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RESEARCH ARTICLE

EFFECTS OF MORINGA OLEIFERA LEAF EXTRACT ON RED AND WHITE BLOOD CELLS COUNTS

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ABSTRACT

Background: Blood is an essential component of the circulatory system and the body at large. It is a fluid connective tissue that performs many functions in the body including regulating and/or transporting medium, maintains its constituents within defined physiological normal range and keeps the body alive. It is composed of cells (erythrocytes, leukocytes and thrombocytes) suspended in plasma. But due to the incidence of poverty and malnutrition and other environmental factors in Africa and other developing countries, some of these disease conditions, especially anaemia, have become prevalent, affecting mostly women and children. Hence every resource, both herbs, plants and drugs, is deployed to curbing this menace. In Nigeria and some other African countries, herbs and plants are used as alternative to drugs due to its availability, affordability, cultural, religious, ethnic and superstitious beliefs.

Aim of Study: The current study examined the blood cell (White and Red cell) counts of Wistar rats fed with ethanolic extract of *M. oleifera* leaves.

Method: Wistar rats (18) were divided into three groups of 6 each: Group 1 served as control (not given extract), Group 2 was fed with 200mg per kg (of body weight) of the leaf extract for 15 days, and Group 3 was fed with 300mg per kg (of body weight) of the leaf extract for the same duration.

The results showed a significant increase ($P<0.05$) in both red and white blood cell counts of rats in groups 2 and 3 as compared to the ones in the control group (group 1) in both low and high doses.

Conclusion: This infers that 200mg per kg and 300mg per kg of *Moringa oleifera* leave extract contain active ingredients required for the formation and maturation of blood cells (red and white blood cells), hence the increase in the blood cells counts.

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INTRODUCTION

Blood Cells

In every physiological system, blood is a fluid connective tissue that performs many functions in the body including regulating and/ or transporting medium, maintains its constituents within defined physiological normal range and keeps the body alive[1] It is composed of cells (erythrocytes, leukocytes and thrombocytes) suspended in plasma. These cells play important roles in the oxygen transport, immune system and blood clotting. Hence abnormalities in its concentration in the blood due to several causative factors may lead to several disease conditions such as leukaemia, polycythaemia, anaemia, thrombocytopenia, leukopenia etc.

It is a specialized connective tissue in fluid form, in which living blood cells are suspended in a non-living fluid matrix known as Plasma[2]. It is a highly differentiated, complex living tissue that pulsates through the arteries to every part of the body, interacts with individual cells via an extensive capillary network, and returns to the heart through the venous system[3]. It provides a means of communication between the cells of different parts of the body and the external environment, (eg) it transports: oxygen and carbon-dioxide between the lungs and tissues, nutrients from the gastrointestinal tract to the tissues, hormones to target glands and tissues, antibodies and other protective substances to sites of infection etc[4]. Collagen and elastic fibres typical of other connective tissue are absent here, but their dissolved fibrous proteins are seen as fibrin strands during blood clotting[2].

Blood consists of a cellular portion, formed elements and a fluid portion, plasma[5, 2]. Plasma is a clear extracellular matrix which is not visible on a prepared slide of blood while the formed element has a visible structure[6]. When centrifuged, the formed elements, which is heavier, settle at the base of the tube leaving the plasma on the top[5]. Erythrocytes, which constitute about 45% of the total volume of blood sample, a percentage known as the Haematocrit value which is 47% in males and 42% in Females, are the densest hence they settle at the bottom, while Leukocytes and Platelets, which total less than 1% or less of the blood volume, form a narrow cream-coloured zone known as buffy coat, just above the Erythrocytes [5, 6]. Many of the functions of blood are undertaken in the capillaries, where the blood flow slows

dramatically, allowing the efficient diffusion and transport of oxygen, glucose, and other molecules across the monolayer of endothelial cells that form the thin capillary walls. In addition to transport, blood and the cells within it mediate other essential aspects of immunity and haemostasis[3]. The constant movement of blood as it flows through the blood vessels keeps its cellular elements rather evenly dispersed within the plasma[7].

Blood is red in colour: arterial colour is scarlet due to the presence of oxygen, while the presence of carbon-dioxide gives venous blood its dark red colour. Blood pH is regulated to stay within the range of 7.35 to 7.45; hence it is slightly alkaline[8,4]. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too basic. Blood pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), and HCO_3^- are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through the respiratory system and the urinary system in order to control the acid-base balance and respiration. It has a specific gravity of 1.052 to 1.061 and is about 7-8% of body weight in a normal 70kg adult. It has an average volume of 5L in women and about 5.5L in men [7].

Composition of blood

Blood consists of a protein-rich fluid known as plasma, in which are suspended cellular elements: white blood cells (leukocytes), red blood cells (erythrocytes) and platelets (thrombocytes). The normal total circulating blood volume is about 5600 mL in a 70-kg man. About 55% of this volume is plasma[9] while the other 45% is composed of the blood cells. When blood is allowed to clot or coagulate, the suspending medium is referred to as serum[3].

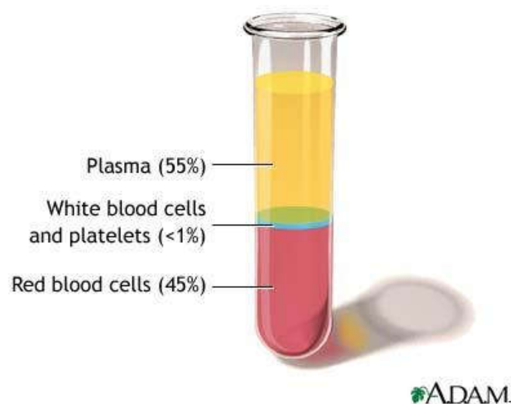

Figure 1 *Compostion of blood* (Beltina.org)

Plasma

Plasma is a straw coloured clear liquid part of the blood composed mostly of 91-92% of water and 8-9% of solids[8] which include various dissolved solutes, including proteins, lipids (fats), carbohydrates, amino acids, vitamins, minerals, hormones, wastes, cofactors, gases, and electrolytes.

The solutes in plasma play crucial roles in homeostasis, such as maintaining normal plasma pH and osmolality[3]. About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid.

Constituents of plasma include:

- Plasma proteins (e.g Serum albumin, Immunoglobulins, Blood-clotting factors)
- Inorganic salts (mainly sodium and chloride)
- lipoprotein particles
- Nutrients, mostly from digested foods
- Waste materials
- Hormones

The term serum refers to plasma from which the clotting protein fibrinogen has been removed[6]. Most of the proteins remaining are albumin and immunoglobulins.

Proteins are the most abundant plasma solute, totaling 6-9g/dL that play roles: clotting, defense and transport of other solutes iron, copper etc[6]. They are normally retained in the blood, because they are too big to escape through the capillary pores into the tissues. They are responsible for creating the osmotic pressure of blood, which keeps plasma fluid within circulation[4]. Plasma viscosity is due to the presence of these proteins. There are three major categories:

Albumins are the smallest and most abundant of the proteins[6] and they are formed in the liver and their main function is to maintain normal plasma osmotic pressure[4]. They act as carriers to transport certain molecules e.g. lipids and steroids. It also buffers the pH of the blood plasma[2, 6] Globulins are divided into α, β and γ from smallest to largest in molecular weight. They play roles in solute transport, clotting and immunity[6]. Most are formed in the liver and the rest are formed in the lymphoid tissue. They function as antibodies (immunoglobulins), transport of some hormones and mineral salts (thyroglobulin and transferrin) and inhibition of some proteolytic enzymes (α_2 macroglobulin) [4]. Fibrinogen is the soluble precursor of fibrin, a sticky protein that forms the fragment of blood clot[6]. It is synthesized in the liver and is essential for blood coagulation[4].

Blood cells (Haemocytes)

A blood cell, also called a haematocyte, is a cell produced by haematopoiesis and normally found in blood. In mammals, these fall into three general categories:

- Red blood cells – Erythrocytes
- White blood cells – Leukocytes
- Platelets – Thrombocytes.

Together, these three kinds of blood cells add up to a total 45% of the blood tissue by volume, with the remaining 55% of the volume composed of plasma, the liquid component of blood[10]. This volume percentage (e.g., 45%) of cells to total volume is called hematocrit, determined by centrifuge or flow cytometry. Hemoglobin (the main component of red blood cells) is an iron-containing protein that facilitates transportation of oxygen and other respiratory gases to tissue

Red blood cells (Erythrocytes)

Erythrocytes are the most numerous cells in blood.
Approximately 2.4 million new erythrocytes are produced per second[11] (Sackmann, 1995). The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells[12]. Human red blood cells take on average 20 seconds to complete one cycle of circulation[13, 14]

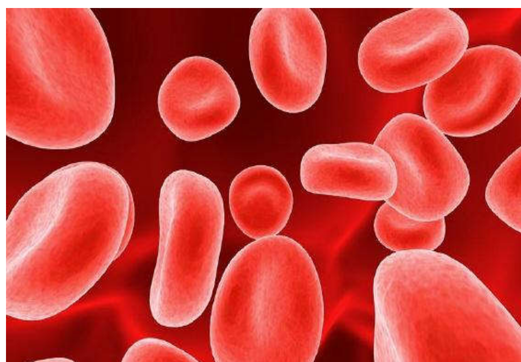

Figure 2 *Erythrocytes* (sciencelink café, 2013)

As red blood cells contain no nucleus, protein biosynthesis is currently assumed to be absent in these cells, although a recent study indicates the presence of all the necessary biomachinery in the cells to do so[15]. The blood's red color is due to the spectral properties of the hemic iron ions in haemoglobin. Each human red blood cell contains approximately 270 million of these hemoglobin biomolecules, each carrying four heme groups; haemoglobin comprises about a third of the total cell volume. This protein is responsible for the transport of more than 98% of the oxygen (the remaining oxygen is carried dissolved in the blood plasma). The red blood cells of an average adult human male store collectively about 2.5 grams of iron, representing about 65% of the total iron contained in the body[16].

Structure of Red blood cells

These biconcave disks lack a nucleus and have a diameter of about 7m and a maximum thickness of 2.5m. The shape of the erythrocyte optimizes its surface area, increasing the efficiency of gas exchange[3]. Normal red blood cells are biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the centre. The average volume of the red blood cell is 90 to 95 cubic micrometers[17]. A typical human erythrocyte has a disk diameter of approximately 6.2-8.2 µm [18] and a thickness at the thickest point of 2-2.5 µm and a minimum thickness in the centre of 0.8-1 µm, being much smaller than most other human cells. These cells have an average volume of about 90 fL [13] with a surface of about 136 μm^2, and can swell up to a sphere shape containing 150 fL, without membrane distension. In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for haemoglobin.

The membrane of the red blood cell plays many roles that aid in regulating their surface deformability, flexibility, adhesion to other cells and immune recognition. These functions are highly dependent on its composition, which defines its properties. The red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer which contains many transmembrane proteins, besides its lipidic main constituents; and the membrane skeleton, a structural network of proteins located on the inner surface of the lipid bilayer. Half of the membrane mass in human and most mammalian erythrocytes are proteins. The other half are lipids, namely phospholipids and cholesterol [19]. The erythrocyte cell membrane comprises a typical lipid bilayer, similar to what can be found in virtually all human cells. Simply put, this lipid bilayer is composed of cholesterol and phospholipids in equal proportions by weight. The lipid composition is important as it defines many physical properties such as membrane permeability and fluidity. Additionally, the activity of many membrane proteins is regulated by interactions with lipids in the bilayer.

Concentration of Erythrocytes in the blood

In normal men, the average number of red blood cells per cubic millimeter is 5,200,000 (±300,000); in normal women, it is 4,700,000 (±300,000). Persons living at high altitudes have greater numbers of red blood cells[17].

Haemoglobin

Haemoglobin, the red, oxygen-transporting protein of erythrocytes, consists of a globin (or protein) portion and four heme groups, the iron-carrying portion. The molecular weight of haemoglobin is about 64,500.

This complex protein possesses four polypeptide chains: two α-globin molecules of 141 amino acids each and two molecules of another type of globin chain each containing 146 amino acid residues. Four types of haemoglobin molecules can be found in human erythrocytes: embryonic, fetal, and two different types found in adults (HbA, HbA2). Each haemoglobin molecule is designated by its polypeptide composition. For example, the most prevalent adult haemoglobin, HbA, consists of two α chains and two β chains. Its formula is given as $\alpha_2\beta_2$. HbA_2, which makes up about 1.5 to 3% of total haemoglobin in an adult, has the subunit formula $\alpha_2\delta_2$. Fetal haemoglobin is the major haemoglobin component during intrauterine life. Its levels in circulating blood cells decrease rapidly during infancy and reach a concentration of 0.5% in adults [3]. Each haemoglobin molecule has four globin chains and four haem units, each with on atom of iron. Since each iron can combine with an oxygen molecule, this means that a single molecule of haemoglobin molecule carries about four atoms of oxygen. An average red blood cell carrries about 280 million haemoglobin molecules, giving each cell an oxygen capacity of over a billion oxygen molecules, theoretically [4]. Haemoglobin binds irreversibly to oxygen to form oxyhaemoglobin. This association is a loose one, hence oxyhaemoglobin releases its oxygen readily, especially under certain conditions [4].

White blood cells (Leukocytes)

White blood cells or leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. The real value of the white blood cells is that most of them are specifically transported to areas of serious infection and inflammation, thereby providing a rapid

and potent defense against infectious agents [17]. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell: Granulocytes (Neutrophils, Eosinophils and Basophils) and Agranulocytes (Lymphocytes and Monocytes) [20]. They live for about three to four days in the average human body. Leukocytes are found throughout the body, including the blood and lymphatic system [21]. The granulocytes and monocytes protect the body against invading organisms mainly by ingesting them-that is, by *phagocytosis.* The lymphocytes and plasma cells function mainly in connection with the immune system [17].

Types of White blood cells

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells

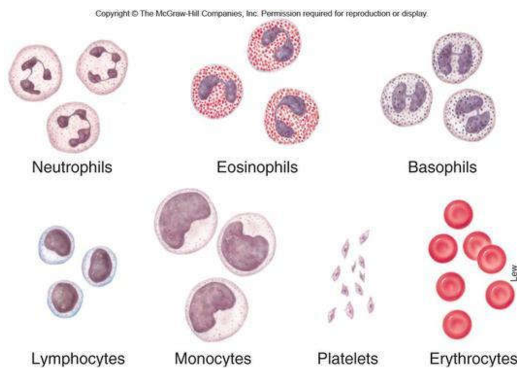

Figure 3 Different kinds of Leukocytes [22]

Granulocytes

These white blood cells have granules and multi-lobed nuclei in their cytoplasm [4]. Leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules (usually lysozymes) are membrane-bound enzymes that act primarily in the digestion of endocytosed particles. During their formation (granulopoiesis), they follow a common line of development through myleoblast to myleocyte prior to their differentiation into neutrophils, eosinophils and basophils[4] Neutrophils

Neutrophils are usually the most prevalent leukocyte in peripheral blood. These dynamic cells respond instantly to microbial invasion by detecting foreign proteins or changes in host defense network proteins. Neutrophils provide an efficient defense against pathogens that have gotten past physical barriers such as the skin [3]. Neutrophils are the most common cell type seen in the early stages of acute inflammation, and make up 60-70% of total leukocyte count in human blood[23]. The life span of a circulating human neutrophil is about 54 days [24]. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytized a few pathogens [25].

Neutrophils are amoeba-like phagocytic cells. Invading bacteria induce neutrophil chemotaxis-migration to the site of infection. Chemotaxis is initiated by the release of chemotactic factors from the bacteria or by chemotactic factor generation in the blood plasma or tissues. Chemotactic factors are generated when bacteria or their products bind to circulating antibodies, by tissue cells when infected with bacteria, and by lymphocytes and platelets after interaction with bacteria. After neutrophils migrate to the site of infection, they engulf the invading pathogen by the process of phagocytosis. Phagocytosis is facilitated when the bacteria are coated with the host defense proteins known as opsonins [3]. Other bactericidal agents and processes operate in neutrophils to ensure efficient bacterial killing. Phagocytized bacteria encounter intracellular defensins, cationic proteins that bind to and inhibit the replication of bacteria. Defensins and other antibacterial agents pour into the phagocytic vacuole after phagocytosis. Agents stored in neutrophil granules include lysozyme, a bacteriolytic enzyme, and myeloperoxidase, which react with hydrogen peroxide to generate potent, bacteria-killing oxidants[3].

Eosinophil

The eosinophils normally constitute about 2 per cent of all the blood leukocytes. Eosinophils are weak phagocytes, and they exhibit chemotaxis [26]. Eosinophils primarily deal with parasitic infections. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. In general, their nucleus is bi-lobed. The cytoplasm is full of granules that assume a characteristic pink-orange colour with eosin stain. As the name implies, the eosinophil takes on a deep eosin colour during polychromatic staining; the large, refractile cytoplasmic granules of these cells stain orange-red to bright yellow. Like neutrophils, eosinophils migrate to sites where they are needed and exhibit a metabolic burst when activated. Eosinophils participate in defense against certain parasites, and they are involved in allergic reactions [4]. Eosinophils attach themselves to the juvenile forms of the parasite and kill many of them. They do so in several ways: (1) by releasing hydrolytic enzymes from their granules, which are modified lysosomes; (2) probably by also releasing highly reactive forms of oxygen that are especially lethal to parasites; and (3) by releasing from the granules a highly larvacidal polypeptide called *major basic protein* [17].

Basophil

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules that hide it. They are characterized by their large blue granules. Basophils are polymorphonuclear leukocytes with multiple pleomorphic, coarse, deep-staining metachromatic granules throughout their cytoplasm. These granules contain heparin and histamine, which have anticoagulant and vasodilation properties, respectively. The release of these and other mediators by basophils increases regional blood flow, facilitating the transport of other leukocytes to areas of infection and allergic reactivity or other forms of hypersensitivity [3].

The basophils in the circulating blood are similar to the large tissue *mast cells* located immediately outside many of the capillaries in the body. Both mast cells and basophils liberate *heparin* into the blood, a substance that can prevent blood coagulation. The mast cells and basophils also release *histamine,* as well as smaller quantities of *bradykinin* and

serotonin. Indeed, it is mainly the mast cells in inflamed tissues that release these substances during inflammation. The mast cells and basophils play an exceedingly important role in some types of allergic reactions because the type of antibody that causes allergic reactions, the immunoglobulin E (IgE) type, has a special propensity to become attached to mast cells and basophils. Then, when the specific antigen for the specific IgE antibody subsequently reacts with the antibody, the resulting attachment of antigen to antibody causes the mast cell or basophil to rupture and release exceedingly large quantities of *histamine, bradykinin, serotonin, heparin, slow-reacting substance of anaphylaxis,* and a number of *lysosomal enzymes.* These cause local vascular and tissue reactions that cause many, if not most, of the allergic manifestations[17].

Agranulocytes

These white blood cells lack granules in their cytoplasm. Their nucleus is large and they constitute about 25-50% of leukocytes. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes [27]. They include: monocytes and lymphocytes.

Lymphocytes

In blood, small lymphocytes are more numerous than larger ones; the latter closely resemble monocytes. Small lymphocytes possess a deeply stained, coarse nucleus that is large in relation to the remainder of the cell, so that often only a small rim of cytoplasm appears around parts of the nucleus. In contrast, a broad band of cytoplasm surrounds the nucleus of large lymphocytes; the nucleus of these cells is similar in size and appearance to that of small lymphocytes [3]. Lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. The blood has three types of lymphocytes:

- B cells make antibodies that bind to pathogens to enable their destruction.
- T cells
- Natural killer cells are able to kill cells of the body that have lost MHC I molecule, as they have been infected by a virus or have become cancerous.

Monocytes

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed, or so that an antibody response may be mounted. Monocytes eventually leave the bloodstream to become tissue macrophages, which remove dead cell debris as well as attacking microorganisms. Neither of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages. Monocytes are phagocytic cells but lymphocytes are not; both participate in multiple aspects of immunity. Monocytes were originally differentiated from lymphocytes based on morphological characteristics. The cytoplasm of monocytes appears pale blue or blue-gray with Wright's stain. The cytoplasm contains multiple fine reddish-blue granules. The monocyte nucleus may be shaped like a kidney bean, indented, or shaped like a horseshoe. Frequently, however, it is rounded or ovoid. Upon activation, monocytes transform into macrophages-large, active mononuclear phagocytes [3].

Moringa oleifera

Moringa oleifera is the most common specie of the genus, *Moringa*. *Moringa*, which is native to parts of Africa and Asia, is the sole genus in the flowering plant family Moringaceae. The name is derived from the Tamil word *murungai* which refer to *M. oleifera* [28]. *Moringa* has about 12 species native to semi-arid habitats from North Africa to Southeast Asia [29] that range in size from tiny herbs to massive trees. In addition to *M. oleifera*, which is a diploid species with 28 chromosomes, several other species of *Moringa* have proven to be useful sources of food, fiber, medicinal, and other products.

Due to the incidence of poverty and malnutrition and other environmental factors in Africa and other developing countries, some of these disease conditions, especially anaemia, have become prevalent, affecting mostly women and children. Hence every resource, both herbs, plants and drugs, is employed to curb this menace. In these geographical regions, herbs and plants are used as alternative to drugs due to its availability, affordability, cultural, religious, ethnic and superstitious beliefs. Over the years, plants have been found to have high amounts of nutrients, vitamins, minerals, protein, fibre etc, which is essential in the body [30]. Several studies have affirmed the presence of certain bioactive chemicals in plants or herbs and mushroom which have nutritional and medicinal benefits [31, 32, 33]. Proximate and phytochemical analysis showed they contained essential nutrients and bioactive compounds (or phytochemicals) that play a role in nutrition (as feed supplement) and as medicines for the treatment of certain diseases [33].

Presently, *Moringa oleifera* is in high demand for its nutritional and medicinal value. Moringa leaves and seeds are used by humans as a good source of vitamins (B and C) and amino acids [34, 35]. It has relatively high crude protein, low anti-nutritional factors and antimicrobial activity [34, 36]. *Moringa oleifera* was also claimed to boost immune systems [33, 37] and its trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular-Trees for Life, Church World Service and Educational Concerns for Hunger Organization-have advocated Moringa as *"*natural nutrition for the tropics." Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce [38].

Morphology

M. oleifera is a fast growing multipurpose, deciduous, drought-resistant shrub or tree. Its tree is slender, about 12m in height and 30cm in diameter [39] with drooping branches that grow approximately 10m in height.

The leaves, which are bipinnate or tripinnate, are about 45 cm long, and are alternate and spirally arranged on the twigs, while the leaflets are finely hairy, green and almost hairless on the upper surface, paler and hairless beneath, and are rounded or blunt-pointed at the apex and short-pointed at the base [40].

The flowers are bisexual and yellowish-white, borne on slender, hairy stalks in spreading or drooping axillary clusters (panicles) 10–25 cm long. Individual flowers are approximately 0.7 to 1 cm long and 2 cm broad, with five unequal yellowish-white, spathulate petals, five stamens with five smaller sterile stamens (staminodes), and a pistil composed of a 1-celled ovary and slender style [41, 42, 43].

The fruits are pendulous, linear, three-sided pods with nine longitudinal ridges, usually 20 to 50 cm long and 2.0 to 2.5 cm broad. The pods, each usually containing up to 26 seeds, are dark green during their development, and take approximately 3 months to mature after flowering [44] . They turn brown on maturity, and split open longitudinally along the three angles, releasing the dark brown, trigonous seeds. Seeds measure about 1 cm in diameter, with three whitish papery wings on the angles. Seed weights differ among varieties, ranging from 3,000 to 9,000 seeds per kilogram [45]. The bark is whitish-gray, thick, fissured and corky, becoming rough. When wounded, the bark exudes a gum which is initially white in color but changes to reddish brown or brownish black on exposure.

Chemical constituents

Generally, *M.oleifera* contains zeatin, quercetin, caffeoylquinic acid [46, 47] b-sitosterol and Kaempferol. It also contains proteins, vitamins, b-carotene, amino acids and phenolics [48]. *M.oleifera* contains in large amounts, calcium (19.3- 22.4mg/g), selenium (27.1µg/g) and phosphorous (2.5-6.3 mg/g), α-linoleate (12.3 mg/g) but less than 1.0 mg/g of linoleate [49].

The leaves contain α-tocopherol (90 mg/kg) [50], and total carotene (1.93 mg/g), b-carotene (0.93 mg/g), ascorbic acid (6.6 mg/g), Iron (0.26 mg/g) and Oxalic acid (11.2 mg/g). Other antioxidants include α- and g-tocopherol, vitamin A, quercetin, kaempferol, flavonoids, anthocyanins [51], nitrite glycosides, niaririn and niazinin, mustard oil glycoside, niazinin Aand niaziminin [52]. The fruits contain protein Fat, Carbohydrate, mineral, fibre, vitamin A, b-nicotinic acid, ascorbic acid, tocopherol, oestrogenic substances and biositosterol [53]. The Root-bark contains the alkaloid moringine and pterygospermin.

Nutrition

The leaves are the most nutritious part of the plant, being a significant source of vitamin B_6, vitamin C, provitamin A as beta-carotene, magnesium and protein, among other nutrients [54]. When compared with common foods particularly high in certain nutrients per 100 g fresh weight, *Moringa* leaves are considerable sources of these same nutrients [55, 56]. Scientific research confirms that leaves of this plant are of significant nutritional value. Gram for gram, *Moringa* leaves contain: seven times the vitamin C in oranges, four times the Calcium in milk, four times the vitamin A in carrots, two times the protein in milk and three times the Potassium in bananas [57]. It also contains Vitamins B1, B2, B3, B6, B7, D, E, and K, and Amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparatic acid, cysteine, and etc. Some of the calcium in moringa leaves is bound as crystals of calcium oxalate which may inhibit calcium availability to the body [58].

Pharmacological Effects

Radio-protective and Immunomodulatory effect

Methanolic extract of *M.oleifera* leaves exerted a radio-protective effect on radiation-induced chromosomal aberrations and micronuclei [59]. Methanolic extract of leaves of *M. oleifera* was found to be more significant than other extracts during the study of immunomodulation due to the presence of flavonoids, polyphenols and terpenoids which may modulate the body's immune-mechanisms. Methanol extract stimulates both cellular and humoral immune systems, hence it plays a plausible role on the body's immunity [60, 61].

Ameliorative effect

Moringa oleifera leaf extract had a protective effect against the induced testicular toxicity induced by administration of chromium which evidenced by improvement in sperm parameters of experimental rats [62].

Anti-Malarial effect

Ethanolic extract of *M.oleifera* was found to have anti-malarial effect on some malaria-induced mice. This was also observed in Cyclophosphamide induced toxicity in mice [51].

Anti-microbial effect

The powder from fresh leaf juice (dissolved in DMSO) has greater antibacterial activity than fresh leaf juice, ethanol and water extracts while fresh leaf juice and ethanol extract of fresh leaves showed higher antibacterial potential than the corresponding water extracts when administered to about ten cultured pathogenic bacteria [63].

Hypoglycemic effect

Administration of M.oleifera extracts (root bark, stem bark and leaves) with hypoglycaemic properties was also observed to have lowered the blood sugar levels and could be used for the management of diabetes [64]. The current study examined the blood cell (White and Red cell) count of Wistar rats fed with ethanolic extract of *M. oleifera* leaves.

MATERIALS/METHODOLOGY

The consent/approval of ethical committee of Bingham University was duly gotten before the commencement f the research

MATERIALS

Eighteen (18) mature Wistar Albino rats (150-200g) of either sex, Three (3) standard cages, Animal feed, *Moringa oleifera* leaf extract, Heparinized Haematocrit tubes, Syringes, Cannula, Hand gloves, EDTA (Ethylene diaminine tetra-acetic acid) bottles, Haemocytometer (Red cell pipette, White cell pipette and counting chamber), Beakers, Cover slips, Electrical binocular Microscope

Reagent solutions/ chemicals

Red cell diluting fluid, White cell diluting fluid

Preparation of Moringa oleifera leaf extract

200gram of crude powdered material was weighed into conical flasks and soaked with 70% ethanol. This was allowed to stand overnight .The content was then shaken for 3hours, warmed and filtered. Several path of rinsing was done and the collective filtrates were evaporated to dryness using a water bath. The dry extract was weighed and preserved in a sample bottle and kept in a desiccator. The phytochemical screening for active chemical constituents was also done.

Animals

18 Wistar rats (100-180g) were purchased from and housed in cages at the Animal house, Bingham University, Karu. Prior to study, they were fed with Starters mash for three weeks and Growers mash subsequently. Food and water were given *ad libitum*. A week before the administration of the Moringa oleifera leaf extract, the rats were weighed and randomly separated into three groups (n=6 in each group).

Experimental design

18 mature Wistar rats of both sexes, weighing between 120-180g, were used for this study, which lasted for 15 days.

Group 1 (Control); no *Moringa oleifera* leaf extract was administered and rats were fed with Growers mash for 15 days.
Group 2 (Experimental 1) rats were administered 200mg/body weight of *Moringa oleifera* leaf extract and fed with Growers mash for 15 days.
Group 3 (Experimental 2) rats were administered 300mg/body weight of *Moringa oleifera* leaf extract and fed with Growers mash for 15 days.

Blood sample collection/preparation

Three days before the commencement of the *Moringa oleifera* leaf extract administration, blood samples were taken from the intra-ocular artery of each rat, using heparinized haematocrit tubes, and stored in labelled EDTA bottles. After 15 days, blood samples were taken from the jugular artery of each rat and stored in EDTA bottles.

Determination of Blood parameters (Haemocytometry)

Determination of Red blood cell count

Blood was drawn into the red cell pipette, from the EDTA bottle, up to the 0.5 mark. The tip of the pipette was wiped with a cotton wool and the diluting fluid was drawn from a beaker into the pipette up to the 101 mark, then the tip of the pipette was wiped again. Both ends of the pipette were firmly closed while the pipette was vigorously shaken for about 2 minutes (to ensure the mixing of the fluid) then left to stand for about 5 minutes. The counting chamber was thoroughly cleaned and a cover slip was placed on it with firm pressure. The suspension was again mixed and two drops were discarded. Then, holding the chamber at an angle of 45^0, with the tip of the pipette touching the cover slip, the chamber was filled with the fluid. The chamber was placed on the microscope stage, using the ×40mm objective lens, the cells were viewed and counted.

Calculations

Let N = the number of cells counted in 80 small squares.
Area of each small square is 1/400 sq.mm. Depth of the chamber is 1/10mm.
:. Volume of fluid over a small square is 1/400 ×1/10 = 1/4000c/mm
If N cells are counted in 80/4000c/mm, :. 1cmm of diluted blood contains:
(N × 4000) × (80 × 200) cells = N ×10,000 cells.

Determination of White blood cell count

Blood was drawn into the white cell pipette, from the EDTA bottle, up to the 0.5 mark. The tip of the pipette was wiped with a cotton wool and the diluting fluid was drawn, from a beaker, up to the 11 mark of the pipette, then the tip was wiped with a cotton wool. Both ends of the pipette were firmly closed while the pipette was vigorously shaken for about 2 minutes, then left to stand for about 5 minutes.

The counting chamber was thoroughly cleaned with distilled water and a cover slip was carefully placed in position on the chamber with firm pressure. The suspension was again mixed and two drops were discarded. Then, holding the chamber at an angle of 45^0, with the tip of the pipette touching the cover slip, the chamber was filled with the fluid. The chamber was placed on the microscope stage, using the ×40mm objective lens, the cells were viewed and counted.

RESULTS

Acute toxicity

LD_{50} was found to be 5000 mg/kg

Effect of administration of ethanolic extract of Moring oleifera on the Red and White blood cell counts

Pre-Administration

Prior to the administration of *M. oleifera* leaves extract, the red and white blood cell count of the rats was normal as shown below in Table 1.

Table 1 Red and white blood cell count before the administration of *M. oleifera* leaf extract

	Control	Group 1	Group 2
Red Blood Cell Count ($\times10^6/mm^3$)	4.48 ± 0.40	4.25 ± 0.19	4.25 ± 0.22
White Blood Cell Count ($\times10^3/mm^3$)	3.32 ± 0.30	4.19 ± 0.61	5.36 ± 0.39

Post-Administration

The summary of the red and white blood cell count of the control and experimental animals are given in Table 2

Effect of M. oleifera leaf extract on Red blood cell count

After fifteen (15) days of administration of *M.oleifera* leaf extract, group 1 and 2 rats, which were given 200mg/kg and 300mg/kg of the extract, had a significant increase ($P< 0.05$) of $6.05\times10^6/mm^3$ and $6.12\times10^6/mm^3$ respectively in their R.B.C count as compared to the $4.82\times10^6/mm^3$ red blood cell count of the control group (Table 2). The RBC count of group 2 rats was considerably higher than that of the group 1 rats, which implies that 300mg/kg of the extract is more effective in the increase of RBC count than 200mg/kg of the extract.

Effect of M. oleifera leaf extract on White blood cell count

The white blood cell count of group 1 rats showed a significant increase ($P<0.05$) of $6.64\times10^3/mm^3$ in white blood cell count, as compared to the $3.98\times10^3/mm^3$ WBC count of the control group. Group 2 rats showed a higher WBC count of $7.12\times10^3/mm^3$ as compared to that of the control (Table 2). Hence, the 300mg/kg dose of the extract is more effective in the elevation of WBC count in the blood.

Table 2 Red and white blood cell count after the administration of *M.oleifera* leaf extract

Parameter	Control	Group 1	Group 2
Red Blood Cell Count ($\times10^6/mm^3$)	4.82 ± 0.45	6.05 ± 0.54*	6.12 ± 0.56*
White Blood Cell Count ($\times10^3/mm^3$)	4.01 ± 0.34	6.64 ± 0.81*	7.12 ± 0.43*

DISCUSSION

M. Oleifera caused a significant increase in RBC count of rats in Groups 1 (given 200mg/kg) and 2 (given 300mg/kg): ($P<0.05$) of 6.05(±0.54) × 10^6mm^3 and 6.12 (±0.56) ×10^6mm^3, respectively, as compared to the 4.82 (±0.45) ×10^6mm^3 of the control group). The formation and maturation of RBC require, majorly, two vitamins: Cynocobalamine (B12) and folic acid, and also β – carotene [17]. The *Moringa oleifera* leave contains: β –carotene and vitamin B_{12}. The presence of the chemicals may have triggered formation and formation of RBC from the bone marrow, consequently causing and increase in the cell count. *M. oleifera* leaves also contain amino acids: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparatic acid, cysteine, etc [58]. These dietry proteins may have also contributed to increase in the red cell count. The study also indicates that the extract can stimulate erythropoiesis as demonstrated by increase in the haemoglobin concentrations, packed cell volume and platelets counts.

The increase in WBC count observed, as shown in table 2, may be as a result of immunological response of the body to the extract as an antigen or to inflammation and necrosis of the various tissues and organs caused by the extract; an attempt by the body mechanism to effect defence and also repair of the damaged tissues[65]. This immunomodulatory effect might be due to the presence of Vitamins A, (which facilitates lymphocyte proliferation), B, C, calcium ions, potassium and traces of carotenoids, saponins, phyates and some phenols.

The dose increase in this work, from 200mg/kg to 300mg/kg of body weight, did not cause any death of experimental animals, and can therefore be said to be non-toxic. However, there are reports that administration of 1600-2000 mg/kg per body weight of aqueous extract of this leaf resulted in 1/6 death in rats, while the surviving rats exhibited transient dullness for about 5 hours.

CONCLUSION

Moringa leave is useful for anaemic patients both in low and relatively high dose**.** The significant increase in the RBC and WBC count with the administration of *M. oleifera* leaves shows that it is not only good as dietary supplement but also medicinal especially for anaemic patients.

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