demonstrated that *C. edulis* at 5 and 20 mg/kg, possess significant anticonvulsant activity in mouse model. These results suggest that *C. edulis* has anticonvulsant activity which supports the ethnomedicinal claim of the use of the plant in the management of epilepsy.

The aqueous root extracts of *C. edulis* have been show to exhibit significant activity against the *Herpes simplex* virus both *in vitro* and *in vivo* for both wild type and resistant strains of HSV (Tolo et al., 2006). The extracts hav been reported to possess potent antiviral activity against Sindbis virus, polio virus, HIV-1 and herpes simplex virus (HSV) (Tylor, 1996).

Extracts of *C. edulis* have demonstrated strong hepato-protective and antioxidant activity in experimental wistar albino rats which was confirmed through measurements of aspertate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, total protein content and
total bilirubin content. Antioxidant action was determined by estimating lipid peroxidation, reduced glutathione, and super oxide dismutase and catalase activity in liver (Chatterjee and Roy, 1965). These results suggest that C. edulis has hepatoprotective and antioxidant activity which are beneficial in the prevention of liver disorders and cancer and hence supports the ethnomedicinal claim of the use of the plant against chronic illnesses. In pharmacological studies, root wood extracts of C.edulis have been reported to induce diuretic effect in rats (Nedi et al., 2004).

The leaf extract of C. edulis reduces blood glucose level in streptozotocin (STZ) diabetic rats, indicating the presence of compounds with hypoglycaemic activity (El-Fiky et al., 1996). Effect of oral administration of 2 g/kg body weight of the ethanolic extract of Carissa edulis leaves on blood glucose levels in diabetic rats. Treatment with C. edulis extract significantly reduced the blood glucose level in diabetic rats during the first three hours of treatment with potency similar to that of the biguanide metformin. On the other hand, in normal rats, it produced insignificant changes in blood glucose levels compared to glibenclamide treatment. It was postulated that, C. edulis contain some hypoglycaemic principles which act probably by initiating the release of insulin from the pancreatic β-cells of normal animals (sulfonylurea-like effect). These results suggest that C. edulis has antidiabetic activity which supports the ethnomedicinal claim of the use of the plant in the management of diabetes (El-Fiky et al., 1996).

The crude extract of C. edulis has high potential of being developed into anti-malarial drug which is cheap and available to the poor (Kebenei et al., 2011). Besides, the results confirmed the correlation between the ethno anti-malarial usage and anti-malarial bioactivity of C. edulis
(Achenbach et al., 1983; Bentley et al., 1984; Achenbach et al., 1985; Ibrahim, 1997). The cardiotonic activity and prolonged blood pressure lowering effect of *C. edulis* was previously reported (Vohra and De, 1963). The cardiac activity of water-soluble fraction has been attributed to the presence of the odoroside glucosides. These results suggest that *Carissa edulis* has blood pressure lowering activity which supports the ethnomedicinal claim of the use of the plant in the management of high blood pressure (Vohra and De, 1963).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant material

Fresh leaves of *Carissa edulis* were collected from Siakago division, Mbeere North Sub-County, Embu County, Kenya. The fresh leaves were identified with the help of local herbalists. The information gathered included vernacular names, plant parts used and the ailment treated. The samples were collected with acceptable bio-conservative methods and were properly sorted out, cleaned, and transported in polythene bags to Kenyatta University, Biochemistry and Biotechnology laboratories for drying and crushing. The plant samples were provided to an acknowledged Taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium, Nairobi, Kenya for future reference. The leaves of *C. edulis* were chopped into small pieces and air dried at room temperature for two weeks until properly dried. They were then ground into fine homogenous powder using an electric mill followed by sieving through mesh sieve and stored at room temperature awaiting extraction.

3.2 Extraction

In this stage, 200g of powder was soaked in 500ml of cold 1:1 mixture of DCM and MeOH and stirred for six hours to extract the active compounds. The successive extract was filtered using whatman’s filter papers and the filtrate concentrated under reduced pressure and vacuum using rotary evaporator. The concentrate was put in an airtight container and stored at -4°C before use in bioassay studies.


3.3 Experimental animals

Male healthy winstar rats (20), aged between two to three months and weighing an average of 150g were used in this study. They were bred in the animal house of the Department of Biochemistry and Biotechnology, Kenyatta University. The rats were housed in cages, maintained under standard laboratory conditions of 12 hour light and dark sequence, at ambient temperature of 25 ± 2ºC and 35-60% humidity. The animals were fed with standard rat pellets obtained from Unga Feeds Limited, Kenya, and water *ad libitum*. Ethical guidelines and procedures for handling experimental animals were followed.

3.4 Experimental design

The rats were randomly assigned into four groups where each group was having five normal rats. The same group of rats were followed for the 21 days of this experimental study. The groups were designated as A-D and were designed as follows: Group A was the control group and received normal saline (1 ml each) for 21 days. The other experimental groups were as follows; Group B animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 50 mg/kgbw for 21 days, Group C animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 75 mg/kgbw for 21 days and Group D animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 100 mg/kgbw for 21 days. The administration was done with the aid of a metal oropharyngeal cannula. Each rat was marked at the tail using a permanent marker pen to distinguish it from the lot. Daily cleaning of the cages was carried out.
Blood from rats in all groups was taken before the commencement of the first oral administration, then on the seventh, fourteenth and twenty-first days. During the entire period of study, rats were allowed free access to mice pellet and water *ad libitum* and observed for any signs of general illness, change in behaviour and/or mortality.

3.5 Preparation of extracts doses for administration

The dose level of 50 mg/kgbw was prepared by dissolving 0.038 g of the extract in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water, the dose level of 75 mg/kgbw was prepared by dissolving 0.056 g in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water while the dose level of 100 mg/kgbw was prepared by dissolving 0.076 g in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water (Erhirhie *et al.*, 2014).

3.6 Collection of blood samples

Blood samples were collected at the start of the experiment, then on the seventh day, the fourteenth day and finally on the twenty-first day from the tails of rats for the determination of hematological parameters. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails pierced. Bleeding was enhanced by gently milking the tail from the body towards the tip. Blood of approximately 2 ml was drawn into BD vacutainer® (BD Plymouth, UK) sample bottles containing anticoagulant (EDTA) for hematological parameters analysis. Another 5 ml of the blood was allowed to clot for 10 minutes at room temperature and thereafter centrifuged at 1282 g for 5 min using Hermle bench top centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and
used within 12 hours of preparation for the lipid assay. On the twenty-first day the animals were euthanized by use of chloroform (Tietz et al., 1994).

3.7 Determination of hematological parameters

Hematological parameters and indices were determined from unclotted blood samples using standard protocols (Tietz et al., 1994). Erythrocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width and platelets, plateletcrit, mean platelet volume, platelet distribution width, white blood cell, neutrophils, monocytes, lymphocytes, eosinophils and basophils were determined using the Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France).

Serum lipid profiles were analysed by use of Roche modular (model P800, Mannheim, Germany) auto-analyser machine. The assay kits for cholesterol, triacylglycerols, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Phillips Healthcare Technologies Ltd. Nairobi, Kenya.

3.8 Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by Harbone (1998) and Kotake (2000). Standard screening tests for detecting the presence of different
chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

3.8.1 Saponins (Froth test)

To test for saponins, 2ml of the plant extract was mixed with a few drops of sodium bicarbonate solution and shaken vigorously (Harbone, 1998; Kotake, 2000). The extract was then allowed to stand for 15-20 minutes and was classified for saponin content as follows:

a) Negative - No froth
b) Weakly positive - Froth less than 1 cm
c) Positive - Froth 1.2 cm high
d) Strongly positive - Froth greater than 2 cm high

3.8.2 Alkaloids

The extracts were tested for alkaloids by first acidifying 5 ml of each extract with 1M HCl. This acidic medium was heated and then treated with Dragendroff’s reagent. The formation of an orange or reddish brown precipitate was regarded as positive for the presence of alkaloids (Harbone, 1998; Kotake, 2000).

3.8.3 Terpenoids (Salkowski test)

To 0.5 g of each of the extract was added 1 ml of ethyl acetate/petroleum ether and then mixed into 2 ml of chloroform. Three mililitres of concentrated sulphulic acid (H₂SO₄) was carefully added alongside to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids (Harbone, 1998; Kotake, 2000).
3.8.4 Flavonoids (Sodium hydroxide test)

Extracts were tested for flavonoids by mixing 2 ml of each extract with 2 ml of diluted sodium hydroxide (NaOH). An intense/golden yellow precipitate indicated positive results (Harbone, 1998; Kotake, 2000).

3.8.5 Cardiac glycosides (Keller-Kilian test)

To test for cardiac glycosides, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drop of 10% ferric chloride (FeCl₃) solution. This was under-layered with 1 ml of concentrated sulphuric acid. A brown, violet or greenish ring at the interphase indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Harbone, 1998; Kotake, 2000).

3.8.6 Steroids

To test for steroids, 0.5 g of each of the extract was dissolved in 2 ml of chloroform. Three millilitres of concentrated H₂SO₄ was carefully added by the sides of the test tube to form a layer. A reddish brown colour at the interface indicates the presence of steroidal ring (Harbone, 1998; Kotake, 2000).
3.8.7 Phenols

The extracts were screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of each extract. Formation of blue to green colour indicated the presence of phenolics (Harbone, 1998; Kotake, 2000).

3.8.8 Data management and analysis

Experimental data on different hematological parameters and serum lipid profiles were obtained from all the animals on the first day and compared with the seventh, fourteenth and twenty-first days for the three dose levels. It was recorded and tabulated on a broad spreadsheet sheet. Results were expressed as Mean ± Standard error of mean (SEM) for analysis. Statistical significance of difference among the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test to separate the means and obtain the specific significant differences among the different groups. The value of P ≤ 0.05 was considered significant. Analysis of the data was done using Minitab statistical software, Version 17.
CHAPTER FOUR

RESULTS

4.1 Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats

Generally, during the twenty-one days of this experimental study, the levels of RBC, HB, PCV, MCV, MCH, MCHC and RDW did not change significantly among the normal rat models in the control group (Table 4.1a).

After fourteen and twenty-one days of administration of the extract at the dose level of 50 mg/kgbw, there was significant increase in the levels of MCV (p<0.01; Table 4.1a). This dose level, however, did not have any significant effect on the levels of RBC, HB, PCV, MCH, MCHC and RDW (p<0.01; Table 4.1a).

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 75 mg/kgbw caused significant increase in levels of MCH after fourteen days of administration (p<0.01; Table 4.1a). This dose level, however, did not have any significant effect on the levels of RBC, HB, PCV, MCHC and RDW (p<0.01; Table 4.1a).

At the dose level of 100 mg/kgbw, there was no significant change in the levels of RBC and all the related parameters during the twenty-one days of administration of the DCM-MeOH leaf extracts of *C. edulis* (p<0.01; Table 4.1a).

When the level of erythrocytes and related parameters were compared on specific day across all dose concentrations, it was evident that there were significant changes (Table 4.1b). After
fourteen days of administration of the DCM-MeOH leaf extracts of *C. edulis*, the levels of MCV significantly increased at all the dose levels (*p*<0.01; Table 4.1b). The levels of RBC significantly increased at the dose level of 50 mg/kgbw, levels of PCV significantly increased at the dose level of 100 mg/kgbw, the levels of MCHC significantly increased at the dose levels of 50 and 100 mg/kgbw and levels of MCH were significantly increased at the dose levels of 75 mg/kgbw after the fourteen days of extract administration (*p*<0.01; Table 4.1b).

After twenty-one days of administration of the DCM-MeOH leaf extracts of *C. edulis*, the levels of RDW significantly increased at the dose level of 100 mg/kgbw (*p*<0.01; Table 4.1b). The levels of RBC, PCV, MCV, MCH and MCHC, however did not significantly increase at all dose levels after the twenty-one days (*p*<0.01; Table 4.1b).
Table 4.1a: Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Control</th>
<th>50 mg/kgbw</th>
<th>75 mg/kgbw</th>
<th>100mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 7</td>
</tr>
<tr>
<td>RBC (×10¹²/l)</td>
<td>6.76±0.</td>
<td>6.43±0.</td>
<td>6.83±0.</td>
<td>6.71±0.</td>
</tr>
<tr>
<td></td>
<td>12a</td>
<td>25a</td>
<td>05a</td>
<td>04a</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>15.76±</td>
<td>15.26±</td>
<td>15.34±</td>
<td>15.71±</td>
</tr>
<tr>
<td></td>
<td>0.45a</td>
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<td>0.13a</td>
<td>0.18a</td>
</tr>
<tr>
<td>PCV (l/l)</td>
<td>49.00±</td>
<td>49.11±</td>
<td>49.56±</td>
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</tr>
<tr>
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</tr>
<tr>
<td>MCV (fl)</td>
<td>71.24±</td>
<td>70.78±</td>
<td>71.65±</td>
<td>71.00±</td>
</tr>
<tr>
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<td>0.13a</td>
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</tr>
<tr>
<td>MCH (pg)</td>
<td>22.00±</td>
<td>22.18±</td>
<td>22.04±</td>
<td>22.04±</td>
</tr>
<tr>
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<td>0.07a</td>
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<tr>
<td>MCHC (g/dl)</td>
<td>30.26±</td>
<td>30.14±</td>
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</tr>
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</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the days with the same extract dose level.
### Table 4.1b: Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Day 7</th>
<th></th>
<th></th>
<th></th>
<th>Day 14</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Day 21</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dose 1</td>
<td>Dose 2</td>
<td>Dose 3</td>
<td>Control</td>
<td>Dose 1</td>
<td>Dose 2</td>
<td>Dose 3</td>
<td>Control</td>
<td>Dose 1</td>
<td>Dose 2</td>
<td>Dose 3</td>
<td></td>
</tr>
<tr>
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<td>0.04^a</td>
<td>0.07^a</td>
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<td>0.25^a</td>
<td>0.02^ab</td>
<td>0.20^b</td>
<td>0.03^ab</td>
<td>0.05^a</td>
<td>1.01^a</td>
<td>0.11^ab</td>
<td>0.08^a</td>
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<tr>
<td><strong>HB (g/dl)</strong></td>
<td>15.76±</td>
<td>15.71±</td>
<td>15.86±</td>
<td>15.12±</td>
<td>15.26±</td>
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<td>0.09^a</td>
<td>0.15^a</td>
<td>0.08^ab</td>
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<td>0.18^a</td>
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<td>0.08^b</td>
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</tr>
<tr>
<td><strong>PCV (l/l)</strong></td>
<td>49.00±</td>
<td>49.00±</td>
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<td>49.86±</td>
<td>49.11±</td>
<td>49.31±</td>
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</tr>
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<td></td>
<td>0.07^a</td>
<td>0.12^a</td>
<td>0.13^a</td>
<td>0.10^ab</td>
<td>0.07^a</td>
<td>0.18^a</td>
<td>0.16^a</td>
<td>0.18^b</td>
<td>0.15^a</td>
<td>0.18^a</td>
<td>0.18^a</td>
<td>0.08^ab</td>
<td></td>
</tr>
<tr>
<td><strong>MCV (fl)</strong></td>
<td>71.24±</td>
<td>71.00±</td>
<td>71.94±</td>
<td>72.10±</td>
<td>70.78±</td>
<td>72.00±</td>
<td>72.79±</td>
<td>72.38±</td>
<td>71.65±</td>
<td>72.50±</td>
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<td>72.42±</td>
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</tr>
<tr>
<td></td>
<td>0.13^a</td>
<td>0.19^a</td>
<td>0.24^ab</td>
<td>0.08^ab</td>
<td>0.22^a</td>
<td>0.15^b</td>
<td>0.18^b</td>
<td>0.20^b</td>
<td>0.16^a</td>
<td>0.16^ab</td>
<td>0.26^ab</td>
<td>0.14^ab</td>
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</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>22.00±</td>
<td>22.04±</td>
<td>22.88±</td>
<td>22.48±</td>
<td>22.18±</td>
<td>22.41±</td>
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<tr>
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<td>0.09^a</td>
<td>0.04^a</td>
<td>0.16^a</td>
<td>0.12^a</td>
<td>0.18^b</td>
<td>0.18^b</td>
<td>0.07^a</td>
<td>0.18^a</td>
<td>0.18^b</td>
<td>0.10^a</td>
<td>0.18^a</td>
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</tr>
<tr>
<td><strong>MCHC (g/dl)</strong></td>
<td>30.26±</td>
<td>31.34±</td>
<td>30.80±</td>
<td>31.28±</td>
<td>30.14±</td>
<td>31.50±</td>
<td>30.99±</td>
<td>31.56±</td>
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<tr>
<td></td>
<td>0.17^a</td>
<td>0.20^ab</td>
<td>0.04^ab</td>
<td>0.18^ab</td>
<td>0.35^a</td>
<td>0.07^b</td>
<td>0.15^ab</td>
<td>0.16^b</td>
<td>2.05^a</td>
<td>0.17^ab</td>
<td>0.18^ab</td>
<td>0.18^a</td>
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</tr>
<tr>
<td><strong>RDW (%)</strong></td>
<td>16.30±</td>
<td>17.06±</td>
<td>16.61±</td>
<td>17.08±</td>
<td>16.26±</td>
<td>16.80±</td>
<td>16.82±</td>
<td>17.25±</td>
<td>16.54±</td>
<td>16.68±</td>
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</tr>
<tr>
<td></td>
<td>0.27^a</td>
<td>0.23^ab</td>
<td>0.22^ab</td>
<td>0.11^ab</td>
<td>0.15^a</td>
<td>0.04^ab</td>
<td>0.21^ab</td>
<td>0.20^c</td>
<td>0.09^a</td>
<td>0.21^a</td>
<td>0.16^a</td>
<td>0.17^b</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50mg/kgbw; Dose 2: 75mg/kgbw; Dose 3: 100mg/kgbw
4.2 Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC count in normal rats

Generally, during the twenty-one days of this experimental study, the levels of WBC, lymphocytes, monocytes, neutrophils, eosinophils and basophils of the normal rats in the control group did not change significantly (Table 4.2a).

After fourteen and twenty-one days of extract administration at the dose level of 50 mg/kgbw, there was significant increase in the levels of WBC, lymphocytes, monocytes, neutrophils and eosinophils (p<0.01; Table 4.2a). The dose level of 50 mg/kgbw, however had no significant effect on the levels of basophils during the entire study (p<0.01; Table 4.2a).

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 75 mg/kgbw caused significant increase in the levels of WBC, lymphocytes, monocytes and neutrophils after fourteen and twenty-one days of extract administration (p<0.01; Table 4.2a). The levels of basophils was increased after twenty-one days of extract administration (p<0.01; Table 4.2a). The levels of eosinophils, however, was not significantly affected after twenty one days of extract administration at the dose level of 75 mg/kgbw (p<0.01; Table 4.2a).

The dose level of 100 mg/kgbw caused significant increase in the levels of WBC, lymphocytes, monocytes and neutrophils after fourteen and twenty-one days of DCM-MeOH leaf extracts of *C. edulis* administration, while the levels of eosinophils also significantly increased after fourteen days of extract administration at the dose level of 100 mg/kgbw (p<0.01; Table 4.2a).
Table 4.2a: Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC counts in normal rats

<table>
<thead>
<tr>
<th>PARAMETE RS</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 7</td>
</tr>
<tr>
<td>WBC (×10^9/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>±0.56a</td>
<td>+0.56a</td>
<td>+0.50a</td>
<td>+0.19a</td>
<td>+0.43a</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>35.06</td>
<td>35.44</td>
<td>35.62</td>
<td>37.40</td>
</tr>
<tr>
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<td>±0.27a</td>
<td>±0.19a</td>
<td>±0.24a</td>
<td>±0.42a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>27.54</td>
<td>27.68</td>
<td>27.72</td>
<td>29.56</td>
</tr>
<tr>
<td></td>
<td>±0.33a</td>
<td>±0.19a</td>
<td>±0.23a</td>
<td>±0.55a</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.86</td>
<td>3.84</td>
<td>3.87</td>
<td>5.68</td>
</tr>
<tr>
<td></td>
<td>±0.30a</td>
<td>±0.29a</td>
<td>±0.25a</td>
<td>±0.32a</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0.67</td>
<td>0.65</td>
<td>0.66</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>±0.02a</td>
<td>±0.02a</td>
<td>±0.03a</td>
<td>±0.07a</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.13</td>
<td>0.14</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>±0.02a</td>
<td>±0.02a</td>
<td>±0.02a</td>
<td>±0.02a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the days with the same extract dose level.
When the levels of total and differential WBC counts in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 4.2b). After seven days of DCM-MeOH leaf extracts of C. edulis administration, there were significant increase in the levels of WBC, monocytes, neutrophils, eosinophils and basophils at all the dose levels while the levels of monocytes increased significantly at the dose level of 50 mg/kgbw (p<0.01; Table 4.2b).

After fourteen days of DCM-MeOH leaf extracts of C. edulis administration, there was significant increase in the levels of WBC at the dose level of 75 and 100 mg/kgbw while the levels of lymphocytes, monocytes and neutrophils increased significantly at all the dose levels (p<0.01; Table 4.2b). The levels of eosinophils and basophils, however, did not significantly change at all dose levels on the fourteenth day (p>0.01; Table 4.2b).

After twenty one days of DCM-MeOH leaf extracts of C. edulis administration, there was significant increase in the levels of WBC at dose levels of 50 and 100 mg/kgbw while the levels of lymphocytes, monocytes and neutrophils significantly increased at all the dose levels (p<0.01; Table 4.2b). The levels of basophils increased significantly at the dose levels of 75 and 100 mg/kgbw (p>0.01; Table 4.2b). The levels of eosinophils, however, did not significantly at all the dose levels on this particular day of the study (p>0.01; Table 4.2b).
### Table 4.2b: Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC counts in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC (×10^9/l)</strong></td>
<td>7.66±0.56</td>
<td>9.12±0.43</td>
<td>9.22±0.23</td>
<td>9.26±0.30</td>
<td>7.76±0.50</td>
<td>12.14±0.45</td>
<td>13.21±0.23</td>
<td>12.20±0.24</td>
<td>7.76±0.50</td>
<td>12.14±0.45</td>
<td>13.21±0.23</td>
<td>12.20±0.24</td>
</tr>
<tr>
<td><strong>Lymphocytes (%)</strong></td>
<td>35.06±0.27</td>
<td>37.40±0.42</td>
<td>35.92±0.95</td>
<td>36.58±0.24</td>
<td>35.44±0.19</td>
<td>40.30±0.34</td>
<td>39.88±0.96</td>
<td>39.50±0.28</td>
<td>35.62±0.24</td>
<td>41.30±0.38</td>
<td>43.32±0.79</td>
<td>41.45±0.39</td>
</tr>
<tr>
<td><strong>Monocytes (%)</strong></td>
<td>27.54±0.33</td>
<td>29.56±0.55</td>
<td>29.06±0.51</td>
<td>29.22±0.12</td>
<td>27.68±0.19</td>
<td>32.94±0.50</td>
<td>33.14±0.51</td>
<td>32.28±0.20</td>
<td>27.72±0.23</td>
<td>34.40±0.65</td>
<td>36.76±0.61</td>
<td>34.28±0.46</td>
</tr>
<tr>
<td><strong>Neutrophils (%)</strong></td>
<td>3.86±0.30</td>
<td>5.68±0.32</td>
<td>6.18±0.46</td>
<td>5.88±0.40</td>
<td>3.84±0.29</td>
<td>8.70±0.30</td>
<td>10.18±0.48</td>
<td>8.26±0.20</td>
<td>3.87±0.25</td>
<td>8.26±0.20</td>
<td>8.30±0.21</td>
<td>8.19±0.15</td>
</tr>
<tr>
<td><strong>Eosinophils (%)</strong></td>
<td>0.67±0.02</td>
<td>0.79±0.07</td>
<td>0.82±0.03</td>
<td>0.81±0.05</td>
<td>0.65±0.02</td>
<td>1.14±0.08</td>
<td>1.17±0.05</td>
<td>0.99±0.08</td>
<td>0.66±0.03</td>
<td>1.39±0.10</td>
<td>1.51±0.34</td>
<td>1.43±0.34</td>
</tr>
<tr>
<td><strong>Basophils (%)</strong></td>
<td>0.13±0.02</td>
<td>0.21±0.02</td>
<td>0.20±0.03</td>
<td>0.25±0.03</td>
<td>0.14±0.02</td>
<td>0.24±0.02</td>
<td>0.29±0.02</td>
<td>0.29±0.06</td>
<td>0.12±0.02</td>
<td>0.24±0.02</td>
<td>0.26±0.02</td>
<td>0.58±0.33</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the extract dose levels with the same day.
4.3 Effects of DCM- MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats

Generally, during the twenty-one days of this experimental study, the levels of platelets, MPV, PCT and PDW of the normal rats in the control group did not show any significant change (Table 4.3a).

Administration of DCM-MeOH leaf extracts of *C. edulis* lead to significant increase in the levels of platelets and related parameter profiles in normal rats after fourteen and twenty-one days at the dose level of 50 mg/kgbw, (p<0.01; Table 4.3a).

Administration of DCM-MeOH leaf extracts of *C. edulis* at the dose level of 75 mg/kgbw led to significant increase in the levels of platelets, MPV, PCT, PDW after fourteen and twenty-one days (p<0.01; Table 4.3a). The levels of PCT were, however, not affected at this dose level.

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 100 mg/kgbw, resulted into significant increase in the levels of platelets, MPV and PDW after fourteen and twenty-one days of administration (p<0.01; Table 4.3a). The levels of PCT were, however, not affected at this dose level.
Table 4.3a: Effects of DCM-MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 7</td>
</tr>
<tr>
<td>PLATELETS (×10^9/l)</td>
<td>794.60 ± 1.72</td>
<td>796.80 ± 1.36</td>
<td>796.40 ± 1.36</td>
<td>852.80 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>±1.36a</td>
<td>±1.36a</td>
<td>±1.36a</td>
<td>±1.93b</td>
</tr>
<tr>
<td>PCT</td>
<td>0.26±</td>
<td>0.28±</td>
<td>0.28±</td>
<td>0.43±</td>
</tr>
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<td>0.01a</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.04a</td>
</tr>
<tr>
<td>MPV</td>
<td>4.72±</td>
<td>4.74±</td>
<td>4.75±</td>
<td>5.27±</td>
</tr>
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<td>0.07a</td>
<td>0.20a</td>
</tr>
<tr>
<td>PDW</td>
<td>17.90±</td>
<td>17.98±</td>
<td>17.92±</td>
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<td>0.44a</td>
<td>0.62a</td>
<td>0.56a</td>
<td>0.23a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the days with the same extract dose level.
When the level of platelets and related parameter profiles in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 4.3b). After seven days of administration of the DCM-MeOH leaf extracts of *C. edulis*, the levels of platelets and PDW were significantly increased at all the dose levels (p<0.01; Table 4.3b). The levels of MPV and PCT also increased significantly at the dose levels of 75 and 100 mg/kgbw (p<0.01; Table 4.3b).

After fourteen days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of platelets, MPV and PDW significantly increased at all the dose levels (p<0.01; Table 4.3b). The levels of PCT, however, did not show any significant change on this day across all the dose concentrations (p>0.01; Table 4.3b).

After twenty-one days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of platelets, MPV, PCT and PDW significantly increased at all the dose levels (p<0.01; Table 4.3b).
Table 4.3b: Effects of DCM-MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dose 1</td>
<td>Dose 2</td>
</tr>
<tr>
<td>PLATELET S($\times10^9$/l)</td>
<td>794.60 ±1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>852.80 ±2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>860.20 ±1.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.26 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ±0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>MPV(fL)</td>
<td>4.72 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.27 ±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.92 ±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>17.90 ±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.42 ±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.54 ±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50 mg/kgbw; Dose 2: 75 mg/kgbw; Dose 3: 100 mg/kgbw
4.4 Effects of DCM-MeOH leaf extracts of C. edulis on serum lipid profiles in normal rats

Generally, during the twenty-one days of this experimental study, the levels of TG, TC, HDL-C and LDL-C of the normal rats in the control group did not show any significant change (Table 4.4a).

After twenty one days of administration of the extract at the dose level of 50 mg/kgbw, the TC levels significantly decreased on day seven, fourteen and twenty one of this study (p>0.01; Table 4.4a). After fourteen and twenty-one days of extract administration at the same dose level, there was a significant decrease in LDL-C levels (p>0.01; Table 4.4a). The concentration of TG and HDL-C, however, did not show any significant increase during all the days of this study at the dose level of 50 mg/kgbw (p<0.01; Table 4.4a).

The DCM-MeOH leaf extracts of C. edulis at the dose level of 75 mg/kgbw caused significant increase in the levels of HDL-C after twenty-one days of administration (p>0.01; Table 4.4a). This dose level however did not cause any significant change in the levels of TG, TC and LDL-C during the twenty-one days of the study (p>0.01; Table 4.4a).

The DCM-MeOH leaf extracts of C. edulis at the dose level of 100 mg/kgbw caused significant decrease in the concentration of TG after seven, fourteen and twenty one days of oral administration (p>0.01; Table 4.4a). The levels of TC and LDL-C significantly decreased after twenty-one days of extract administration (p>0.01; Table 4.4a). The HDL-C concentration was increased significantly on day fourteen and twenty-one after administration of this dose level (p>0.01; Table 4.4a).
When the level of lipids and related parameter profiles in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 4.4b). After seven days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p>0.01; Table 4.4b). Concentration of HDL-C increased significantly at all the dose levels after seven days of administration of the extract (p<0.01; Table 4.4b), while the levels of LDC-C decreased significantly at the dose levels of 75 mg/kgbw and 100 mg/kgbw (p>0.01; Table 4.4b).

After fourteen days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG, LDL-C and TC decreased significantly at all the dose levels (p>0.01; Table 4.4b). The concentration of HDL-C increased significantly at all the dose levels after fourteen days of extract administration (p<0.01; Table 4.4b).

After twenty-one days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p>0.01; Table 4.4b). The levels of LDL-C decreased significantly at the dose level of 100 mg/kgbw (p>0.01; Table 4.4b). The concentrations of HDL-C, however, increased significantly at all the dose levels after twenty-one days of extract administration (p<0.01; Table 4.4b).
Table 4.4a: Effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS (mmol/l)</th>
<th>Control</th>
<th>50mg/kgbw</th>
<th>75mg/kgbw</th>
<th>100mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 7</td>
</tr>
<tr>
<td>TG</td>
<td>1.84±0.05</td>
<td>1.72±0.02</td>
<td>1.92±0.10</td>
<td>1.30±0.09ab</td>
</tr>
<tr>
<td>TC</td>
<td>1.81±0.13</td>
<td>1.84±0.09a</td>
<td>2.00±0.15a</td>
<td>1.45±0.07c</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.52±0.06</td>
<td>0.57±0.10a</td>
<td>0.50±0.14a</td>
<td>1.13±0.04a</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.42±0.14a</td>
<td>1.37±0.08a</td>
<td>1.46±0.13a</td>
<td>1.38±0.09b</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the days with the same extract dose level.
### Table 4.4b: Effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats

<table>
<thead>
<tr>
<th>PARAMETERS (mmol/l)</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dose 1</td>
<td>Dose 2</td>
</tr>
<tr>
<td>TG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.84±0.05</td>
<td>1.30±0.09a</td>
<td>1.33±0.08a</td>
</tr>
<tr>
<td>TC</td>
<td>1.81±0.13b</td>
<td>1.45±0.07a</td>
<td>1.27±0.02a</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.52±0.06a</td>
<td>1.13±0.04b</td>
<td>1.28±0.05b</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.42±0.14b</td>
<td>1.38±0.09b</td>
<td>1.20±0.02a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50 mg/kgbw; Dose 2: 75 mg/kgbw; Dose 3: 100 mg/kgbw.
4.5 Phytochemical screening

Qualitative phytochemical screening of the DCM-MeOH leaf extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, phenolics, terpenoids and traces of steroids. However, saponins and cardiac glycosides were absent in the leaf extracts.

Table 4.5: Phytochemical composition of DCM-MeOH leaf extract of *Carissa edulis*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaf extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ (trace)</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
</tr>
</tbody>
</table>

Present phytochemicals are denoted by (++) sign, absent phytochemicals are denoted by (-) sign while + (trace) denote slightly present phytochemicals.
5.1 Discussion

Assessment of hematological parameters can be used to explain hematological functions of a chemical compound or plant extracts in an organism (Yakubu et al., 2007). Blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents (Olafedehan et al., 2010). In the present study, the DCM-MeOH leaf extracts of *C. edulis* demonstrated varying degrees of hematological and serum lipid profile changes in normal rats at the dose levels of 50, 75 and 100 mg/kg bw.

The extract of *C. edulis* significantly increased the levels of RBC, Hb, PCV, MCV, MCH, MCHC and RDW in normal rats. The observed increases in RBC, Hb and PCV levels upon administration of DCM-MeOH leaf extract of *C. edulis* suggests that the extract could have stimulated erythropoietin release in the kidney, which is the humoral regulators of RBC production (Degruchy, 1976). Expectedly, increase in RBC counts on administration of *C. edulis* extract resulted in increase in MCV levels while increase in Hb levels resulted to increase in MCH and MCHC profiles. Since MCHC, MCH and MCV profiles relate to individual red blood cell count while hemoglobin and hematocrit profiles relate to the total population of red blood cells in the blood, it could thus imply that though the extract may stimulate the production of red blood cells and hemoglobin, it could have an inhibitory effect on hemoglobin incorporation into red blood cells and a consequent reduction in oxygen exchange (Adebayo et al., 2005).

It was also shown that the extract contained some phytochemicals such as tannins that can stimulate the formation of erythropoietin which is a glycoprotein hormone that stimulates
stem cells in the bone marrow to produce red blood cells (Ohlsson et al., 2006). The increase in
the above parameters is also due to improvement in erythrocyte membrane integrity through
the antioxidant potential of the extract phytochemicals such as flavonoids, thereby reducing
haemolysis (Naaz et al., 2007).

The mechanism leading to the increase in erythrocyte count was probably mediated by the
anti-oxidant property of the extracts which enhanced maintenance of erythrocyte membrane
integrity. The presence of phytochemicals like flavonoids, tannins and terpenes in the
dichloromethane; methanolic leaf extracts of C. edulis may be responsible for the haemopoietic
stimulating effects. This is in line with previous research that showed that prophylactic and
therapeutic oral administration of antioxidant supplements of plant extracts significantly
increased cells of hematopoietic origin in animals exposed to potentially lethal dose of
radiation (Chris et al., 2008). Flavonoids, tannins and terpenes have been found to protect
erythrocytes from oxidative damage (Grassmann, 2005). Further, Ren et al. (2003) and Chahar et
al. (2011) reported that flavonoids have various benefits for human health due to their
antioxidant and free-radical scavenging activities as well as anti-inflammatory, antiviral and anti-
cancer properties.

Administration of DCM-MeOH leaf extract of C. edulis led to significant increase in the levels
of white blood cells and all related indices in normal rats. This shows that the DCM-MeOH
extracts of C. edulis may have immune boosting properties. The increase in WBC count may
have been due to enhancement in the rate of entry of leucocytes into the blood from the
bone marrow and a diminished rate of removal from circulation. Granulocyte-macrophage
colony stimulating factor, macrophage colony stimulating factor, interleukins (IL-2, IL-4
and IL-5) regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBCs (Ganong, 2001). Therefore, such increase in WBC counts may be due to increased production of haematopoietic regulatory elements by the stromal cells and macrophages in the bone marrow (Chang-Gue et al., 2003). These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extracts. Saponins, alkaloids, tannins, phenolic compounds and flavonoids have generally been reported as immunostimulants (Lakshmi et al., 2003; Dashputre and Naikwade, 2010).

Differential white blood cell counts are indicators of the ability of an organism to eliminate infection. Neutrophils attack and destroy pathogens such as parasites in the blood (Dacie et al., 1995). The increased neutrophil counts improve the phagocytic activity in the animals. Lymphocytes are the main effector cells of the immune system. The increase in the lymphocyte levels in the present study may help improve the effector cells of the immune system. Similarly, increased levels of eosinophils and basophils observed in the present study may suggest positive effect on the immune system. Since monocytes have been shown to increase in cases of infection, the increase in monocytes observed with the extract in this study may be ascribed to challenges on the immune system.

Platelets are the blood cells involved in Coagulation (Williams et al., 2005). Coagulation of blood requires that the platelets should be in sufficient size, number and function. The increase in the platelet levels observed in this study may be explained by stimulatory effect on thrombopoietin (Li et al., 1999). Bone marrow is responsible for the production of red blood cells, white blood cells and platelets (Grossmann et al., 1996). The significant increase in platelets and related parameter profiles after oral administration of DCM-MeOH leaf extracts
of *C. edulis* suggests that the extracts contain compounds and phytochemicals that may have stimulated thrombopoietic process in normal rats.

The significant increase in platelet count may indicate that the extracts have the potential to be developed as plant based therapeutic agents for thrombocytopenia. This is in line with Subenthiran *et al.* (2013), who reported that leaf juice of *Carica papaya* consumed during the course of dengue infection had the potential to induce platelet production. An increase in platelet count after seven days of oral administration DCM-MeOH leaf extracts of *C. edulis* extract may indicate that the extract had a megakaryopoietic stimulatory activity. This is in line with finding of Choi *et al.* (1995) that platelets are produced from megakaryocytes within 4 to 6 days under normal healthy body conditions.

Generally, the significant increase in platelets, plateletcrit and MPV profiles after oral administration of DCM-MeOH leaf extracts of *C. edulis* may be attributed to presence of tannins, which have been shown to confer antihemorrhagic properties in animals. This is consistent with the findings of Asquith and Butler (1986) that the sap of *Musa paradisiaca* is used for the treatment of fresh wounds, cuts and insect bites.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides could avail useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Yakubu *et al.*, 2008). Administration of DCM-MeOH leaf extracts of *C. edulis* caused significant reduction in the serum levels of total cholesterol,
triglycerides and LDL-C and increased the level of HDL-C in normal rats. This suggests protective potential of the extract against hyperlipidemia, which may be beneficial in preventing diabetic complications and cardiovascular disorders.

High blood cholesterol concentration is one of the important risk factors for cardiovascular diseases (Abebayo et al., 2005). Thus the reduction in serum total cholesterol concentration effected by the DCM-MeOH leaf extracts of *C. edulis* is beneficial and may reduce the risk of cardiovascular disease. Agents that have the ability to lower cholesterol concentration in the blood have been reported to reduce vascular resistance by improving endothelial function (Abebayo et al., 2005). Similar alterations in lipid as well as haematological profiles were reported in various other plant extracts such as *Bulbine natalensis*, *Bougainvillea spectabilis* leaves (Adebayo et al., 2005) and *Fadogia agrestis* stem (Yakubu et al., 2007).

The extract may have achieved this by blocking cholesterol biosynthesis (Tanabe et al., 1993), probably through binding and reducing the production of bile acids or by increased generation of propionate, which has been shown to reduce cholesterol levels (Spiller, 1993). Further, the reduction of the hyperlipidemia by DCM-MeOH leaf extracts of *C. edulis* may have been mediated by a control of lipid metabolism (Cho et al., 2002). Preliminary phytochemical screening of the plant revealed the presence of flavonoids, alkaloids saponins and tannins. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia (Nemenibo, 2003). The lipid lowering activity of DCM-MeOH leaf extracts of *C. edulis* may also be attributed to its phytochemical constituents. These findings suggest that *C. edulis* may be effective in ameliorating cardiac disease complications seen in hyperlipidemia situations. The prevention of oxidation of low density
lipoprotein-cholesterol by the antioxidant compounds like poly-phenolics and flavonoids is also important in the prevention of cardiovascular diseases. These phytochemicals are helpful in treating various other diseases (Nemenibo, 2003).

The dose sensitive increase in the concentration of HDL-cholesterol and reduction of the VLDL-cholesterol in this study showed that \textit{C. edulis} can be used to treat cardiovascular diseases and coronary heart diseases. This justifies its use in folk medicine for the treatment of cardiovascular diseases. Further to this is the presence of flavonoids and other poly-phenolic compounds in the DCM-MeOH leaf extracts of \textit{C. edulis}, which have the ability to scavenge free radicals, therefore, acting as antioxidants (Nemenibo, 2003). It has been established that free radical scavengers help prevent cardiovascular diseases by interfering with the oxidation of the VLDLs and LDLs, which are key drivers of atherosclerosis.

5.2 Conclusions

In conclusion, the present study showed that oral administration of DCM-MeOH leaf extract of \textit{C. edulis} in normal rats;

i. Resulted in a significant increase in the levels of erythrocytic parameter profiles. This may suggest that the extract possess erythropoietin stimulating activity and phytochemicals that also improve haematopoeitic activity of the cells and the improvement in erythrocyte membrane integrity through the antioxidant potential. That the significant increase in total white blood cell and differential white blood cell counts in normal mice after oral administration of the extract shows that the DCM-MeOH leaf extracts of \textit{C. edulis} may promote the immune-stimulatory activities. That the
significant increase in platelet and related parameter profiles in normal mice after oral administration of the DCM-MeOH leaf extracts of *C. edulis* shows that the extract has the potential to stimulate thrombopoietin production and can thus be used to manage hemostatic capacity of blood.

ii. The significant reduction in the levels of TG, TC and LDL-C as well as significant increase in the levels of HDL-C shows that the extracts have potential to selectively regulate the components of serum lipid profiles. This is attributed to the phytochemical compounds which have the ability to scavenge for free radicals, and also regulate the lipid metabolism processes.

iii. That the DCM-MeOH leaf extract of *C. edulis* was confirmed to contain various phytochemicals whose actions were proved to result into changes in the levels of hematological and serum lipid profiles of normal rat models.

The present study, therefore, scientifically confirms and supports the traditional use of leaves of *C. edulis* in enhancing hematological parameters, altering the levels of serum lipid profiles and generally improving health. In this study, the alternative hypothesis is hence accepted.

### 5.3 Recommendations and suggestions for further studies

i. This study recommends use of DCM-MeOH leaf extracts of *C. edulis* in improvement of hematological parameters and serum lipid profiles after being subjected to various stipulated assays, moderation and approvals.

ii. Further research should be done in order to elucidate the actual mechanisms underlying the erythropoietin, immune-stimulation and thrombocytopoietic promoting activity and
serum lipid profiles alteration activity of the DCM-MeOH leaf extracts of *C. edulis* in animal models.

iii. A study to assess the toxicological potential of the DCM-MeOH leaf extracts of *C. edulis* is suggested.

iv. Trials should be done in cases where extracts of this plant are administered to animals that suffer from hematological disorders.
REFERENCES


