IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 4, Issue 6 (Nov. – Dec. 2018), PP 34-52
www.iosrjournals.org

Effect of Ethanol Extract of Moringa Oleifera Leaves in Protecting Anaemia Induced in Rats by Aluminium Chloride

Ameh, S.S., Alafi, O. F.,
Federal University of Technology Minna
Department of Biochemistry
Corresponding Author: Ameh, S.S

Abstract: *Moringa oleifera leaf is a nutritious vegetable with variety of the rapeuticuses. This study was carried out to determine the effectiveness of ethanol leaf extract of Moringa oleifera in the treatment of anaemia induced in albino rats by administration of AlCl3. Phytochemical screening was carried out on M.oleifera leaf extract according to standard procedure. Fifty albino rats were divided into six groups each consisting of eight animals. The control group (group1) received normals a line solution only, group2 was the negative control group and was administered 0.5mg/kg of AlCl3. Group3, 4 and 5 were the treatment groups and in addition to 0.5mg/kg body weight of AlCl3 received M.oleifera extract of 500, 1000 and 2000 mg/kg respectively. Group 6 was the extract control group and were given 500 mg/kg body weight of M.oleifera. The phytochemical analysis of the ethanol extract of M.oleifera leaf showed the presence of tannins, saponins, glycosides, terpenoids, steroids, flavonoids and alkaloids. The result showed that AlCl3led to significant decrease (P<0.05)inred blood cell count (RBC), hemoglobin (Hb), and hematocrit (PCV) compared with the positive control rats (not administered AlCl3). The result also showed that administration of M.oleifera leaf extract at doses of 500, 1000 and 2000 mg/kg caused significant dosed ependent decrease in serum AST and ALT activities compared with then egative control group (administered AlCl3 without treatment). However, no significant difference (P>0.05) was observed in serum AST and ALT activities of the extract control group (administered 500 mg/kg of extract without AlCl3) compared with the normal control group while administration of M.oleifera leaf extract at doses of 500, 1000 and 2000 mg/kg caused significant dose dependent increase in serum total proteins. No significant difference (P>0.05) was observed in serum total proteins of anaemic rat administered M.oleifera extract at doses of 500 mg/kg compared with the negative control group (AlCl3). The normal control group and the extract group had progressive increase in body weight throughout the study period (28days) while the group of rats given AlCl3 with M.oleifera extract at doses of 500, 1000 and 2000 mg/kg showed aninitial decrease in body weight for 2 weeks of treatments and a significant improvement in body weight after the third week. This study has shown that M.oleifera leaf extract has the potential to ameliorate the damaged blood parameters in AlCl3 induced anaemia in rats.*

Date of Submission: 12-11-2018 Date of acceptance: 27-11-2018

I. Introduction

Anaemia is a major public health problem in developing countries associated with an increased risk of morbidity and mortality especially in pregnant women and young children. The global estimate of childhood anaemia indicates that 293.1 million children under five years, approximately 43%, are anaemic worldwide and 28.5% of these children are residing in Sub Saharan Africa (McLean, Cogswell, Egli, Wojdyla and Benoist, 2009). Anaemia is caused by both nutritional (vitamin and mineral deficiencies) and non-nutritional (infection) factors. (Brandy, 2007). The risk factors for anaemia vary in different settings; they include intestinal worms, malaria, HIV infection, nutritional deficiencies, haematological malignancies and chronic diseases like sickle cell disease. *Plasmodium falciparum* infection related to anaemia contributes significantly to maternal and child mortality, hence prevention and treatment of anaemic pregnant women and young children who are at risk is of major importance. Hookworm infections and schistosomiasis cause blood loss and contribute to the burden of anaemia. HIV/AIDS is an increasing cause of anaemia which is recognized as an independent risk factor for early death among HIV/AIDS infected people. Anaemia in childhood can result not only from events in childhood but also from socioeconomic status and maternal factors, like iron deficiency (Schellenberg, Schellenberg, Mushi, Savigny and Mgalula, 2003).

Anaemia can also result from a decreased ability of the red blood cells to provide adequate oxygen to the body tissues. This may be due to a decreased number of red blood cells, a decreased amount of haemoglobin in the red blood cells which transports oxygen, a decreased volume of red blood cells, failure of red cell proliferation, defective maturation of red blood cells, haemolysis, blood loss and by other mechanisms like

direct toxicity of the bone marrow resulting in decreased bone marrow production of red blood cells. It has serious negative consequences including increased mortality in women and children, decreased capacity to learn, and decreased productivity in individuals. symptoms of anaemia are often vague and may include: feeling tired, weakness, shortness of breath, confusion, feeling like one is going to pass out, increased thirst, fatigue, headache, faintness, increased sensitivity to cold, tinnitus, black spots before the eyes, irritability, lack of power of concentration, fever, pallor, dyspnoea, tachycardia, systolic murmurs, cyanosis, nausea, vomiting and abdominal pain (Johnson and Rubenstein, 2013).

The plant *Moringa oleifera* is the most widely cultivated species of a monogetic family called Moringaceae that is native to the Sub-Himalayan part of India, Pakistan, Bangladesh and Afghanistan (Palada and Chang, 2003). The Root, bark, pods, flowers, seeds, gum and leaves of this plant are used in traditional medicine for the treatment of various human diseases (Lowell, 2002). The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months without any major loss of its nutritional value (Arabshahi *et al*, 2007). The mineral contents and bioavailability of *Moringa oleifera* has been a subject of tremendous studies. There are however limited reports on the influence of variation in geographical locations or agro-ecology of *Moringa oleifera* on the mineral composition in various organs of the plant in Nigeria. Confirmation of mineral content of plant materials across varied agro-ecologies is necessary in the selection and formulation of plant based mineral supplement in animal and human nutrition (Anjorin *et al.*, 2010). The leaves of this plant contain a profile of important trace elements and are good sources of proteins, beta-carotene, amino acids and various phenolics. The leaves contain phytochemicals that include alkaloids, anthraquinone, coumarins, falvones, phenols, quinines and tannins (Kasolo *et al.*, 2011). *Moringa oleifera* is reported to prevent malnutrition because of the high protein and micronutrient content of the leaves (Anjorin *et al.*, 2010). Leaves of *Moringa oleifera* serves as a goodsource of vitamins (vitamin A and B-vitamins) and minerals (calcium, copper, sulphur, iron) (Lowell, 2002). The leaves are believed to contain approximately 46 types of antioxidants, 90 nutrients, 18 amino acids (among which 8 are the essential ones). It is on record that a gram of moringa leaf powder contain more vitamin C than oranges, more vitamin A than carrots, more calcium than milk, more potassium than banana, more iron than spinach; and the protein quality can equate with those in milk and eggs (Ndon, Katsumata and Sand, 2007). It is estimated that 100g dried leaf powder of *Morimga oleifera* contains about 28.29 mg of iron (Oduro*et al*., 2008).

The plant has been said to be a promising remedy for anaemia especially iron deficiency anaemia. The plant has been used to combat malnutrition, especially among infants and nursing mothers. Countries like Senegal, India, Benin and Zimbabwe are now using Moringa leaves for programmes to fight malnutrition (Fahey, 2005). This present study aims to evaluate the effectiveness of the ethanol extract of Moringa leaf powder as a remedy to protect and treat anaemia.

1.1 Justification For The Study

The increased risk of maternal and child mortality due to anaemia has been reported in various studies Carried out to ascertain the impact of anaemia (Scholl and Hediger, 1994). Medicinal plants have been used and recognized all over human history and have provided treatment measures for various ailments since antiquity. A medicinal plant as defined by the world health organization (WHO) is a plant in which one or more part of it contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Ogamba *et al*., 2011). Recently many study in plant research has been conducted to analyse the efficacy of *Moringa oleifera* in both humans and animals and has found the plant to be safe for human consumption. If Moringa is proved to have anti- anaemic property, it may open new research efforts that will lead to the design of new drugs for effective treatment of anaemia.

1.2 Aim And Objectives

1.2.1. Aims Of The Study

The aim is to determine the anti-anaemic potential of ethanol extract of *M. oleifera* leaves against aluminum chloride induced anaemic rats.

1.2.2. Objectives Of The Study

The specific objectives of this study were to:

- Extract dried Moringa oleifera leaf powder using ethanol;
- Determine the effect of ethanol extract of *M. oleifera* leaf on haematological parameters (PCV, RBC and WBC count) of aluminum chloride induced anaemic rat;
- Determine the effect of ethanol extract of *M. oleifera* leaf on serum enzymes (AST, ALT and ALP) of aluminum chloride induced anaemic rats; and
- Determine the effect of ethanol extract of *M. oleifera* leaf on body weight and blood glucose level of aluminum chloride induced anaemic rats.

II. Literature Review

2.1 *MORINGA OLEIFERA*

Moringa oleifera Lamarck tree which is commonly called ben oil or drumstick tree. It is now widely cultivated in Africa, Central and South America, Sri Lanka, India, Malaysia and the Philippines. It is a fast growing deciduous shrub or small tree up to 12m tall and 30cm in diameter with an umbrella shaped open crown (Anjorin *et al.*,2010). In Nigeria it is mostly grown in the northern part and locally known as Zogeli among the Hausa speaking people. The Yoruba of South-West Nigeria call it ewe ile or igi iyaanu (because of its many medicinal uses). *M. oleifera* is considered one of the world's most useful trees, as almost every part of the tree can be used for food, or has some other beneficial property and extracts from all parts of the plant show pharmacological properties, recognized by popular use and corroborated by the scientific community (Oliveira *et al.*, 1999). It is an exceptionally nutritious vegetable tree with a variety of potential uses (Ram, 1994). The leaves can be eaten fresh, cooked or stored as dried powder for several months and the pods when young can be cooked and eaten like beans. Its oil and micronutrients have been reported to contain antitumour, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters (Hsu, 2006). The crude protein, crude lipids and ash values of 26.4%, 6.5% and 12%, respectively for the unextracted leaves have been reported (Gupta *et al*., 1998).

2.1.1 Botanical Description

Moringa is a slender tree ranging in height from 5-12m with an open umbrella shaped crown, straight trunk (10-30cm thick), drooping branches that grows to approximately 10 m in height and a corky whitish bark. The tree comes with a lax crown of graceful airy foliage, whose feathery effect is due to the finely trip innate division of the leaves. The leaves are densely crowded at the tops of the branchlets and depending on climate, the foliage is evergreen or deciduous. The Flowers are insect pollinated and require a large number of insect visitations with carpenter bees being the most common guests (Bhattacharya, *et al.*, 2004). The fruits are initially light grey-pen, slim and tender, eventually turning dark green and firm. Depending on genotype, they are up to 120cm long. Fully mature, the dried seeds are surrounded by a lightly wooded shell with three Papery wings .The plant (depending on climate) has leaflets 1-2cm in diameter and 1.5-2.5cm in length. The tree produces a tuberous tap root which explains its tolerance to drought conditions. Moringa is adaptable to a wide range of environmental conditions, from hot, dry, humid, and wet conditions. The tree is tolerant to light frosts, but does not survive as a perennial under freezing conditions. Moringa grows more rapidly, reaching higher heights, when found in well-drained soils with ample water, but tolerates sandy soils, heavier clay soils and water limited conditions. The tree can be established in slightly alkaline soils up to pH 9 as well as acidic soils as low as pH 4.5 (Fahey, 2005).

Figure: 2.1*Moringa oleifera* leaf

Source: (Azubuogu , 2012)

2.1.2 Taxonomic Classification
Kingdom: Plantea
Sub kingdom: Tracheobionta
Super Division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Dilleniidae
Order: Capparales
Family: Moringaceae
Genus: Moringa
Species: Oleifera

2.1.3. Related Species
The National Research Council (2006) noted that, out of the 14 Moringa species, only M. oleifera has been accorded research and development. The other 13 species which remain unknown to science are:

Moringa drouhardii(Madagascar), *Moringa concanensis*(mostly India), *Moringa arborea*(northeastern Kenya), *Moringa hildebrandtii*(Madagascar), *Moringa oleifera*(India), *Moringa borziana*(Kenya and Somalia), Moringa ovalifolia(Namibia and extreme southwestern Angola), Moringa peregrina(Horn of Africa, Red Sea, Arabia), *Moringa longituba* (Kenya, Ethiopia, Somalia), *Moringa stenopetala* (Kenya and Ethiopia), *Moringapygmaea* (northern Somalia), *Moringa rivae* (Kenya and Ethiopia), *Moringa ruspoliana* (Kenya).

2.1.4. Occurrence of *Moringa Oleifera*

According to Wikiepedia (2009), *Moringa oleifera* is the most widely cultivated variety of the genus Moringa. However, it normally is cut back annually to one meter or less, and allowed to re-grow, so that pods and leaves remain within reach. Moringaceae is a single genus family with 14 known species. *Moringa oleifera* is a most widely known and utilized species and a native of the sub-Himalayan regions of north-west India, *Moringa oleifera* is now indigenous to many countries in Africa, Arabia, South East Asia, the Pacific and Caribbean Islands; and South America. Rajangam (2001) reported that India is the largest producer of Moringa with an annual production of 1.1 to 1.3 million tonnes of tender fruits from an area of 380km². Among the states, Andhra Pradesh leads in both area and production (156.65 km²) followed by Karnataka (102.8 km²) and Tamil Nadu (74.08 km²). In other states, it occupies an area of 46.13 km².

2.2. Phytochemistry

Phytochemicals are chemicals produced by plants which may have an impact on health, or on flavor, texture, smell,or color of the plants, but are not required by humans as essential nutrients. An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds like fully acetylated glycosides bearing thiocarbamates, carbamates or nitriles (Fahey *et al*., 2001). In particular, this plant family is rich in compounds containing the simple sugar, rahmnose, and it is rich in a fairly unique group of compounds called Glycosides containing isothiocyanates, malonates and flavonoids glucosinolates (Miean *et al.*, 2001). Some of the compounds that have been isolated from Moringa preparations which are reported to have hypotensive, anticancer and antibacterial activity include 4-(4'-*O*-acetyl-a L-rhamnopyranosyloxy)benzyl isothiocyanate**,** 4-(a-L-rhamnopyranosyloxy)benzyl isothiocyanate**,** niazimicin, pterygospermin**,** benzyl isothiocyanate**,**and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate (Daxenbichler *et al*., 1991). Flowers of Moringa have been reported to contain flavonoid pigments such as quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin (Nair and Subramanian, 1962). According to Foidl *et al.,* (2001) extracts of Moringa leaf in 80% ethanol contain cytokinine-type hormones.The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in moringa leaf. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in moringa leaves (Siddhuraju and Becker, 2003). The extracts of Moringaleaf (Siddhuraju and Becker, 2003) also appear to have cancer preventive effect, when assayed by the differentiating activity against human promyelocytic leukaemia cells.

2.3. Other Uses of *Moringa Oleifera*

M. oleifera produces fruit between April to June in Pakistan. It is considered one of the world's most useful tree, as almost every part of the Moringa tree can be used for food or has some other beneficial property. In the tropics, it is used as forage for livestock, and in many countries moringa micronutrient liquid, a natural anthelmintic (kills parasites) and adjuvant (to aid or enhance another drug) is used as a metabolic conditioner to aid against endemic diseases in developing countries (Foidle *et al.,* 2001). It is the most nutrient rich plant yet discovered and it provides a rich and rare combination of nutrients, amino acids, antioxidants, antiaging and

anti-inflammatory properties used for nutrition and healing. *M. oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and vitamin C (Fahey, 2005). According to researchers it has the potential to combat vitamin A and other micronutrient deficiencies (Nambiar, 2006). The whole seeds can also be eaten green, roasted or steamed in tea and curries (Fahey, 2005).

2.3.1. Water Purification

Several chemical coagulants have been used in conventional water treatment processes for portable water production that includes inorganic, synthetic organic polymer and naturally occurring coagulants (Okuda *et al.,* 2000). Generally, alum (Aluminum sulphate), an inorganic coagulant and its synthetic polymeric derivatives are widely used in water treatment (Najim *et al.,* 1998). However, there is a fear that aluminum may induce Alzheimer's disease and it possesses strong carcinogenic properties (Malleavialle *et al.,* 1984). On the other hand, there is much evidence that the use of extracts from plant species possessing both coagulating and antimicrobial properties are safe for human health (Akinnibosun *et al.,* 2009). Among the large number of plant materials that have been used over the years, the seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities (Doer, 2005). Folkard *et al.,* (1993) reported that while aerating well water in rural areas of Sudan for the reduction of carbon dioxide prior to softening, numerous complaints of red water in hot water systems were received even when aeration was continued and the carbon dioxide neutralized with lime in the regular plant treatment process. This complain ceased and did not reoccur as Moringa seeds was used. Moringa Seed powdered is able to clarify even the most turbid water. The powder joins with the solids in the water and sinks to the bottom. This treatment also removes 90-99% of bacteria contained in water and purifies the water by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titer (Ghasi *et al*., 2000).

2.3.2. Alternative Medicine for Human Pathogens

Escalating antibiotic resistance by pathogenic bacteria has been observed since last decade and the adverse effects of conventional antibiotics calls for a friendly alternative. Out of the 250,000 to 500,000 species of plants on earth (Cowan, 1999), moringa is one of the 10% which have a profound potential in pharmaceutical industry as a source of bioactive constituents for drug development. However, in another study to determine the antimicrobial potential of different plant seed extracts against Multidrug Resistant Methicillin Resistant Staphylococcus aureus (MDR – MRSA), it was established that *Moringa oleifera* seeds had a synergistic potential to restore the effectiveness of B – lactam antibiotics against MRSA (Suarez, Haenni, Canarelli, Fisch, Chodanowski, Servis C, *et al.*, 2005), This synergistic properties could be attributed to β – lactamase inhibition by the Flo peptide (a specific polypeptide found in *Moringa oleifera* that is both a flocculent and a biocide). The cationic Flo peptide supposedly serves as a highly efficacious immunity response, interacting with the anionic cell membranes of bacterium (Abdulmoneim and Abu, 2011). This interaction destabilizes the bacterial membrane, causing leakage of cytoplasmic content and killing the bacterial cell. Recently, an invitro antimicrobial activity of *Moringa oleifera* leaf extracts prepared in aqueous and organic solvents against Staphylococcus aureus, Bacillus subtilis, Escheriachia coli, Pseudomonas aeruginosa, Aspergillus niger and Candida albicans exhibited antimicrobial properties (Fluck, 1995). Recent study on the antibacterial effect of *Moringa oleifera* leaves extracts demonstrated its activity against clinical samples and environmental samples of *Proteus mirabilis,* a small
Gram negative facultative anaerobe that commonly cause urinary tract infections and formation of stones in the urinary tract. (Arun and Rao, 2011).

2.4. Pharmacological Actions of *Moringa Oleifera*

2.4.1. Antioxidant Activity

Ashok, (2003) investigated the antioxidant potential of *Moringa oleifera* on hepatic marker enzymes, lipid peroxidation, and antioxidants. The antioxidant property was investigated during antitubercular drug (isoniazid, rifampicin, and pyrazinamide) induced toxicity in rats. Enhanced hepatic marker enzymes and lipid peroxidation of antitubercular drug treatment was accompanied by a significant decrease in the levels of vitamin C, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase. Administration of *Moringa oleifera* extract and silymarin significantly decreased the hepatic marker enzymes and lipid peroxidation with a simultaneous increase in the level of antioxidants (Kumar *et al*., 2003).

2.4.2. Synthesis of Nanoparticles

The possibility of using Moringa in nanotechnology is being explored for useful products. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. Metal nanoparticles which possess a high specific surface area and a high fraction of surface atoms have been studied extensively because of their unique physiochemical characteristics such as catalytic activity, optical

properties, electronic properties, antibacterial properties and magnetic properties (Howey, Bowsher, Brumelle and Woodworth, 1994). Nanoparticles have a long list of applications in improving human life as well as the environment. The technology has come into spotlight due to its benefits such as shorter development periods and lower costs compared to the development of a new drug (Kang, Brange, Burch, Volund and Owens, 1991). Drug can be dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending on the method of preparation, nanoparticles, nanospheres or nano capsules can be obtained (Bhadra, Bhadra and Jain, 2002). It has been established that the use of plant leaves extract is cheaper as it reduces the costs and does not require any special culture preparation and isolation techniques. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provides advancement over chemical and physical method as it is cost effective and eco-friendly. It can be easily scaled up for large scale synthesis and there is no need to use high pressure, energy, temperature and toxic chemicals. In this regard, investigations in the biofabrication of Ag nanoparticles using *M. oleifera* leaves extract revealed the leaves to have the potential of producing Ag nanoparticles extracellularly by rapid reduction of silver ions (Ag^+ to Ag^0) which were quite stable in solution (Khanuja, Arya and Saikia, 2005). In subsequent testing for antimicrobial activity against a number of pathogens, this Ag nanoparticles suspended hydrosol showed considerable antimicrobial activity in comparison to chloramphenicol and ketoconazole antibiotics.

2.4.3. Cardiovascular Activity

The isolation of two nitrile glycosides, niazirin and niazirinin from the ethanol extracts of *Moringa oleifera* leaves and three mustard oil glycosides was reported by Faizi *et al.,* (1994). Compounds such as 4-[(4'-O-acetyl-alpha-L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B showed hypotensive activity. Most of these compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, thiocarbamates showed hypotensive activity.The hypocholesterolemic effect of the crude leaf extract of *Moringa oleifera* was investigated by Ghasi *et al.,* (2000) and it was found to be statistically significant but no significant effect on serum total protein was observed. The preventive effects of *Moringa oleifera* on hyperlipidemia caused by iron deficiency in male Wistar rats was tested by Ndong *et al.,* (2007). Ara *et al.,* (2008) reported comparative effects of ethanol extracts of the leaves of *Moringa oleifera* with atenolol on serum cholesterol level, serum triglyceride level, blood glucose level, heart weight and body weight of adrenaline induced rats.. The *Moringa oleifera* leaf extract made significant changes in each cardiovascular parameter after proper investigation.

2.5. Hematology

2.5.1. Blood and its Constituents

Blood accounts for 7% of the human body weight (Elert and Glenn , 2012) with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3 (Shmukler and Michael, 2004). The average adult has a blood volume of roughly 5 litres which is composed of plasma and several kind of cells (Alberts and Bruce, 2012). Blood is one of the

most common biological samples whose constituents are used for diagnosing a large number of diseases. Blood consists of three kinds of cells; these blood cells (which are also called corpuscles or "formed elements") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%. Blood is primarily composed of Plasma which makes up more than half of the blood's volume (55%) and consists of about 92% of water and a number of other proteins, ions and hormones. RBCs constitute 99% of the blood cells and are doughnut shaped with a disk diameter of 6-8 μm and a thickness of 2 μm. WBCs are divided into 6 subcategories namely: neutrophils, basophils, eosinophils, lymphocytes, monocytes and macrophages. They are normally spherical in shape with their size ranging from 7 to 20 μm in diameter (Bonetta, 2005). These cells play an important role in defending the body against infections and hence constitute an important part of the body's immune system. The total count of WBC's and the differential count of their subtypes is therefore an important indicator for a number of immunological diseases (Rogers, 2001).

Platelets also known as thrombocytes are irregularly shaped blood cells which are 2-3 μm in diameter and play an important role in clotting of blood at the site of a cut or an injury. In particular, WBC's or their subtypes constitute different aspects of human immune system and an increase or decrease in their count from the normal level usually indicates an immunological disorder (Sell, 2001). Patients afflicted with these diseases need to undergo WBC count tests at regular intervals (few days to months) to ascertain the efficacy and to accordingly modify the treatment regiwwment to check the proliferation of the disease.

2.5.1.1. Red Blood Cell

Red blood cells (RBCs) are the most common type of blood cell and the body's principal means of delivering oxygen (O_2) to the body tissues by the blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries. The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network in humans. Mature red blood cells are flexible and oval biconcave disks; they lack a cell nucleus and most organelles in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second in human adults (Erich, 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 (Pierige, serafini, rossi and magnani, 2007).

2.5.1.2. White Blood Cell

White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders. All leukocytes are produced and derived from a 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, Anthea, Jean and Charles, 1993). Five different and diverse types of leukocytes exist (LaFleur and Brooks, 2008). These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic.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 × 109/L. In the US this is usually expressed as 4,000–11,000 white blood cells per microliter of blood. They make up approximately 1% of the total blood volume in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia.

2.5.1.3. Platelets

Platelets, also called "thrombocytes", is a component of the blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries (Laki, 1972). They are fragments of the cytoplasm which are derived from the megakaryocytes (Machlus, Thon and Italiano, 2014) of the bone marrow which enters the circulation and they have no cell nucleus. Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells. The main function of platelets is to contribute to hemostasis (the process of stopping bleeding at the site of interrupted endothelium). They gather at the site and unless the interruption is physically too large, they plug the hole. Firstly, platelets attach to substances outside the interrupted endothelium (adhesion). Secondly, they change shape, turn on receptors and secrete chemical messengers (activation). Thirdly, they connect to each other through receptor bridges (aggregation) (Yip, Shen, Berndt and Andrews, 2005). Formation of this platelet plug (primary hemostasis) is associated with activation of the coagulation cascade with resultant fibrin deposition and linking (secondary hemostasis). Low platelet concentration called thrombocytopenia is due to either a decreased production or increased destruction. Elevated platelet concentration called thrombocytosis is either congenital, reactive (to cytokines), or due to unregulated production.

2.5.2. Packed Cell Volume

Packed cell volume (PCV), is the ratio of the proportion of erythrocytes which is expressed as a percentage of the volume of the whole blood per given sample (Kusiluka and Kambarage, 1996). Determination of PCV value is fundamental in diagnosing the various pathological and metabolic disorders (Grunwaldt *et al*., 2005). A low packed cell volume value can depict anaemia, haemorrhage, bone marrow failure, leukaemia, malnutrition or specific nutritional deficiency, multiple myeloma and rheumatoid arthritis. Packed cell volume values higher than the reference values could indicate dehydration due to diarrhoea, erythrosis and polycythermia. Packed cell volume is influenced by altitude (Tibbo *et al*., 2004), age (Grunwaldt *et al.,* 2005), health status, ambient temperature, and physiological status (excitement, muscular exercise, pregnancy, estrus, parturition, water balance and transportation (Osaer *et al*., 2000). Reduced oxygen tension in mountainous regions results to an elevated production and release of a glycoprotein called erythropoietin which stimulates erythropoiesis as a coping or adaptive mechanism to low oxygen level in such an environment (Tibbo *et al.,* 2004).

2.6. Aluminium Chloride

Aluminium, (Al), is ubiquitous in the environment and its extensive industrial utilization has stimulated considerable interest in the possible environmental toxicity of this metal. However, little is known about possible effects of Al as a trace element in animals and humans in normal conditions. It has recently become clear that when Al is mobilized from soil by acid rain, it poses a hazard to all exposed organisms (Beavon, 2004; Birchall *et al.,* 1989). The sources of aluminium are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, ware and containers. Also, it is also added to drinking water for purification purposes (Davis and Littlewood, 2012). The toxic effect arising from the absorption and accumulation of aluminium have well been documented and include a progressive encephalopathy which eventually leads to dementia. Al contributes to a variety of cognitive impairments in mice and rabbits, (Muller *et al.,* 1990). Studies on workers exposed to Al dust in industrial environments demonstrate similar effects (Akila *et al*., 1999). Many researchers have found elevated Al levels to be associated with a decline in visual memory, attention, concentration, frontal lobe function and lower vocabulary scores in hemodialysis patients (Bolla *et al.,* 1992). Salts of aluminium may bind to DNA, RNA; inhibit such enzymes as hexokinase, acid and alkaline phosphates, phosphodiesterase and phosphooxydase. Aluminium exposure caused impairments in glucose utilization, agonist

imulated inositol phosphate accumulation, free radical mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation (Tefferi, 2003).

2.6.1. Mechanism Behind Aluminum Chloride-Induce Anaemia

Studies have shown that daily injections of aluminum into rats result to severe anaemia within 2-3 weeks. This anaemia resulted from a decreased heme synthesis, decreased globulin synthesis and increased hemolysis (Skillen *et al*, 1989). Aluminum chloride may have an effect on the metabolism of ion by influencing the absorption of iron through the intestine. It also hinders the transport of iron in the serum and displaces iron from binding to transferrin. Transferrin plays the essential role of iron uptake and transport but is also the major serum binding protein for aluminum. Studies has shown that aluminum binds to serum transferrin to form a complex called aluminum-transferrin which interact with the same receptors as iron-transferrin. An exposure to a high dose of aluminum can change iron metabolism in different animal species. (Al-Hashem *et a.l,* 2009).

2.4 Diagnostic Enzymes

Enzymes are present in cells at a much higher concentrations than in plasma. Normal plasma enzyme concentrations reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation. The activity of an enzyme in plasma may be elevated above normal due to cell proliferation, increase in the rate of cell turnover or damage or induction and reduced clearance from plasma. A lower enzyme activity may occur due to a reduced synthesis or congenital deficiency (Crook, 2006). Typical examples of enzymes which are commonly used as diagnostic markers are alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT, γGT), etc. The activities of these enzymes in the plasma or serum are often measured in drug studies since the toxicity of drugs and chemicals often manifest in organ damage (Prouilleta *et al*.,2004).

ALP hydrolyses organic phosphates at high pH. They are present in most tissues but are particularly in high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. ALP activities may be increased due to pregnancy and growth related factors, bone disease, liver disease, inflammatory bowel disease or intestinal obstruction and malignancy.

The aminotransferases (ALT and AST) are involved in the transfer of an amino group from amino acid to oxoacid. GOT (glutamate oxaloacetate aminotransferase) are present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Elevated levels are caused by acute viral or toxic hepatitis, malignant infiltration of the liver, skeletal muscle disease, cirrhosis, cholestatic jaundice, severe haemolytic episodes as well as various drugs. GPT (glutamate pyruvate aminotransferase) is present in high concentrations in the liver and to a lesser extent, in skeletal muscle, kidney and heart. Elevated levels may be caused by similar circumstances as for AST and also by certain drugs.

Gamma-glutamyltransferase (GGT) has been demonstrated in various human and animal organs, including kidneys, liver, prostate, pancreas and spleen. It is also present in various body fluids, such as saliva, serum, bile and urine (Nemesanzky and Lott, 2005). Elevated levels of GGT may be caused by induction of enzyme synthesis by drugs (or alcohol). Many drugs are implicated (anticonvulsants, phenobarbitone, phenytoin), cholestatic liver disease, and hepatocellular damage. Very high plasma GGT activities, out of proportion to those of the aminotransferases, may be due to alcoholic hepatitis, induction by anticonvulsant drugs or by alcohol intake, cholestatic liver disease, hypertriglycridaemia, and fatty liver (Crook, 2006).

III. Material And Method

3.1 Materials

3.1.1 Plant Collection

The leaves of *Moringa oleifera* were obtained in september, 2014 from Mallam Musa's garden in his compound, Minna, Niger state. Nigeria.

3.1.2 Reagents

All the chemicals used were products of Sigma Chemical Co., USA. Aluminum chloride, hydrochloric acid, sodium hydroxide, iron 3 chloride, Conc. ammonium hydroxide, methanol hexane, Conc. H_2S0_4.

3.1.3 Apparatus And Materials

Test tubes, Beakers, Conical flasks, Round Bottom flasks, Measuring cylinders, Masking tape, Volumetric flasks, Separating funnels, pipette, What man Filter paper (No. 1), cuvette, water bath, systex haematology analyser, Electronic weighing balance, Foil paper and electronic blending machine.

3.2. Sample Preparation And Extraction Procedure

The collected fresh leaves of *Moringa oleifera* were destalked, washed with clean-water, dried at room temperature and finally grounded using an electric blender. Extraction of the powdered plant material was performedusing reflux extraction methodby weighing 50g of the powder plant sampleinto 300ml of ethanolat temperature between 60^0C-70^0C. The process was run for two hours each for four extractions and afterwards a rotary evaporator was used to evaporate the solvent and then later concentrated in a water bath to obtain the extract. The extract was then kept in well labelled sterile bottles and stored in the Refrigerator until required. The ethanol Extract of the plant weighed 53.59g (19.14%).

3.3 Management Of Experimental Animals

A total of fifty albino rats were obtained from the animal farm at Zungeru state Polytechnic, in Niger state weighing 75 to 170g. They were housed in plastic cages and given commercial diet (rat pellet) and water *ad-libitum* and were maintained under standard laboratory conditions.

3.4. Acute Toxicity

Lorke's method(1983)was used to study the acute toxicity effect of the ethanol extract of *Moringa oliefera* leaf in white albino rats. The study involved oral administration of different doses of the extract to 12 rats of four groups consisting of 3 animals each of different sexes. Signs accompanying toxicity and possible death of animals were monitored for twenty four hours and the median lethal dose (LD_{50}) of the extract were determined and compared with values from those of the control group. The$LD_{50}$of the plant extract was found to be > 2600 mg/Kg body weight.

3.5. Experimental Design

After an acclimatized period of two weeks, the rats were divided into six groups each consisting of eight animals in accordance to their body weight and the experiment was run for twenty eight days. Rats were administered their respective doses of *Moringa oleifera* each day 10 minute before the administration of aluminum chloride for 28days and administration were as follows:

Group1:- Normal saline (control)
Group2:- Aluminum chloride 0.5 mg/kg body weight
Group3:- Aluminum chloride 0.5 mg/kg body weight +*M. oleifera* (500 mg/kgbody weight)
Group 4:-Aluminum chloride 0.5 mg/kg body weight +*M. oleifera* (1000 mg/kgbody weight)
Group 5:- Aluminum chloride 0.5 mg/kg body weight +*M. oleifera* (2000 mg/kgbody weight)
Group 6:- 0.5mg/kg body weight of the Extract.

3.6. Blood Sample Collection

The tips of the rats tail were cut inorder to collect blood in heparinized capillary tube and sealed with hematocrit sealer (plasticine), the tubes were then centrifuged using a micro-haematocrit centrifuge at 10000 rpm for 5minutes and the packed cell volume was read using micro-haematocrit reader. Rats were anaesthetized in slight chloroform and blood sample collected into a clean dry centrifuge tubes. The blood sample was allowed to stand for 10minutes at room temperature and then centrifuged at 1000 rpm for about 15minutes. Two layers were seen after spinning, the whole blood was used for haematological parameters while serum was removed by pipetting which was used for the assay of Aspartate aminotransferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP) activities and total protein.

3.7. Biochemical Parameters

3.7.1. Determination Of Aspartate Amino Transferase (AST)

This is an enzyme usually found inside the liver cells. When a blood test detects high levels of this enzyme in the blood, it usually means the liver is injured in some way . However AST can also be released if the heart or skeletal muscle is damaged. For this reason AST is usually considered to be more specifically related to liver problems.

Principle

α-Ketoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction uses the oxaloacetate for a kinetic determination of NADH consumption.

L-aspartate + α-Ketoglutarate $\xrightarrow{\text{AST}}$ L-glutamate + oxalacetate

Oxalacetate + NADH $\xrightarrow{\text{MDH}}$ Malate + $sNAD^+$

Procedure

The working reagent was prepared by mixing four volume of reagent 1 (R_1) with one volume of R_2. 100 ul of the reagent was mixed with 100 ul of the serum, incubated for one minute and change in absorbance was read for three minutes.

3.7.2. Determination Of Alanine Aminotransferase (ALT)

This is an enzyme that helps to process proteins. Large amounts of ALT occur in liver cells. When the liver is injured or in flamed (as in hepatitis), the blood level of AL T usually rises. Serum elevations of ALT activity are rarely observed except in parenchymal liver disease, since ALT is a more liver-specific enzyme than asparate aminotransferase (AST).

Principle

α-Ketoglutarate reacts with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The pyruvate is used in the indicator reaction for a kinetic determination of the reduced form of nicotinamide adenine dinucleotide (NADH) consumption.

L-alanine + α-Ketoglutarate $\xrightarrow{\text{ALT}}$ L-glutamate + Pyruvate

Pyruvate + NADH (H^+) $\xrightarrow{\text{LDH}}$ Lactate + NAD^+

Procedure

The working reagent was prepared by mixing four volume of reagent 1 (R1) with one volume of R2. 100 ul of the working was mixed 100 ul of the serum incubated for one minute and change in absorbance was read for three minutes.

3.7.3. Determination of Alkaline Phosphatase (ALP)

This enzyme occurs mainly in the liver cells next to bile ducts, and in bone. The blood level is raised in some types of liver and bone diseases. Increased ALP activity is associated with two groups of diseases: those affecting liver function and those involving osteoblastic activity in the bones. In hepatic disease, an increase in ALP activity is generally accepted as an indication of biliary obstruction.

Principle

In the presence of magnesium ions, p-nitrophenylphosphate is hydrolyzed by phosphatases to phosphate and p-nitrophenol. The rate of p-nitrophenol liberation is proportional to ALP activity.

Procedure

The working reagent was prepared by mixing 4 volume of reagent 2 (R1) with 1 volume of reagent 2 (R_2). 100ul of the working reagent was mixed with 20ul of serum, incubate for one minute and measure the change in absorbance per minutes during three minutes.

IV. Results

4.1. Qualitative Secondary Metabolites Analysis of *M.Oleifera* Leaf Extract

PHYTOCONSTITUENTS	QUALITATIVE PRESENCE INFERENCE	INFERENCES
Alkaloids	+	Brown precipitate
Cardiac glycosides	+	Brown ring colouration
Phlobatannins	_	Red precipitate
Saponins	+	Frothing
Anthraquinones	+	Yellow colouration
Steroids	+	Blue colouration
Terpenes	+	Blue colouration
Tannins	+	Blue black colouration
Flavonoids	+	Yellow colouration
Phenols	+	Blue black colouration

KEY: + (present), - (absent)

4.1 Biochemical Parameters

4.1.1. Aspartate Amino Transaminase Activity (AST)

Figure 4.1 shows the effect of administration of *Moringa oleifera* leaf extract on serum AST activities in $AlCl_3$ induced anaemic rats. The initial serum AST activities of all experimental groups were not significantly different (P>0.05). After 28 days of the experiment, the negative control group (rats administered only $AlCl_3$) showed significant increase in serum AST activities compared to the normal control group (not administered $AlCl_3$). Administration of *Moringa oleifera* leaf extract at doses of 500, 1000 & 2000mg/kgbody weight caused significant dose dependent decrease in serum AST activities compared with the negative control group. However, no significant difference (P>0.05) was observed in serum AST activities was observed in the extract group (rats administered 500mg/kg*Moringa oleifera* leaf extract only) compared with the normal control rats.

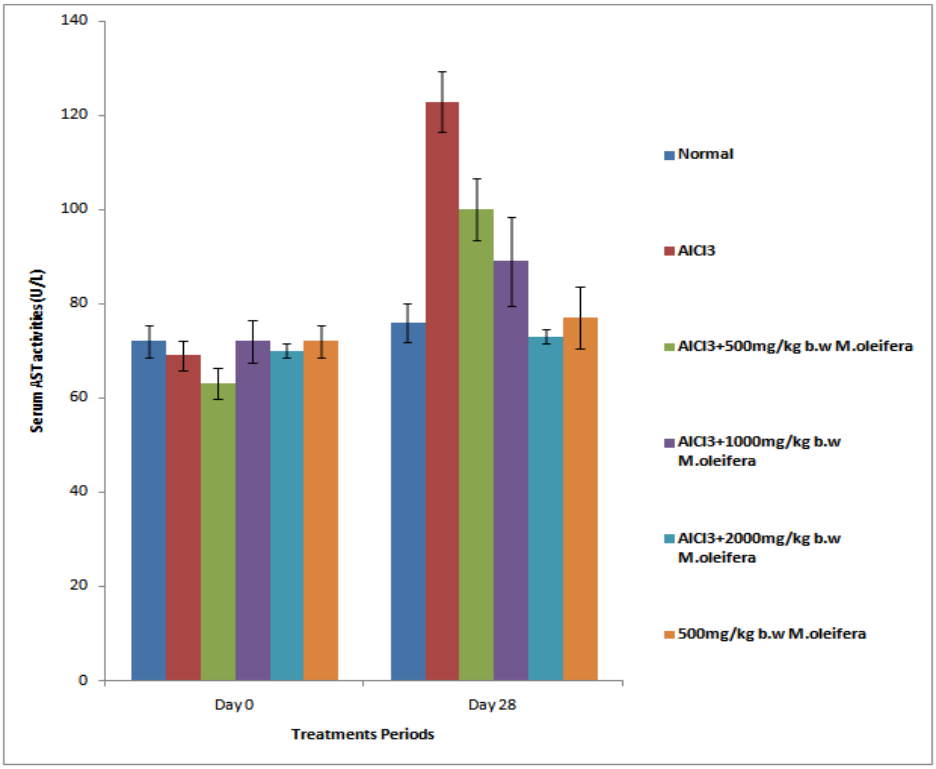

Figure 4.1: Effect of administration of *Moringa oleifera* leaf extract on serumAspartate amino transaminaseactivities in $AlCl_3$ induced anaemic rats.

4.1.2. Alanine Amino Transaminase (ALT)

Figure 4.2 shows the effect of administration of *Moringa oleifera* leaf extract on serum ALT activities in $AlCl_3$ induced anemic rats. The initial serum ALT activities of all experimental groups were not significantly different (P>0.05). After 28 days of the experiment the negative control group (rats administered only $AlCl_3$) showed significant increase in serum ALT activities compared to the normal control group (rats not administered$AlCl_3$). Administration of *Moringa oleifera* leaf extract at doses of 500, 1000 & 2000mg/kgbody weight caused significant dose dependent decrease in serum ALT activities compared with the negative control group. However, no significant (P>0.05) differences was observed in serum ALT activities of the extract group (rats administered 500mg/kg of*M. oleifera* leaf extract only) compared with the normal control rats.

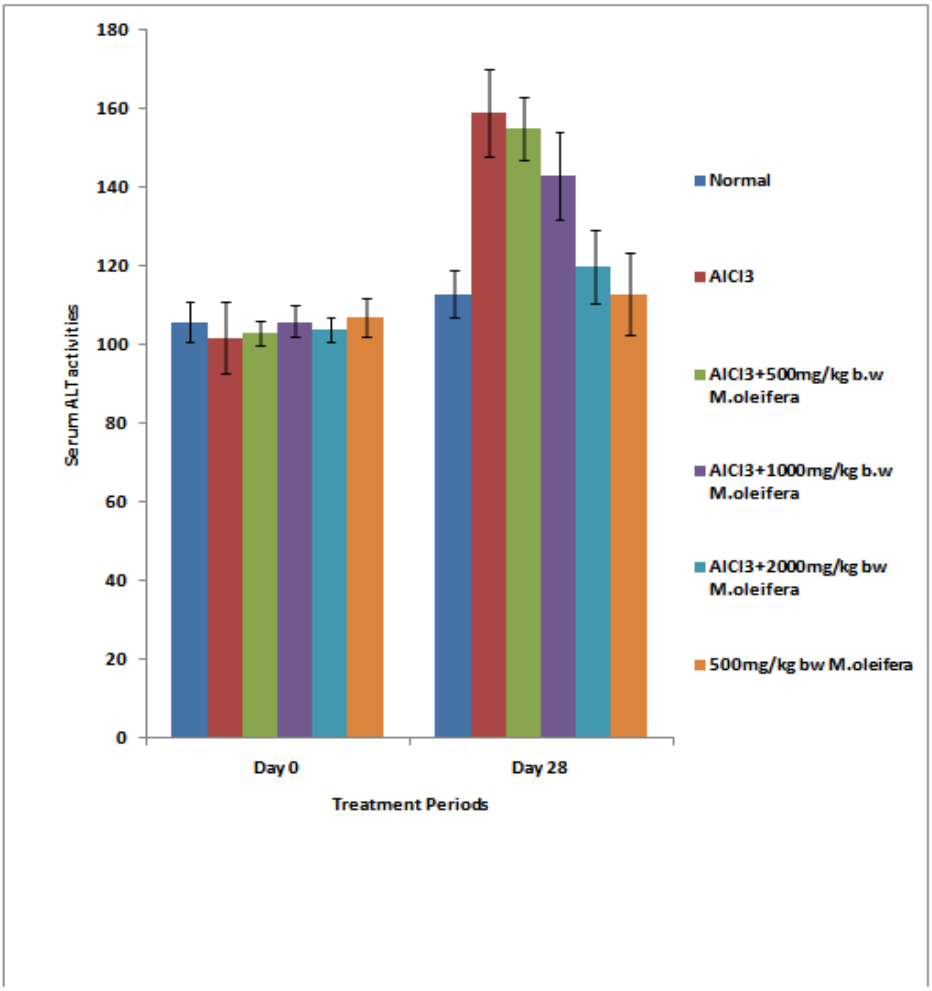

Figure 4.2: Effect of administration of *Moringa oleifera* leaf extract on Serum Alanine amino transaminase activities in $AlCl_3$ induced anemic rats

4.1.3. ALKALINE PHOSPHATASE (ALP)

Figure 4.3 shows the effect of administration of *Moringa oleifera* leaf extract on serum ALP activities in $AlCl_3$ induced anemic rats. There was no significant difference in serum ALP activities of all the experimental groups throughout the study period. However the extract group (rats administered only 500 mg/kg body weight of *M. oleifera* leaf extract) showed significant decrease in serum ALP activities compared with the negative control group (rats administered $AlCl_3$only).

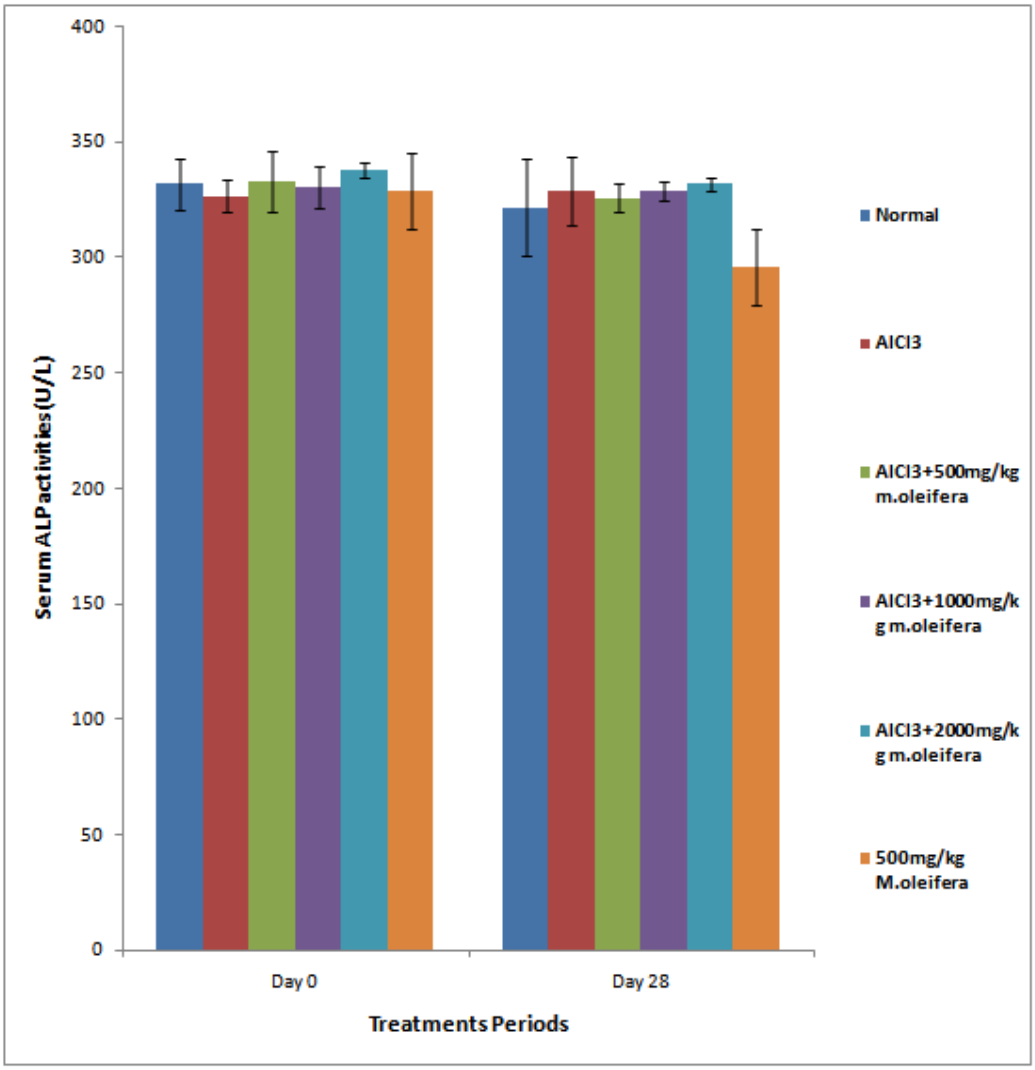

Figure 4.3: Effect of administration of *Moringa oleifera* leaf extract on Serum ALP activities in $AlCl_3$ induced anemic rats

4.1.4. Total Proteins

Figure 4.4 shows the effect of administration of *Moringa oleifera* leaf extract on serum total proteins in $AlCl_3$ induced anemic rats. The initial level of total proteins of all experimental groups were not significantly different ($P>0.05$). After 28 days of the experiment the negative control group (rats administered only $AlCl_3$) showed significant decrease in serum total proteins compare to the normal control rats. Administration of *Moringa oleifera* leaf extract at doses of 1000 and 2000mg/kg body weight caused significant dose dependent increase in serum total proteins compared with the negative control group. However, no significant ($P>0.05$) differences was observed in serum total proteins of anemic rats administered *M. oleifera* leaf extract at 500mg/kg body weight compared with the negative control group.

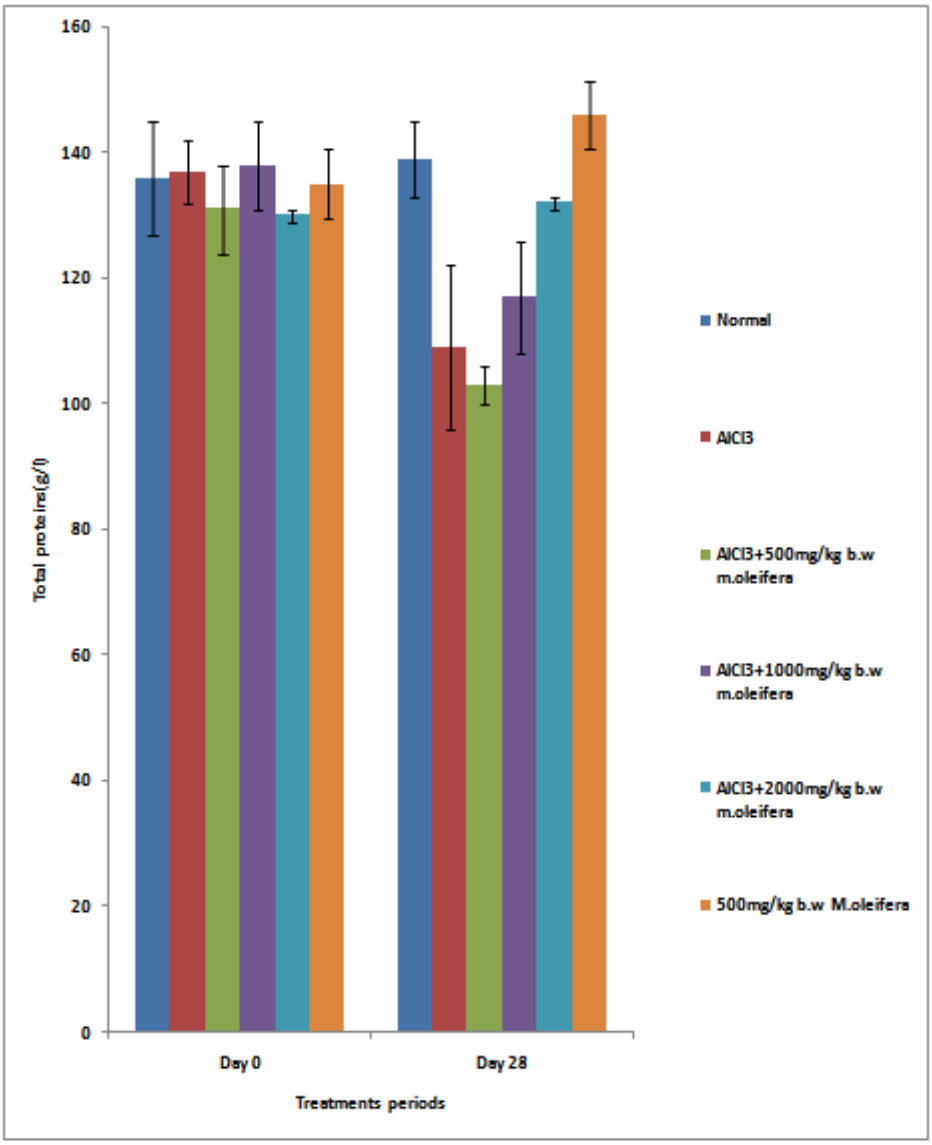

Figure 4.4: Effect of administration of *Moringa oleifera* leaf extract on serum total proteins in $AlCl_3$ induced anemic rats

4.2. WEIGHT CHANGES

Table 4.2 shows the effect of administration of *Moringa oleifera* leaf extract on body weight change of $AlCl_3$ induced anemic rats. $AlCl_3$ administration for four weeks caused progressive decrease in weight of rats. The normal control group and the extract group (rats administered only M. *oleifera extract* at 500mg/kg body weight) had progressive increase in body weight throughout the study period while the treatment group administered $AlCl_3$in addition with *M.oleifera* extract at doses of 500, 1000 & 2000mg/kg body weight showed initial decrease in body weight after 2 weeks of treatments but significant improvement in body weight were observed at the third and fourth week compared with the negative control group (rats administered $AlCl_3$only).

Table 4.2: Effect of administration of *Moringa oleifera* leaf extract on body weight of$AlCl_3$ induced anemic rats

Groups	**period (week)**			
	1	2	3	4
Normal	77.33±6.88[a]	78.33±5.84[a]	95.00±1.10[a]	97.00±1.5[b]
AlCl$_3$	96.00±15.52[a]	93.66±15.64[a]	91.66±15.40[a]	88.66±14[b]
AlCl$_3$+500mg/kgb.w*M.oleifera*	77.66±3.33[a]	70.00±2.64[a]	70.33±3.48[a]	78.66±3.3[b]
AlCl$_3$+1000mg/kgb.w*M.oleifera*	127.00±20.55[a]	123.67±20.4[b]	125.00±20.8[a]	134.33±21[a]

$AlCl_3$+2000mg/kgb.w*M.oleifera*	166.67±7.78[b]	168.00±7.56[b]	168.00±7.86[b]	173.00±4.5[a]
500mg/kgb.w*M.oleifera*	170.33±9.08[b]	174.67±7.76[b]	180.67±8.00[b]	188.67±9.9[a]

4.3. Blood Glucose

Table 4.3 shows the effect of administration of *Moringa oleifera* leaf extract on blood glucose in $AlCl_3$ induced anemic rats. The blood glucose level of rats that received only$AlCl_3$ and those that receivied$AlCl_3$and *Moringa oleifera* leaf extract at doses of 500, 1000 and 2000mg/kg body weight were not significantly different from the control rats. However, the group of rat that received only *Moringa oleifera* leaf extract at doses of 500mg/kg body weight alone showed significant ($P<0.05$) decreases in blood glucose level when compared with the control rats.

Table 4.3: Effect of administration of *Moringa oleifera* leaf extract on blood glucose level in $AlCl_3$ induced anemic rats

	Period (week)			
Groups	1	2	3	4
Normal	85.66±3.33[a]	79.66±0.33[a]	71.33±0.66[b]	88.00±2.00[b]
$AlCl_3$	87.66±2.66[a]	89.33±4.33[a]	74.66±9.66[b]	77.66±2.66[b]
$AlCl_3$+500mg/kgb.w*M.oleifera*	78.00±7.00[a]	89.66±3.33[a]	82.00±2.00[b]	76.66±4.33[b]
$AlCl_3$+1000mg/kgb.w*M.oleifera*	88.00±8.00[a]	89.66±3.66[a]	87.33±6.66[b]	78.66±4.33[b]
$AlCl_3$+2000mg/kgb.w*M.oleifera*	88.33±0.33[a]	86.66±1.33[a]	72.00±0.00[b]	76.66±0.33[b]
500mg/kgb.w*M.oleifera*	87.33±4.33[a]	82.33±0.33[a]	51.66±3.17[a]	51.00±2.00[a]

4.4. Packed Cell Volume (PCV)

Table 4.4 shows the effect of administration of *Moringa oleifera* leaf extract on packed cell volume (PCV) in $AlCl_3$ induced anemic rats. The initial PCV of all experimental groups were not significantly different ($P>0.05$). The negative control group (rats that received only$AlCl_3$)showed significant decrease in PCV compared to the normal control group throughout the study period. After two weeks of treatments, the PCV of the treatment groupadmistered $AlCl_3$ in addition to*M.oleifera extract* at doses of 500, 1000 & 2000mg/kgbody weight were not significantly different from that of the negative control group. However at the second and third week of treatment, the PCV of the treatment groups (rats administered$AlCl_3$and *M.oleifera extract* at doses of 500, 1000 and 2000mg/kgbody weight) showedsignificant difference compared with the negative control group. The extract group(rats that received*M.oleifera* alone at 500mg/kgbody weight) showed significant increase in PCV compared with the negative control group and the experimental groups.

Table 4.4: Effect of administration of *Moringa oleifera* leaf extract on PCV in $AlCl_3$ induced anemic rats

	period (week)			
Groups	**1**	**2**	**3**	**4**
Normal	43.75±2.12[a]	45.70±2.34[b]	45.90±0.21[b]	47.30±0.97[b]
$AlCl_3$	44.75±0.65[a]	33.56±0.99[a]	21.60±0.55[a]	16.09±0.62[a]
$AlCl_3$+500mg/kgb.w *M.oleifera*	45.96±1.79[a]	35.09±1.09[a]	40.25±0.60[b]	43.31±1.09[b]
$AlCl_3$+1000mg/kgb.w*M.oleifera*	44.51±2.54[a]	37.98±1.98[a]	44.89±1.09[b]	47.45±2.11[b]
$AlCl_3$+2000mg/kgb.w*M.oleifera*	45.02±1.09[a]	36.03±0.98[a]	40.90±1.43[b]	48.25±2.98[b]
500mg/kgb.w*M.oleifera*	44.98±1.98[a]	48.87±1.93[b]	51.29±2.91[c]	55.34±0.98[c]

4.5. Red Blood Cell, White Blood Cell And Hemoglobin

$AlCl_3$ administration for four weeks significantly ($P>0.05$) decreased red blood cell counts, hemoglobin, and significantly increased white blood cell counts compared with the normal control group.

Table 4.5: Effect of administration of *Moringa oleifera* leaf extract on red blood cell, white blood cell count and hemoglobin in $AlCl_3$ induced anemic rats

Groups	RBCs ($x10^{12}$/L)	WBC ($x10^9$/L)	Hb (g/dl)
Normal	5.52 ± 0.45^{bc}	4.56 ± 0.05^{a}	15.7 ± 1.84^{b}
$AlCl_3$	3.57 ± 0.44^{a}	7.21 ± 0.11^{c}	5.43 ± 1.10^{a}
$AlCl_3$+500mg/kgb.w *M.oleifera*	4.53 ± 0.64^{b}	6.34 ± 0.28^{b}	14.43 ± 1.49^{b}
$AlCl_3$+1000mg/kgb.w*M.oleifera*	4.77 ± 0.19^{b}	6.88 ± 0.11^{b}	15.73 ± 1.10^{b}
$AlCl_3$+2000mg/kgb.w*M.oleifera*	$5.12 \pm 0.67b^{c}$	$5.55 \pm 0.19a^{b}$	16.08 ± 1.23^{b}
500mg/kgb.w*M.oleifera*	6.64 ± 1.24^{c}	4.66 ± 0.44^{a}	18.44 ± 0.95^{c}

V. Discussion

Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status (Muhammad et al., 2000). Assessment of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal (Ashafa *et al.*, 2009). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu et al., 2003).

Several mechanisms have been proposed for the aluminum-induced anaemia, but the exact mechanism of aluminum-induced anaemia is unknown. The proposed mechanisms appear to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization (Han *et al.,* 2000). The present study thus revealed that $AlCl_3$admininisterd rats showed lower than normal haematological and biochemical parameters. Some of these abnormalities might be due to destruction of matured red blood cells leading to the low Hb counts accompanied by the fall in the RBC and PCV (Muhammad and Oloyede, 2009). The decrease in hemoglobin could result not only from decreased red blood cell count but from impaired heme biosynthesis in the bone marrow. The significant increase in white blood cell count of the negative control group (rats administered $AlCl_3$ only without treatment) might have resulted from an activation of the immune system or a normal cell-mediated immune response (El-Demerdash 2004).

Administration of the extract elicited increase in the haematological parameters of the $AlCl_3$induced rats. Thus, increase in RBC and Hemoglobin by the extract were an indication of its ameliorative effect on $AlCl_3$induced anaemia. The result of the present study is similar to the findings of Savage *et al*., (2000), who reported alteration in haematological parameters following $AlCl_3$. Hefound that patients with anemia caused by aluminum toxicity often have increased reticulocytes counts, decreased meancorpuscular volume, and mean corpuscular hemoglobin concentration administration to rats (Savage *et al*., 2000). The ameliorative effect of *M. oleifera* on $AlCl_3$ induce alteration in hematological parameters is not suprising as *M. oleifera*, has been reported to treat Alzheimer's disease that was caused by Al accumulation (Obulesu and Dowlathabad, 2011). The leaves of this plant which contain vitamins and Fe in significant amount were mentioned to improve iron and blood status of rats (Dhar and Gupta, 1982). The measurement of activities of various enzymes in the body fluids plays significant role in disease investigation and diagnosis (Malomo, 2000), damage on the organs or tissues and to a reasonable extent the toxicity of the drug (Yakubu *et al., 2*003). Biomarker enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Akanji *et al.*, 1993).

ALT and AST are known as cytosolic marker enzymes and are used as indicator for hepatic damage. The significant increase in the activities of these enzymes following $AlCl_3$ administration may be attributed to increased membrane permeability or hepatocellular necrosis and cytosol leakage into the serum (Teppema *et al.,* 2002).

ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al.,* 1993). The non significant ($P>0.05$) effect differences in the activity of ALP in $AlCl_3$administered rats compared with the normal control rats suggested that the integrity of the plasma membrane has not been compromised. However, improvement towards normalization of the biomarker enzymes following administration of *M. oleifera* leaf extract suggested that the extract had some functions in preserving structural integrity of hepatocellular membrane during $AlCl_3$intoxication.

The present findings also revealed that $AlCl_3$ had no effect on serum glucose levels even though aluminium is known to play specific role in carbohydrate metabolism (Thirunavukkarasu and Sakthisekaran 2003). The concentrations of total protein are useful markers of secretory, synthetic and excretory functioning of

the liver and kidney (Yakubu and Musa, 2012). The observed decrease in total protein suggested a compromise of the synthetic ability of the liver arising from the administration of $AlCl_3$. This findings agrees with the previous study of Chinoy and Memon (2001) and Newairy *et al*. (2009) who found that exposure to $AlCl_3$caused necrosis to the liver with the subsequent release of AST from the injured hepatic cells to the plasma and $AlCl_3$could also exacerbate reactive oxygen species (ROS) formation which has been found to cause oxidative damage of proteins and DNA. The decrease in protein content might be attributed to higher intracellular concentration of Al in the liver which could result in reduced protein synthesis as well as reduced enzymes of protein synthesis (Tripathi *et al*., 2009). Such decrease in total protein could, however, lead to hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals. Treatment with *Moringa oleifera* extract after administering $AlCl_3$ significantly ameliorated the decline in the plasma protein content probably by scavenging the free radicals and improving the antioxidative status and in turn the process of protein synthesis.

VI. Conclusion & Recommendation

In conclusion, the present has shown that oral administration of $AlCl_3$ at a dose of 50 mg/kg daily for 28 days caused significantly alteration to the level of haematological & biochemical parameters in rats. However, ethanol leaf extract of *Moringa oleifera* had significant and dose dependent ameliorative effect on $AlCl_3$ induced alteration in the biochemical and hematological parameters.

References

[1]. Abdulmoneim, M.S., Abu, I.E (2011). An in vitro antimicrobial activity of *Moringa oleifera* Microbiol L. seed extracts against different groups of microorgamisms.*Australian Journal of Basic and Applied Sciences;***5** :129 - 134.

[2]. Akanji, M.A., Olagoke, O.A. and Oloyede, O.B. (1993). Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system*;Journal of Toxicology*, **81** : 173-179.

[3]. Akila, R., Stollery B.T and Riihimaki, V (1999). Decrements in cognitive performance in metal inert gas welders exposed to aluminum. *Journal of Occupational and Environmental Medicine*;**56** : 632-639.

[4]. Akinnibosun, F.I, Akinnibosun, H.A and Ogedegbe, D (2009). Investigation on the antibacterial activity of Aqueous and ethanolic extracts of leaves of Boerhavia diffusa. *Science World Journal;***4** (2):15-18

[5]. Al-Hashem, F. (2009). Camel's Milk Protects Against Aluminum Chloride-Induced Normocytic Normocromic Anaemia, Lipid Peroxidation and Oxidative Stress in Erythrocytes of White Albino Rats. Ame*;Journal of Biochemisrty and Biotechnology*. **5** (3):126-136.

[6]. Anjorin, S.T, Ikokoh, P.S. Okolo, A. (2010). Mineral Composition of *Moringa oleifera* Leaves, Pods and Seeds from Two Regions in Abuja. *Nigeria International Journal of Agriculture and Biology;* Vol 12 No. **3** : 1814–9596.

[7]. Arabshahi, D.S., Devi, D.V and Urooj, A.A (2007). Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. *Journal of Food Chemistry;***100** : 1100-1105.

[8]. Arun, T and Rao C.H (2011). Phytochemical screening and antibacterial activity of *Moringa. oleifera* Lam. against Proteus mirabilis from urinary tract infected patients. *International Journal of ParmTechResearch;***3** : 2118 – 2123

[9]. Ashafa, O.T., Yakubu, M.T., Grierson, D.S. and Afolayan, A.J. (2009). Toxicological evaluation of the aqueous extract of Felicia muricata Thunb. leaves in Wistar rats. *African Journal of Biotechnology;***8** :949-954.

[10]. Brady, P.G. (2007). Iron Deficiency Anaemia: A Call for Aggressive Diagnostic Evaluation. *Medical Journal;***100** (10):966-967.

[11]. Chinoy, N.J. and Memon, M.R. (2001). Beneficial effects of some vitamins and Calcium on fluoride and aluminum toxicity on gastrocnemius muscle and liver of male mice. Fluoride **34** :21-33.

[12]. Cowan, M.M (1999). Plant products as antimicrobial agents. *Journal of Clinical Microbiology;* **12** : 564 – 582

[13]. Davis, L.F and Littlewood, T.J (2012). The investigation and treatment of secondary anaemia. *Blood review Journal;***2 :**65-71.

[14]. Dhar, B. and Gupta, O.P. (1982). Nutritional value of *Moringa oleifera* Lam. *Bull Medical Ethnobotany Research journal;***3**(4):280 - 288.

[15]. Doer, B.A (2012). Field Guides for Emergency water Treatment with Moringa oleifera.

[16]. "Volume of Blood in a Human".

[17]. Davis, L.F and Littlewood, T.J (2012). The investigation and treatment of secondary anaemia. *Blood review Journal;***2 :**65-71.

[18]. Erich, R.B and Sackmann, E.V (1995). Biological Membranes Architecture and Function., Handbook of Biological Physics.

[19]. Fahey, J.W, Zalcmann, A.T and Talalay (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants.*Journal of Phytochemistry;**56** (1)*: 5-51.

[20]. Fahey, J. (2005). *Moringa oleifera*: A review of the Medical Evidence for its Nutritional, Therapeutic and Prophylactic Properties Part 1, *Trees for Life Journal*.

[21]. Fluck, H. (1995). The influence of climate on the active principles in medicinal plants. *Journal of Pharmacy and Pharmacology;***7** : 361 -383.

[22]. Foidle, H.P., Makkar and Becker (2001). The potential of *moringa oleifera* for agricultural and industrial uses. p. 45-76. InL. Fuglie (ed.) The miracle tree.

[23]. Folkard, G.K, Sutherland, J.P and Shaw, R. (2008), Water clearification using Moringa oleifera seed coagulant.

[24]. Gupta, K., Barat, G.K., Wagle, D.S. and Chawla, H.K (1989). Nutrient contents and anti-nutritional factors in conventional and non-conventional leafy vegetables.*Journal of Food Chemistr;***31** : 105-116

[25]. Han, J., Han, J. and Dunn, M.A. (2000). Effect of dietary aluminum on tissue non-heme iron and ferritin levels in the chick. *Journal of Toxicology;***142** (2):97-109

[26]. Howey, D.C, Bowsher, R,R, Brumelle R.L, Woodworth, J.R (1994). Human insulin: a rapidly absorbed analogue of human insulin, Diabetes, **43** :396-402.

[27]. Hsu, R, Midcap S and Lucienne A.L (2006). *Moringa oleifera*, Medicinal and Socio-Economic uses. *International Journal on Economic Botany;***23** :1-25.

[28]. Jain, N.C(1975). "A scanning electron microscopic study of platelets of certain animal species". **33** (3): 501–517.

[29]. Johnson, R.L, Rubenstein, S.D (2013). "Anaemia in the emergency department: evaluation and treatment.". *Emergency medicine practice***15** (11): 1–15.
[30]. Johnson, S., Warner, M.Q., LaHart, D., and Wright, J. D., (1997). *Journal of Human Biology and Health*.
[31]. Kang, S, Brange, J, Burch A, Volund A, Owens D.R (1991). Comparison of subcutaneous soluble human insulin and insulin analogues on meal- related plasma glucose excursions in type I diabetic subjects, **14** : 571 –577. 81.
[32]. Khanuja., P.S., Arya J.S., Tiruppadiripuliyur, S.K., Saikia, D, Kaur, H.T and Singh, M.C. (2005). Nitrile glycoside useful as a bioenhancer of drugs and nutrients, process of its isolation from *Moringa oleifera*. United States Patent **6** :858,588.
[33]. Kumar, N.A and Pari, L. (2003). Antioxidant action of *Moringa oleifera* Lam (drumstick) against antitubercular drugs induced lipid peroxidation in rats. *Journal of Medical Food;* **6** (3), 255-259.
[34]. Laki, K. (1972). "Our ancient heritage in blood clotting and some of its consequences". Annals of the New York Academy of Sciences **202**: 297–307.
[35]. Machlus,K.R., Thon, J.N., Italiano, J.E. (2014). "Interpreting the developmental dance of the megakaryocyte: A review of the cellular and molecular processes mediating platelet formation". *British Journal of Haematology***165** (2): 227–236.
[36]. Mallevialle, J. Bruchet, A and Fiessinger, F. (1984). How safe are organic polymers in water treatment. *Jounal of American Water Works Association* ***7*6**(6):431-436
[37]. Malomo, S.O. (2000). Toxicological implication of ceftriaxone administration in rats. *Nigerian Journal of Biochemistry and Molecular Biology*.; **15** (1): 33-38.
[38]. Maton, Anthea, Jean, Hopkins, Charles, William, McLaughlin, Susan Johnson, Maryanna Quon,Warner, David LaHart and Jill D. (1993).*Journal of Human Biology and Health*.
[39]. McLean, E., Cogswell, M., Egli I., Wojdyla D and Benoist B (2009). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System *Journal of Public Health Nutrition;***12** 444-454.
[40]. Muhammad, N.O. and Oloyede, O.B. (2009). Haematological parameters of broiler chicks fed Aspergillus niger-fermented Terminalia catappa seed meal-based diet. *Global Journal of Biotechnology and Biochemistry;***4**:179 – 183.
[41]. Muller, G.V., Bernuzzi, D., Desor, M.F., Hutin, D., Burnel and Lher, P.R. (1990). Developmental alteration in offspring of female rats orally intoxicated by aluminum lactate at different gestation periods. *Neurotoxicology and Teratology Journal;***42** :253-261.
[42]. Najm, I., Tate, C., and Selby, D., (1998). Optimizing enhanced coagulation with PAC a case study, *Journal of American Water Works Association*;**90** (10):88-95.
[43]. Nambiar, V.S. (2006). Nutritional potential of drumstick leaves: an Indian perspective. Proc. Moringa and other highly nutritious plant resources: National Academies Press.
[44]. Ndong, M.U., Katsumata and K.S (2007). Preventive effects of *Moringa oleifera* Lamo hyperlipidemia and hepatocyte ultra structural changes in iron deficient rats. Bioscience Biotechnology and Biochemistry Journal;**71** : 1826-1833.
[45]. Newairy, A.A., Salama, A.F., Hussien, H.M. and Yousef, M.I. (2009). Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. *Journal of Food and Chemical Toxicology;***47** :1093–98.
[46]. Oduro, I., Ellis, W.O and Owusu D. (2008). Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Science Reseach and Assiociate;***3** (2): 57-60.
[47]. Okuda, T., Baes, A.U., Nishitimas, W and Okada, M. (2001). Isolation and characterization of coagulant extracted from *Moringa Oleifera* seed by salt solution. *Journal of Water Resources*;**35** (2):405-410
[48]. Oliveira, J.T., Silveira, S.B., Vasconcelos, K.M., Cavada, B.S. and Moreira R.A. (1999). Compositional and nutritional attributes of seeds from the multiple purpose tree of *Moringa oleiferaLamarck*. *Journal of the Science of Food and Agric;***79** (6):815-820.
[49]. Pierigè, F., Serafini, S., Rossi, L and Magnani, M. (2008). "Cell-based drug delivery". *Advanced Drug Delivery Journal*;**60** (2): 286–95.
[50]. Prouilleta, C.; Mazie`reb, J.; Mazie`reb, C.; Wattela, A.; Braziera, M.; and Kamela, S.(2004) Stimulatory effect of naturally occurring flavonolsquercetin and kaempferol on alkaline phosphatase activity in MG-63 human osteoblasts through ERK and estrogen receptor pathway. *Journal of Biochemistry and Pharmacology;***67**, 1307–1313.
[51]. Rajangam,J., Azahakia-Manavalan, R.S., Thangaraj, T., Vijayakumar, A and Muthukrishan, N. (2001). Status of Production and Utilisation of Moringa in Southern India.
[52]. Schellenberg, D., Schellenberg, J.R., Mushi, A., Savigny, D., Mgalula, L., Mbuya, C and Victora, C.G (2003). The silent burden of anaemia in Tanzanian children: a community-based study. Bulletin of the World Health Organization.**81** :581-590.
[53]. Scholl, T.O and Hediger, M.L (1994). Anaemia and iron-deficiency anaemia: compilation of data on pregnancy outcome. *The American journal of clinical nutrition;***59** (2): 492S-500S.
[54]. Teppema, L.J., Nieuwenhuijs, D., Sarton, E., Romberg, R., Olievier, N.C., Ward, D.S. and Dahan A. (2002). Antioxidants prevent depression of the acute hypoxic ventilatory response by subanaesthetic halothane in men. *Journal of Physiology*;**544** : 931-938.
[55]. Tefferi, A. (2003). Anaemia in Adults: A Contemporary Approach to Diagnosis. *MayoClinic Proceedings Journal;***78** 1274–80.
[56]. Vinay, Kumar, Abul, K., Abbas, Nelson Fausto, Richard N and Mitchell (2007). Robbins Basic Pathology.
[57]. Yakubu, M.T. and Musa, I.F. (2012). Liver and Kidney Functional Indices of Pregnant Rats Following the Administration of the Crude Alkaloids from Sennaalata (Linn. Roxb) Leaves. *Iranian Journal of Toxicology*;**6** :16, 615-625
[58]. Yip, Shen, Berndt, M.C and Andrews, R.K. (2005). "Primary platelet adhesion receptors".(International Union of Biochemistry and Molecular Biology: Life)**57** (2): 103

APPENDIX

Blood Glucose Level

Sample		glucose1	glucose2	glucose3	glucose4
Normal	Mean	85.6667	79.6667	71.3333	68.0000
	Std. Error of Mean	3.33333	.33333	.66667	2.00000
$AlCl_3$	Mean	97.6667	89.3333	74.6667	67.6667
	Std. Error of Mean	2.66667	4.33333	9.66667	2.66667
$AlCl_3$+500mg/kg	Mean	78.0000	89.6667	82.0000	56.6667
	Std. Error of Mean	7.00000	3.33333	2.00000	3.33333
$AlCl_3$+1000mg/kg	Mean	88.0000	99.6667	107.3333	58.6667
	Std. Error of Mean	8.00000	3.66667	6.66667	4.33333
$AlCl_3$+2000mg/kg	Mean	61.3333	121.6667	72.0000	66.6667
	Std. Error of Mean	.33333	1.33333	.00000	.33333
M.oleifera	Mean	87.3333	72.3333	51.6667	51.0000
	Std. Error of Mean	4.33333	.33333	3.17980	2.00000
Total	Mean	83.0000	92.0556	76.5000	61.4444
	Std. Error of Mean	3.22470	3.93698	4.37555	1.82256

Body Weight

Sample		weight1	wejght2	weight3	weight4
Normal	Mean	77.3333	78.3333	95.0000	97.0000
	Std. Error of Mean	6.88799	5.84047	1.10151E1	1.15326E1
$AlCl_3$	Mean	96.0000	93.6667	91.6667	88.6667
	Std. Error of Mean	1.65227E1	1.56454E1	1.54092E1	1.47460E1
$AlCl_3$+500mg/kg	Mean	77.6667	70.0000	70.3333	78.6667
	Std. Error of Mean	3.33333	2.64575	3.48010	3.38296
$AlCl_3$+1000mg/kg	Mean	1.2700E2	1.2367E2	1.2500E2	1.3433E2
	Std. Error of Mean	2.05508E1	2.04151E1	2.08407E1	2.13411E1
$AlCl_3$l+2000mg/kg	Mean	1.6667E2	1.6800E2	1.6800E2	1.7300E2
	Std. Error of Mean	4.05394E1	4.01497E1	4.01497E1	3.86307E1
M.oleifera	Mean	1.7033E2	1.7467E2	1.8067E2	1.8867E2
	Std. Error of Mean	9.02466	7.75314	8.00694	9.90511
Total	Mean	1.1917E2	1.1806E2	1.2178E2	1.2672E2
	Std. Error of Mean	1.16746E1	1.21115E1	1.20651E1	1.23245E1

Initial weight of animals at day zero

Groups	**Weight**
Normal	71.24 ± 5.20
$AlCl_3$	90.30 ± 3-33
$AlCl_3$ + 500mg/kg	74.32 ± 2.60
$AlCl_3$ + 1000mg/kg	123.57 ± 7.30
$AlCl_3$ + 2000mg/kg	159.40 ± 4.45
M.oleifera (500mg/kg)	165.48 ± 3.48

Ameh,S.S.” Effect of Ethanol Extract of Moringa Oleifera Leaves in Protecting Anaemia Induced in Rats by Aluminium Chloride." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.6 (2018): 35-52.