AN ASSESSMENT OF THE EFFECT OF *MORINGA OLIFERA* LEAF POWDER AS A NUTRITIONAL SUPPLEMENT IN THE DIET

BY

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DECLARATION

I hereby declare that this work has not been done and presented anywhere for a degree. All works and resources used have been duly credited.

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ABSTRACT

The *Moringa oleifera* (MO) leaf powder can be used as vegetable in the main diet or as a nutritional supplement. The MO plant is a good source of nutrients, antioxidants and phytochemicals, and these properties have been implicated in alleviating various cellular disorders. This work assesses the use of the MO leaf powder in the diet as a nutritional supplement.

Three different concentrations of MO leaf powder supplemented diet (10% MOD, 20% MOD and 40% MOD) were fed to apparently healthy rats for 30, 60 and 90 days. Also low-protein diet and standard diet were supplemented with MO leaf powder respectively and fed to malnourished mice for 21 days. The effects of the MO supplemented diet on body weight, biochemical and haematological status were analyzed. There were no significant changes in the body weights.

There were varied changes in the biochemical and haematological parameters measured for the different MO concentrations at the various time lines. Significant observation were measured, at day 30 in the levels of alkaline phosphatase (ALP) increased, triglyceride decreased, monocyte increased, red blood cell (RBC) increased, haemoglobin (HB) increased, haematocirt (HCT) increased, mean corpuscular volume (MCV) increased and mean corpuscular haemoglobin (MCH) decreased; while at day 60 in the levels of urea and white blood cell (WBC) increased and likewise at day 90 in the levels of fasting blood sugar (FBS) decreased, aspartate aminotransferase (AST) increased, monocyte decreased and platelet decreased. Similarly, the levels of serum ascorbic acid increased and malondialdehyde (MDA) decreased significantly in the 21 day.

The results shows that dietary supplementation with MO leaf powder is relatively safe to the liver and kidneys, may be beneficial in the treatment of anaemia, boost ascorbic acid antioxidant activity and help prevent lipid peroxidation processes that are detrimental to body tissues. However, moderation is needed in the use of the MO leaf powder to prevent hypoglycaemia and spontaneous bleeding.

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DEDICATION

This work is dedicated to my parents, Rev. Paa Kwesi N. Esubonteng and Mrs. Beatrice N. A. Esubonteng for their prayers, counsel and love. May God bless you exceeding abundantly.

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5NT	5’-nucleotidase
αGST	Glutathione-S-transferase
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BASOS	Basophils
Ca	Calcium
CHE	Serum cholinesterase
CREAT	Creatinine
CSRPM	Centre for Scientific Research into Plant Medicine
Cu	Copper
D. BIL	Direct bilirubin
EDTA	Ethylene diamine tetraacetic acid
EOSINOS	Eosinophils
FBS	Fasting blood sugar
Fe	Iron
GAPFA	Greater Accra Poultry Farmers Association
GGT	γ -glutamyltransferase
GHAFCO	Ghana Agro Food Company
GLD	Glutamate dehydrogenase
HB/Hb	Haemoglobin

HCN	Cyanide
HDL-C	High-density lipoprotein cholesterol
I. BIL	Indirect bilirubin
ICR	Imprinting control regions
K	Potassium
KNUST	Kwame Nkrumah University of Science and Technology
LDL-C	Low-density lipoprotein cholesterol
LPD	Low protein diet
LPD-MO	Low protein diet plus *Moringa oleifera*
LYMPHS	Lymphocytes
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
Mg	Magnesium
Mn	Manganese
MO	*Moringa oleifera*
MOD	*Moringa oleifera* diet
MONOS	Monocytes
MPV	Mean platelets volume
Na	Sodium
NEUTROS	Neutrophils
P	Phosphorus
PUFA	Polyunsaturated fatty acids
RBC	Red blood cells

RDW	Red blood cell distribution width
ROS	Reactive oxygen species
SEM	Standard error of the mean
SDR	Sprague-Dawley rats
SD	Standard diet
SD-MO	Standard diet plus *Moringa oleifera*
SOD	Superoxide dismutase
T. BIL	Total bilirubin
T. CHOL	Total cholesterol
TCA	Trichloroacetic acid
TRIG	Triglyceride
WBC	White blood cells
WHO	World Health Orginization
Zn	Zinc

CHAPTER ONE

INTRODUCTION

Nutrition is a science that studies nutrients and other substances in foods and in the body and the way these nutrients relate to health and disease. Nutrients are the nourishing substances in food that provide energy and promote the growth and maintenance of the body. In addition, nutrients aid in regulating body processes such as heart rate and digestion and in supporting the body's optimum health. Nutrients are organized into six main groups; carbohydrates, proteins, lipids, vitamins, minerals and water (Drummond and Brefere, 2007).

Nutritional status is now recognized as a major determinant in health and disease. The course of virtually all chronic diseases is affected by the nutritional status of the ill person, and treatment and outcome of a disease can be markedly improved if the patient complies with recommended diet changes (Bronner, 2006).

It must be emphasized, however, that both nutrient deficiencies and excesses impair the function of the immune system and make an individual vulnerable to infectious disease and other disorders (Bronner, 2006).

1.1 Dietary Supplement

Nutritional supplements are used mainly to fill nutritional insufficiencies or inadequacies in the diet. Nutritional supplements contain ingredients (such as a vitamins, minerals, amino acids, or enzymes) that are either common or uncommon to natural foods. These substances are either extracted from a natural food or they are

made in a laboratory and are provided in many forms such as pills and drinks (Longe, 2008; Wildman, 2009).

1.2 Nutraceutical and Functional foods

Nutraceuticals are substances found in natural foods that seem to have the potential to prevent disease or be used in the treatment of various disorders. Meanwhile, functional foods are the foods in which one or more nutraceuticals can be found. Nutraceutical substances include some of the more recognized nutrients such as vitamins (antioxidants), minerals, fiber and phytochemicals. A nutraceutical may be an essential nutrient; however, the neutraceutical properties of certain essential nutrients may not be why they are essential in the first place. For instance, vitamin C is essential for making important molecules in our body such as collagen, yet its nutraceutical roles may be more related to its antioxidant activities, such as helping to prevent degenerative eye disorders (Wildman, 2009).

1.2.1 Antioxidants

The Oxford Dictionary of Biochemistry and Molecular Biology, defines antioxidant as any substance, often an organic compound, that opposes oxidation or inhibits reactions brought about by dioxygen or peroxides (Smith, 2000). Usually the antioxidant is effective because it can itself be more easily oxidized than the substance protected. The term is often applied to components that can trap free radicals, such as α-tocopherol, thereby breaking the chain reaction that normally leads to extensive biological damage. Some few other examples include vitamin A, vitamin C, threonine, selenium, glutathione and zinc.

1.2.2 Phytochemicals

They are, in the strictest sense, chemicals produced by plants. Commonly, though, the word refers to only those chemicals 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 for example niazimicin and pterygospermin (Fahey, 2005).

Fruits and vegetables are good sources of nutraceuticals when present in the diet. The Moringa oleifera (MO) leaf is used as vegetable in the main diet or as a nutritional supplement. When used as a nutritional supplement the dried leaf powder is preferable. The MO plant is also a good source of antioxidants and phytochemicals and these properties have been implicated in alleviating various cellular disorders.

1.3 The *Moringa oleifera* plant

The Moringa oleifera plant is a member of the family Moringaceae and it is known by various names by different cultures and peoples. Some call it the Horseradish tree, Benzolive tree, Kelor, Marango, Mionge, Moonga, Mulangay, Nebeday, Saijhan, Sajna or Ben oil tree. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands (Fahey, 2005).

1.3.1 Uses of *Moringa oleifera* plant

The MO plant is a multipurpose plant and has been used and consumed by humans. The many uses for MO include alley cropping, animal forage, biogas, fencing, fertilizer, gum, medicine (all plant parts), biopesticide and water purification.

Fahey (2005) in his review of the medical evidence for its nutritional, therapeutic, and prophylactic properties states many benefits of the plant. The MO trees have been used to combat malnutrition, especially among infants and nursing mothers. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Doerr and Cameron, 2005). It 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.

The following medicinal uses have been observed from the use of various parts of the plant. The leaves have anthelmintic, aphrodisiac properties and cures hallucinations, hiccough and asthma (Mehta and Agrawal, 2008; Maroyi, 2010). The fruit are used to treat biliousness, pain, leucoderma and tumor. The seed cures eye diseases and head complaints. The oil is useful in leprous ulcers and as external application for rheumatism. The roots and seeds are prescribed for the treatment of snakebites and scorpion stings. The roots have laxative, expectorant, diuretic properties and good for treating inflammations, sore throat, bronchitis, piles, stomatitis, urinary discharges and obstinate asthma. The root bark is useful in treating heart complaints, eye diseases, inflammation, dyspepsia, and enlargement of spleen. The root and bark have abortifacient effect (Rupjyoti *et al.*, 2003; Fahey, 2005; Kamal and Jawaid, 2008; Jameel *et al.*, 2010).

1.3.2 Nutritional value of *Moringa oleifera* leaves

A large number of reports on the nutritional qualities of MO now exist in both the scientific and the popular literature. Gram per gram, MO leaves contain more Vitamin

A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, more potassium than bananas and the protein quality rivals that of milk (Fahey, 2005).

In determining the nutritional potential of the leaves using standard analytical methods, Oduro *et al.,* (2008) reported that crude protein was 27.51%, crude fibre was 19.25%, crude fat was 2.23%, ash content was 7.13%, moisture content was 76.53%, carbohydrate content was 43.88%, and the calorific value was 1296.00 kJ/g (305.62 cal/g). Elemental analysis of the leaves indicate calcium and iron content in mg/100 g dry matter (DM) were 2009.00 and 28.29, respectively.

Table 1.1 Nutrient comparison in 100g

Nutrient	**Common Foods (mg)**	**Fresh Leaves (mg)**	**Dried Leaves (mg)**
Vitamin A	Carrots 1.8	6.8	18.9
Calcium	Milk 120	440	2003
Potassium	Bananas 88	259	1324
Protein	Yoghurt 3.1	6.7	27.1
Vitamin C	Orange 30	220	17.3

Source: *Moringa oleifera* (www.info@dolcas-biotech.com, 2008).

This work assesses the use of the MO leaf powder in the diet as a nutritional supplement. The nutritional status of malnourished model, biochemical and

haematological status of laboratory rodents were determined to establish the safety and the health benefits in the long-term use of the powdered matter.

1.4 Justification

Recently, more scientific research has been conducted to determine its efficacy and effects in humans and animals alike. Most of these results have proved that there are more benefits to be derived from this plant. However, most of the work done with MO has been with the use of the extract of various parts of the plant (Ghasi *et al.*, 2000; Adedapo *et al.*, 2009; Jaiswal *et al*., 2009; Nandave *et al.*, 2009), but not the leaf matter. The LD_{50} of leaf extract showed that it is safe at levels as high as 2000 mg/Kg dose (Adedapo *et al.*, 2009).

As previously stated, the MO is an easy to cultivate plant that does not require any technical know-how and grows well in most parts of the world especially in the hot climates. It is also abundant in many households as fences and not as a food or nutritional material. Meanwhile, individuals with nutritional deficiencies can use the MO leaves as an inexpensive source of nutritional supplement and at the same time benefit from it reported medicinal properties.

Interaction between certain substances in food material can deplete or prevent the bioavailability of other nutrients in the diet. This is observed in cereals, where the phytate binds to some minerals thereby making them unavailable for use in the body. Some prescribed and non-prescribed food supplements have nutrient depleting effects and the use of MO has been suggested to alleviate and compensate for these losses.

Consequently, the health of individuals taking such medications is overall improved (Johnson, 2005).

1.5 Aim and objectives

The aim of the study was to assess the long-term effects of the un-extracted MO leaf powder on rats and mice development as a dietary supplement. In health, the body organs function by utilizing and/or eliminating foreign substances to maintain a normal internal environment. In the disease state there is a dysfunction of some of these organs. The liver which is the major metabolic organ of the body is involved in metabolism of nutrients while the kidneys eliminate the waste products of metabolism through the urine. The specific objectives were to study:

- The toxic effects on vital metabolic organs of the body including the liver and kidneys in apparently healthy rats.
- The toxic effects on blood constituents in apparently healthy rats.
- The blood levels of the antioxidant Vitamin C and lipid peroxidation product malondialdehyde in mice.
- The effects on the weight changes of the animals.

Hepatic or liver assessment is normally done to measure its major (synthetic and excretory) functions. The tests conducted were namely glycaemic index (fasting blood glucose), liver function tests (bilirubin, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase) and lipid profile (total cholesterol, low density lipoprotein, high density lipoprotein, triglyceride).

Renal assessment measures the kidneys' ability to filter and excrete waste metabolites as it should. Serum urea and creatinine concentrations are used to monitor renal insufficiency. The test performed measured serum creatinine and urea concentrations.

Heamatological assessment measures the level of blood components and a full blood count test performed was to measure red blood cell count and red blood cell indices, white blood cell count and white blood cell differentials, platelets and mean platelets volume.

The measurement of antioxidant activity in the blood would be determined by measuring the vitamin C and malondialdehyde (MDA) concentrations.

Conclusion

As the incidence of poor diet and inactivity increase due to adoption of western lifestyle, more people are at risk irrespective of their age of developing cardiovascular disease, obesity and diabetes. Morbidity caused by these conditions will lead to low economic development and inability to meet health costs; finally death rates will increase. The dietary supplementation of the abundant, easily cultivated MO leaf might help minimize the occurrence of nutritional diseases and deaths leading to healthy population and improve economical developments. It is hoped that the use of MO in the diet will provide valuable information including its possible toxicity to body organs to support the development of MO-based diet and the campaign for the use of MO leaf powder and it products as nutritional supplements. This in itself is more natural compared with synthetic nutritional supplements and hopefully will be inexpensive to use. It will also be a platform to help develop exclusively medicinal

product to manage various nutritionally related disorders. Finally the body of knowledge in the use of MO leaf based diet will also be scientifically enhanced.

CHAPTER TWO

LITERATURE REVIEW

Good health has been the concern of people of all levels including governments, health service providers and the individual. All possible ways to achieve optimum health are sought for and tried to ascertain advantages and disadvantages through pilot programmes and then implemented or practised if the long term benefits far outweigh the converse. To attain this most practitioners in health or nutritionists will usually employ dietary regimes and physical activity. As dietary intake is so important and with the return to most underutilized tropical plants, MO as a long-term food supplement product in various preparations is appropriate for study. Knowing its previous and current usage based on its nutritional and medicinal qualities reported, it gives a good perspective as to how well it could be used and the duration of usage. It is used as a vegetable in diet because of the similar health benefits normally afforded by fruits and vegetables. The MO leaves in the dried form added to the food of malnourished children, weaned children and pregnant and lactating women because of the high nutritional contents proved highly effective (Fahey, 2005; Price, 2007).

2.1 Fruits and vegetables

It is advocated that fruit and vegetables be consumed because of their numerous benefits. Research findings are strongly suggestive of a potential for fruit and vegetables to reduce the risk of type 2 diabetes mellitus. Several short-term, carefully conducted clinical trials show that the consumption of fruits and vegetables can help achieve or maintain a healthy body weight. The findings show that diets rich in fruit

and vegetables significantly reduce the risks of ischaemic heart disease and stroke (FAO/WHO, 2004).

Fruits and vegetables are recommended in the diet because they contain fibre that has physiological properties in health. There are two types of fibre in fruits and vegetables mainly soluble and insoluble fibre. Soluble fibre in the gastrointestinal tract absorbs water and swells; this slows the movement of chyme. The soluble fibre also binds cholesterol and inhibits its intestinal absorption until it is eliminated, thus reducing plasma cholesterol level. When the diet is high in soluble fibre it reduces rate of absorption of glucose. Insoluble fibre cannot absorb water but increases the motility in the intestine and helps with regular bowel movement. Fibre-rich diets help to reduce the incidence of diabetes mellitus, atherosclerosis, cancer and obesity (James, 2004; Sullivan, 2009).

Some examples of fruits and vegetables rich in fibre include apple (with skin), banana, orange cabbage, lettuce, spinach.

2.2 Dietary supplementation

Dietary supplements serve a wide range of purposes. Some of these are medically appropriate, while others may do nothing or harm the individual. Reasons for taking dietary supplements include: replacing a necessary substance not found in large enough quantities in the diet, preventing or decreasing the risk of developing a disease or condition, boosting the immune system and improving general health, boosting energy levels, improving mental or physical performance, stimulating weight loss and reducing symptoms of a disease or health condition (James, 2004; Longe, 2008).

There has been an increase in the use of dietary supplements and this has necessitated that individuals consult with their physicians so as to get the appropriate supplement without any detrimental effects (Balluz *et al.*, 2000).

2.3 Cultivation of *Moringa oleifera* plant

Moringa oleifera is believed to be native to sub-Himalayan tracts of northern India but is now found worldwide in the tropics and sub-tropics. It grows best in direct sunlight under 500 meters altitude. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 5 - 9), well-drained sandy or loamy soil. Minimum annual rainfall requirements are estimated at 250mm with maximum at over 3,000mm, but in waterlogged soil the roots have a tendency to rot. (In areas with heavy rainfall, trees can be planted on small hills to encourage water run-off). The presence of a long taproot makes it resistant to periods of drought. Trees can be easily grown from seed or from stem cuttings. Temperature ranges are 25-35 degrees Celsius (0-95 degrees Fahrenheit), but the tree will tolerate up to 48 degrees in the shade and it can survive a light frost. The seeds have no dormancy period, so they can be planted as soon as they are mature and they will retain viability for up to one year. It flowers and fruit annually and in some regions twice annually. During its first year, the tree grows up to five meters in height and produces flowers and fruits. If untrimmed, the tree can eventually reach 12 meters in height with a trunk 30cm wide; however, the tree can be annually cut back to one meter from the ground. The tree quickly recovers and produces leaves and pods within easy reach. Within three years a tree will yield 400-600 pods annually and a mature tree can produce up to 1,600 pods (Annenberg, 2005; Price, 2007).

2.4 Nutrient composition of the MO leaves

It is believed that the chemical compositions of the MO leaf will have or result in observed scientific or clinical effects such as nutritional and medicinal activities (Shanker *et al*., 2007; Nikkon *et al.*, 2009; Sashidhara *et al.*, 2009). As such researchers demonstrate the action of the component(s) or group of components in both animal and humans experiments. The amount of these components varies depending on the agro-climatic condition of a place. Though the MO plant can grow in most soil and without any special technical skill, however, for the best nutritional and compound contents the agro-climatic requirement must be considered as the table below shows this effect.

Table 2.1 Vitamin C content of MO leaves from three locations

Location	**Vitamin C content (g/kg dry matter)**
Nicaragua	9.18
India	8.36
Niger	6.78

Source: The potential of *Moringa oleifera* for agricultural and industrial uses (Foidl *et al.*, 2001) .

It is reported to be a good source of carbohydrate, lipids, proteins, minerals and vitamins. All these nutrients are said to be of the highest value (Fahey, 2005; Price, 2007).

2.4.1 Carbohydrates

Carbohydrates are aldehyde or ketone compounds with multiple hydroxyl groups. They serve as energy stores, fuels, and metabolic intermediates. They form part of the structural framework of DNA and RNA, and also major structural elements in the cell walls of bacteria and plants. All carbohydrates contain carbon, hydrogen, and oxygen. They are categorized by size: monosaccharides; disaccharides; and polysaccharides or complex sugars. Carbohydrates are consumed as part of the diet and then digested in the gastrointestinal tract to produce glucose. Galactose and fructose are also released and then absorbed in the small intestine; they are used to eventually produce energy. The body uses glucose to generate energy in the form of ATP. The brain cells and RBC almost entirely use glucose as fuel (Marieb and Katja, 2006) while other body tissues may use lipids as substitute for energy production. When the body has high amounts of glucose in circulation the excess is stored as glycogen in the liver and skeletal muscles. Fibre (soluble and insoluble) is a complex carbohydrate; soluble fibre helps decrease low-density lipoprotein (LDL) cholesterol while the main function of insoluble fibre is to bind bile acids, which reduces fat and cholesterol absorption (Longe, 2008). Increased fibre in the diet decreases the risk of developing obesity, diabetes mellitus, atherosclerosis, haemorroids and colorectal cancer (Sullivan, 2009). Whole grains, legumes, wheat bran, fruits and vegetables are good sources of carbohydrate and fibre.

2.4.2 Lipids

The lipids are a large and heterogeneous group of substances of biological origin that are easily dissolved in organic solvents such as methanol, acetone and chloroform (Koolman and Roehm, 2005). By contrast, they are either insoluble or only poorly

soluble in water. At room temperature, the solid form is called fat while the liquid form is called oil. Over 90% of dietary fats are triglycerides (Longe, 2008; Sullivan, 2009). Other dietary fats include cholesterol. Lipids provide the most concentrated source of energy in the body; about twice of the amount provided by carbohydrate. Lipids are stored in the adipose tissues and this provides insulation from heat loss and protection for vital structures in the body. They play many roles in the body including forming the structure of cell membranes, involved in the transport, breakdown and excretion of cholesterol, transport of fat soluble vitamins and the building blocks for many important compounds such as hormones, blood clotting agents, and compounds involved in immune and inflammatory responses. The MO leaf unlike the seed is not high in lipids. The leaf powder contains 2.3g per 100g of fats (www.info@dolcas-biotech.com, 2008) and for diet that needs to be low in lipids this will be a good substitute.

2.4.3 Proteins

A protein is a linear polymer of amino acids linked by peptide bonds in a specific sequence. There are twenty different kinds of amino acids used in forming proteins and out of these eight are called essential amino acids, because the human body cannot synthesize them. These eight amino acids are tryptophan, methionine, valine, threonine, lysine, leucine, histidine, and isoleucine. Because humans cannot make them, they must be supplied in the diet. If they are not supplied, proteins cannot be made, which results in a protein deficiency. The quality of protein depends on the level at which it provides the nutritional amounts of essential amino acids needed for overall body health, maintenance, and growth (James, 2004).

Animal proteins, such as eggs, cheese, milk, meat, and fish, are considered high quality, or complete, proteins because they provide sufficient amounts of the essential amino acids. Plant proteins, such as grain, corn, nuts, vegetables and fruits, are lower-quality or incomplete, proteins because many plant proteins lack one or more of the essential amino acids, or because they lack a proper balance of amino acids.

Much of the structure and function of our body is based on proteins. Thus, protein and individual amino acids must function in our body in a number of ways. For instance, proteins can function as enzymes, structural proteins, contractile proteins, antibodies, transport proteins, protein hormones, clotting factors and receptors on cells (Wildman, 2009). The MO leaf powder has a high concentration of complete proteins and many nutritional benefits have accrued from its use in malnourished people in most parts of the world; especially in Africa. It contains all the essential amino acids (Makkar and Becker, 1996; Foidl *et al.*, 2001) and thus can be used as an alternative for other sources of protein in the diet. It has been used to combat kwashiorkor and other protein malnutrition deficiencies (Fahey, 2005) with rapid recovery.

2.4.4 Minerals

Minerals are inorganic elements that originate in the earth and cannot be made in the body. They play important roles in various body functions and are necessary to sustain life and maintain optimal health, and thus are essential nutrients (Longe, 2008). Some minerals such as sodium, potassium, and chloride function as electrolytes, while other minerals, such as copper, zinc, iron, chromium, selenium, and manganese can be incorporated into enzyme molecules. Some minerals such as calcium, phosphorus, and fluoride play a vital structural role in strengthening bones

and teeth. Minerals are grouped into two main classes based on their contribution to body weight. The classes are the major minerals and the minor or trace minerals.

The terms major and minor/trace, however, do not reflect the importance of a mineral in maintaining health, as a deficiency of either can be harmful (Rosenthal and Glew, 2009). Some examples of the major minerals are sodium, calcium, phosphorus, potassium and the minor minerals are iron, zinc, copper, iodine.

The MO plant is richer in some important minerals as compared to some fruits and vegetable. Comparatively, weight per weight, the dried MO leaf powder has its potassium higher than banana, more calcium than milk, more iron than spinach (Fahey, 2005). Aslam *et al.*, (2005) in the comparative mineral composition analysis for MO leaf and pod from different regions in Panjab, Pakistan found that the leaves contained high amounts of Ca (1292-1837), Mg (98.2-109), K (19732-24397), Mn (76.9-112.8), P (1180-1450), Zn (20.9-34.1), Na (1635-2721), Cu (7.3-11.2) and Fe (205-573) mg/kg. In Ghana the Ca and Fe content of the MO leaf was found to be 2,009.00 and 28.29 mg/100g respectively (Oduro *et al.*, 2008). This makes the leaves a good source as a mineral supplement. Children and pregnant and non-pregnant women stand the risk of developing mineral deficiencies; especially those in the developing countries.

2.4.5 Vitamins

Vitamins are defined as non-caloric organic substances and are required in very small amounts, typically microgram to milligram quantities. Vitamins either can't be made in the body or are not made in sufficient quantities to meet our needs. Thirteen

different vitamins have been identified as necessary for humans. The body can make small quantities of two of these vitamins, vitamins D and K. All other vitamins must be obtained either from food or from dietary supplements. Each of the 13 vitamins has specific functions, and taken together vitamins play a role in almost every function in the body. They help convert food to energy, and are involved with processes as diverse as blood clotting, vision, reproduction, and transmission of nerve impulses. The MO leaf is rich in vitamins including Vitamins A, C, D, E, K and B-complex (B1, B2, B3, B6 and B7) (www.info@dolcas-biotech.com, 2008; www.moringasupplements.com, 2009; www.moringasource.com/nutrition.php, 2010). The vitamin C content of the dried leaves is far less compared to the fresh ones; this is due to the oxidation that occurs on drying.

2.5 Phytonutrients and phytochemicals

Phytonutrients unlike the macro and micro nutrients are needed for growth and metabolic activities are considered non-essential. The absence of phytochemicals in the diet has no detrimental effects on the nutritional status. However, research has shown that they play important roles related to better health when they are in the diet (James, 2004; Wildman, 2009). They may impart flavour to the food and are all plant based; they may be derived from plant foods such as fruit and vegetables. In nutrition phytochemicals are put into three main classes namely phytochemicals, medicinal plants, herbs and spices (Longe, 2008).

Some of the phytochemicals found in the MO leaves with health benefit include the carotenoids, flavonoids, terpenes and phytosterols.

2.5.1 Carotenoids

Carotenoids are fat-soluble plant pigments found in certain fruits and vegetables such as carrots and are responsible for the red, orange and yellow colours, some of which are important to human health. The most common carotenoids in the diet are alpha-carotene, beta-carotene, beta cryptoxanthin, lutein, zeaxanthin, and lycopene. It was shown in comparative studies of the nutritional quality of four species of the genus that MO has the highest beta-carotene content (Yang *et al.,* 2006). Though the role carotenoids play in the human body are not well understood, they also act as an antioxidants scavenging free radicals (Longe, 2008; Wildman, 2009). In this way the damage that would be caused by free radicals to cell membranes through oxidation is prevented. The most abundant carotenoid is the beta-carotene of carrots and sweet potato, together with alpha-carotene and beta-cryptoxanthin are converted to retinol (vitamin A) in the body. Vitamin A effects are important for maintaining good vision, a healthy immune system, and strong bones (James, 2004). Vitamin A also helps turn on and off certain genes (gene expression) during cell division and differentiation. Lutein, zeaxanthin and lycopenes are not converted to vitamin A.

Table 2.2 Carotenoids in different parts of MO

Carotenoid	Part (mg/kg DM)		
	Leaves	**Stem**	**Seed**
Alpha-carotene	6.5	N.D.	N.D.
Beta-carotene	401	N.D.	3.8
Echinenon	N.D.	N.D.	N.D.
Fucoxanthin	N.D.	N.D.	N.D.
Leutin	702	21.8	4.0
Myxuxanthophyll	N.D.	N.D.	N.D.
Neoxanthin	219	5.9	N.D.
Violaxanthin	76.5	1.3	N.D.
Zeaxanthin	19.4	N.D.	N.D.
Xanthophylls	83.1	1.6	N.D.
Carotenoids	1508	34.4	4.0
Chlorophyll	6890	271.1	N.D.

Source: The Potential of *Moringa oleifera* for Agricultural and industrial uses. What development potential for Moringa products? (Foidl *et al*., 2001)
Note: N.D. denotes not detected

2.5.2 Flavonoids

Flavonoids are phenolic derivatives and they include flavones, flavonols, isoflavones, catechins, quercetins and anthocyanidins. Phytochemical screening of MO leaves indicated the presence of flavonoids in its ethanolic extract (Rastogi *et al.*, 2009) and a quantitative analysis with hydroalcoholic extract estimated at 27µg/mL of flavonoid content (Rajanandh and Kavitha, 2010). Most often than not, many flavonoids have diverse biochemical roles and act as antioxidants. Nutritionally, they help in preventing cardiovascular and cancerous conditions. Some of these flavonoids may act to decrease the level of total and LDL-cholesterol in the blood, while others may

decrease free-radical activities, thereby protecting LDL from oxidation as well as helping to protect the walls of the arteries (Longe, 2008). This is why substances abundant in flavonoids (green tea, onions, chocolate and red wine) are recommended to be beneficial in health.

2.5.3 Terpenes

Terpenes are cyclic compounds with 10, 15, 20 or correspondingly more carbon atoms. They also act as antioxidants and are important in cancer prevention though they may inhibit some enzyme activities (Heldt and Heldt, 2005). Terpenes are constituents of the essential oil fractions which occur in the leaves of MO and have been implicated in the antifungal activity against dermatophytes (Chuang *et al.*, 2007) and also are found to be efficacious antibacterial and antifungal agents (Kekuda *et al.*, 2010). These antioxidant and antimicrobial properties are benefits that may be gained from the use of the leaf powder.

2.5.4 Phytosterols

Phytosterols are known to lower LDL thus reducing the risk of developing heart diseases. Phytosterols appear to block the absorption of cholesterol in the digestive tract, which in turn lowers the level of total and LDL cholesterol in the blood (Longe, 2008). The MO leaf contains the phytosterol called beta-sitosterol a plant sterol with close chemical resemblance to cholesterol which enables it to block the absorption of cholesterol by competitive inhibition (Pasha *et al.*, 2010; Rajanandh and Kavitha, 2010). The beta-sitosterol of the MO leaf was concluded to be responsible for its hypolipidaemic and as well as antioxidant properties (Rajanandh and Kavitha, 2010).

2.6 Free radicals/reactive oxygen species (ROS)

Free radicals are chemical particles with an odd number of electrons. The free radical form of the molecule O_2 is $^{\cdot}O_2^-$. (superoxide anion) and this anion reacts with other molecules that initiate a chain reaction of more free radical production (Wildman, 2009). Free radicals in higher levels may react with bio-molecules, including nucleic acids, proteins, and lipids; causing cancer, myocardial infarction, heart tissue death and brain damage. This damage resulting from free radicals is termed “oxidative injury” (Longe, 2008). The production of free radical is part of the normal metabolic activity of the body system but because of its deleterious effect it should be neutralized. The body is able to get rid of free radicals by varying mechanisms such as the use of the enzyme superoxide dismutase (SOD) which breaks down superoxide anion to oxygen and hydrogen peroxide, it also employs antioxidants as neutralizers (Rao, 2006).

Antioxidants are good neutralizers or scavengers of free radicals therefore any source of antioxidant more especially dietary (fruits and vegetables) are highly advised (Longe, 2008; Wildman, 2009). Examples of dietary antioxidants are vitamin C, vitamin E, selenium, carotenoids and flavonoids.

2.7 Antioxidants present in MO leaves

The importance of various classes of phytonutrients/phytochemicals has been discussed above and the roles they play are well appreciated in relation to nutrition. However, the identified phytochemicals of MO leaves and their reported activity are presented below here.

The Ugandan people used the MO leaves mostly as a medicinal remedy rather than nutritive supplement. With the reported health benefits of the MO plant the leaf extracts were studied for phytochemicals and their preventive and curative properties. It showed the presence of gallic tannins, catechol tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars that have health benefits (Kasolo *et al.*, 2010). The following compounds are reported to have various medicinal properties, 4-(4'-O-acetyl-α-L rhamnopyranosyloxy)benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Fahey, 2005). Atawodi *et al.;* (2010), also reported the presence of the polyphenols and antioxidants such as quercetin glucoside, quercetin rhamnoglucoside (rutin), kaempferol rhamnoglucoside and chlorogenic acid in the MO leave). Extract from various parts (leaves, pods and seeds) of the plant strongly exhibited antioxidant and radical scavenging activities in vitro using different evaluation models (Atawodi *et al.*, 2010). The leaves both in vivo and in vitro, due to their high phenolic content, exhibit their greatest radical scavenging property. Oxidative stress is thus prevented by the use of the MO and this may justify its use for health purposes (Nandave *et al.*, 2009; Singh *et al.*, 2009).

2.8 Secondary metabolites and antinutritional factors

Secondary metabolites are chemical substances found in plants with no apparent direct metabolic or biological activity (Heldt and Heldt, 2005). They may play certain important roles for the plant such as pathogen inhibitor, natural pesticide, attract animals for pollination and seed dispersal. In animals and humans, however, these substances may be toxic to the body. They include substances such as lectins,

proteinase inhibitor, amylase inhibitor and cyanogenic glycoside or glucosinolates. They may be deactivated or decomposed when cooked as in the common example of cassava leaves and tuber but most secondary metabolites are not destroyed by this action.

High concentrations of the secondary metabolites are carcinogenic. It has been estimated that in industrialized countries more than 99% of all carcinogenic substances that humans normally consume with their diet are plant secondary metabolites that are natural constituents of the food. However, it has been shown that the human metabolism usually provides sufficient protection against these harmful natural substances (Heldt and Heldt, 2005; Kasolo *et al.*, 2010).

Cyanogenic glycoside, saponins, tannins, phytates and alkaloids are some of the secondary metabolites that occur in the MO plant; their advantage and disadvantages are discussed.

2.8.1 Cyanogenic glycosides

Cyanogenic glycosides are rapidly degraded in the plant cell to corresponding aldehyde or ketone, cyanide and sugar and it is the cyanide (HCN) portion that is toxic to the human system. This is an anion that blocks the electron transport chain by reacting with the ferric form of cytochrome aa3 and thus acts as an inhibitor of respiration. The symptoms of toxicity include headache, salivation nausea, anxiety, confusion, vertigo, convulsions, paralysis, unconsciousness coma, cardiac arrhythmias, hypotension, and respiratory failure (Timbrell, 2009). A report in Nigeria of people poisoned from consuming cassava product stated that they presented with

vomiting, abdominal pain, became comatose with acute renal failure and death due to cardio-pulmonary arrest (Vetter, 2000). However, the MO leaves are absolutely free from cyanogenic glycoside (Makkar and Becker, 1997) thus assuring the absence of HCN poisoning on consuming the leaf product as food. Negligible amounts were detected in the twig and stem of the plant.

2.8.2 Saponins

Saponins are a class of glucosylated steroids named after their soap-like property. They act as detergents because of the hydrophilic polysaccharide and hydrophobic steroid side chains thereby dissolving the cell membrane of fungi and causing haemolysis of red blood cells in animals. They are used in the pharmaceutical industry as component in synthesizing contraceptives and also as medicine against heart disease in low doses. Saponin content of 80 g/Kg was found in the leaves of MO and this did not produce any haemolytic effects (Makkar and Becker, 1997).

2.8.3 Tannins

Tannins, a collective term refer to a variety of plant polyphenols used in the tanning industry. The phenolic group of tannins binds strongly to proteins through hydrogen bonding and this bond cannot be cleaved by digestive enzymes. This prevents the bioavailability of nutritional protein in such diets to the body. They also have a very sharp and unpleasant taste thereby reducing the acceptability of the diet. Tannins occur in innocuous amount of 1.4% in the MO leaf with no condensed tannins. Some methods of detection did not show its presence in the leaf if any at all (Foidl *et al.*, 2001).

2.8.4 Phytates

Phytate is the major phosphorus compound in plants and is important for phosphate storage in cereal grains, especially as its calcium-magnesium salt, phytin. It occurs in many plant seeds, grains, fruits and vegetables. The minerals in these products are therefore not bio-available when consumed thus can lead to mineral deficiencies of calcium, magnesium, manganese, copper, iron and zinc (Dashti *et al.*, 2000; Eklund-Jonsson *et al.*, 2006; Kumar *et al.*, 2009). A phytate content of 3.1% was reported to be present in the MO leaves (Makkar and Becker, 1997; Foidl *et al.*, 2001). The leaves are rich in minerals and the bioavailability may be reduced due to phytate interaction.

2.8.5 Alkaloids

Alkaloids are synthesized from amino acids and contain heterocyclic rings with one or several N atoms. They can be used as stimulants, narcotics, poison and medicine in the extracted form. They are classified based on the ring structure present with cocaine (tropane), morphine, caffeine, atropine and nicotine. Because of the effects they have in the body, the amount in a plant product is of biological interest. Some of these alkaloids are used in the synthesis of pain killers while other people use it because of the euphoric effect they give. Without tolerance, the alkaloids on initial ingestion or use result in nausea, dizziness, and muscular weakness (Heldt and Heldt, 2005).

The bark of MO is also a repository of tannins used in the tanning industry. The root-bark was commonly used as a condiment or garnish but this practice is not recommended due to certain toxic components. The alkaloids moringine and

moringinine have been identified in the root-bark of the plant. It also contains a bacteriocide called spirochine (Maroyi, 2006). Moringinine acts as cardiac stimulant, produces rise of blood-pressure, acts on sympathetic nerve-endings as well as smooth muscles all over the body, and depresses the sympathetic motor fibres of vessels in large doses only (Palaniswamy, 2004). Traces of alkaloids are reported to be present in the flower (Anwar *et al.*, 2006).

Though some secondary metabolites are toxic especially for human consumption, there are some that have beneficial roles in the body; the carotenoids, flavonoids and phytosterols. Parts of the MO plant have been used widely on many experimental grounds with appreciable results. Some of these experiments sought to exploit the fundamental function of the secondary metabolites produced by the plant. For example, the seed powder of the plant has been used as coagulating agent in water purification where it binds particles and bacteria. This water is not harmful for drinking (Price, 2007). It has been used to spray crops *(Foidl et al.*, 2001) and as an anti-bacterial agent against human pathogens (Rahman *et al.*, 2009; Kekuda *et al.*, 2010).

The MO leaves have some amounts of tannins, saponins, phytate but no proteinase inhibitor, amylase inhibitor, lectins and cyanogenic glucoside (Vetter, 2000; Foidl et al., 2001). Nonetheless the pod, seed, flower, stem and roots contain various amounts of biologically important compounds (Vetter, 2000; Fahey, 2005; Kumar et al., 2007).

2.9 The liver

The liver is the largest organ in the body and has numerous functions. It is an accessory organ of the digestive system that works with the small intestine. It regulates carbohydrate, protein, and lipid metabolism and detoxifies body wastes and drugs that have entered the body. In addition, the liver eliminates bilirubin, a waste product of dead red blood cells, by incorporating it into bile.

2.10 Liver function tests

Liver function tests based on its specific functions are done routinely in the laboratory. In addition measurement of serum enzymes specific to liver is helpful in assessing liver damage.

2.10.1 Liver enzyme activity

In the absence of liver disease, enzymes are present in the blood as a result of normal wear and tear of the cell. Several enzymes are released from diseased hepatocytes. They may be cytosolic, mitochondrial or membrane associated enzymes. Type of enzyme released depends on severity and the type of liver condition. The amount of enzyme in plasma is thus altered in liver diseases. Such enzyme tests are useful in evaluation of liver function as well as diseases affecting the liver. In liver assessment, the main liver enzymes whose activities are measured include alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), 5'-nucleotidase (5'-NT), serum cholinesterase (CHE), and glutamate dehydrogenase (GLD). The aminotransferases (ALT, AST) and ALP are the most widely used in investigations. These clinically important enzymes are routinely measured in clinical chemistry laboratories (Rao, 2006; Burtis *et al.*,

2008). A new enzyme to this set is the enzyme α-glutathione-S-transferase (α GST), believed to be an appropriate enzyme marker for liver damage. The reliability of this enzyme in assessing liver function has been proved both in the clinical and laboratories setup (Sidlova *et al.*, 2003).

2.10.2 Liver synthetic activity

The liver synthesizes many plasma proteins, blood clotting factors, lipoproteins and urea. Synthesis of these compounds may be affected in pathological conditions. Hence their concentration in plasma may decrease. However, due to their long half life and regenerating capacity of the liver the decrease may be apparent only on long standing liver diseases (Rao, 2006).

2.10.3 Liver secretory or excretory activity

This involves secretion of bilirubin and bile acids. Bilirubin is produced in the liver, spleen, and bone marrow and is also a by-product of hemoglobin breakdown. The measurement of bilirubin in serum and urine and serum bile acids is helpful in assessing liver damage. Measurement of urine urobilinogen is also a useful test of liver function. In differentiating pre-hepatic and post-hepatic jaundice, increases and decreases in urobilinogen measurements is employed (Marshal and Bangert, 2004; Rao, 2006).

2.10.4 Liver xenobiotic or clearance activity

The liver clears several xenobiotics rapidly from blood stream. Some of the xenobiotics eliminated by the liver include food additives, certain drugs, pesticides, poisons and toxins. Very little, if any, is cleared by other organs. Therefore

elimination of these xenobiotics from the blood stream depends on functions of liver. The liver takes up these molecules by active transport mechanism involving a carrier molecule and excretes it later in bile (Rao, 2006; Rao, 2008).

CHAPTER THREE

METHODOLOGY

3.1 Materials

3.1.1 Animals

Male and Female Sprague-Dawley rats (SDR) averagely weighing 220g were purchased from the Centre for Scientific Research into Plant Medicine (CSRPM Mampong-Akuapem) and housed in plastic cages at 12 hours day and night cycle. The animals were allowed to get acclimatized to the animal house conditions for two weeks during which they were fed the control diet.

Imprinting control regions (ICR) developed by the Institute for Cancer Research (Fox Chase Cancer Center, Philadelphia, USA) mice bred by the Department of Pharmacology, KNUST were also used. Twenty pups between 3 to 4 weeks old averagely weighing 6g were weaned and housed in plastic cages at 12 hours day and night cycle.

3.1.2 Chemicals and diagnostic kits

ACCUCARE™ kits (Lab-Care Diagnostics, Mumbai, India) supplied by Nelisam Ghana Limited, 100 meters from the Abosey Okai Central Mosque in Accra, were used for conducting fasting blood sugar, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, creatinine, urea, cholesterol, high density lipoprotein, low density lipoprotein and triglyceride tests. Trichloroacetic acid (TCA), thiobarbituric acid (TBA) dinitrophynyl hydrazine (DNPH) for ascorbate and

malondialdehyde assays were from Sigma-Aldrich (Germany) and were provided by Department of Pharmacology, KNUST, Kumasi.

3.1.3 Test tubes

Nelisam Ghana Limited, 100 meters from the Abosey Okai Central Mosque in Accra supplied sodium fluoride tubes, gel clot activator tubes, K_3 EDTA tubes and micro-tubes.

3.1.4 Instruments

Universal 320 centrifuge (Hettich Zetrifugen) supplied by DJB Labcare; DJB Labcare Ltd, 20 Howard Way, Interchange Business Park, Newport Pagnell, Buckinghamshire, England, AMS Ellipse Chemistry Analyzer supplied by Analyser Medical Systems; SpA Via E. Barsanti, 17/A, 00012 Guidonia, Rome (Italy), CELL-DYN 3700 Haematology analyzer supplied by Abbott Diagnostics; Abbott GmbH & Co. KG, Max-Planck-Ring 2, 65205 Wiesbaden, Germany, Optima SP-300 spectrophotometer bysupplied by Optima Inc.; Rogie Bldg. 5F, 1-48-11 Itabashi-ku, Tokyo 173-0004 Japan.

3.1.5 *Moringa oleifera* leaves

Freshly harvested *Moringa oleifera* leaves collected from the Mamprobi locality in Accra were washed in tap water and air dried under shade. The leaves were turned over to prevent mould growth and heat build-up that may change leaf colour. The completely dried and crispy leaves were ground in a domestic corn-mill and packaged into black polyethylene bags for storage until they were ready to be used in the *Moringa oleifera* diet (MOD) production.

3.2 Experimental design

Two sets of experiments were carried out. Experiment 1 involved the use of rats for toxicity assessments. Experiment 2 involved the use of mice for anti-oxidative properties assessments.

3.2.1 Experiment 1

3.2.1.1Grouping and feeding

The rats were selected and put into 4 groups containing 15 rats; 5 rats per cage. The four were labeled as the control, 10% MOD, 20% MOD and 40% MOD groups respectively which were fed over a 90 day period. All rats except the control group received the graded MOD; the animals were fed *ad-libetum*. At 30, 60 and 90 days, 5 rats from each group were sacrificed.

3.2.1.2 Blood sampling

To carry out biochemical and haematological analyses the rats were sacrificed under anaesthesia to collect the jugular vein blood. For biochemistry samples gel clot activator and sodium fluoride tubes were used to collect blood and K_3 EDTA tubes were used to collect blood for the haematology samples. Samples were analyzed on the day of collection.

3.2.1.3 Dietary formulation

The feed for the rats were provided by Ghana Agro Food Company (GHAFCO Tema). The standard all-purpose-feed in the mashed form was used as control diet. The MOD was prepared by the proportions formula below:

Mass of MO = (x%/100%) x 35 Kg = Y Kg

Total feed weight per group = 35Kg.

Where x = needed percentage of *Moringa oleifera* leaf powder in the diet.

Y = mass of *Moringa oleifera* leaf powder added.

These quantities were weighed and thoroughly mixed in a chemical mixer to produce the needed percentage MODs. No heat treatment was applied.

3.2.2 Experiment 2

3.2.2.1 Grouping and feeding

The mice were selected and put into 4 groups of 5 each. All groups were fed low-protein diet (LPD) for 2 weeks. The first group continued feeding on LPD for another 3 weeks. The second group was fed low-protein supplemented with MO leaf powder diet (LPD-MO) for another 3 weeks. The third group was fed standard diet (SD) for another 3 weeks and fourth group was fed the standard diet supplemented with MO leaf powder (SD-MO) for 21 days.

The mice were fed *ad-libetum* daily with appropriate diet. They were allowed free access to fresh tap water each day.

3.2.2.2 Blood sampling

To carry out biochemical and haematological analyses the mice were sacrificed under anaesthesia to collect the jugular vein blood. For biochemistry samples gel clot activator and sodium fluoride tubes were used to collect blood and K_3 EDTA tubes were used to collect blood for the haematology samples. Samples were analyzed on the day of collection.

3.2.2.3 Dietary formulation

The feed for mice was produced in the laboratory by simple proportional measurements. The SD diet was purchased from the Greater Accra Poultry Farmers Association (GAPFA) Nima, with 15% crude protein content. LPD diet was obtained by adding milled maize to SD in a ratio of 6:1 to produce a 3% crude protein content. LPD-MO was obtained by adding both LPD and MO leaf powder in a ratio of 6:1 to give a 15% crude protein content. Likewise, SD-MO was obtained by adding LPD, MO and SD in a ratio of 1:1:8 to obtain high crude protein content of at least 15%.

3.2.3 Centrifugation

The blood samples used in biochemical analyses were centrifuged at 2146xg for 3 minutes to collect the plasma or serum.

3.2.4 Glucose

Measurement was based on the colorimetric end point test method.

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine product.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of the sample absorbance/standard absorbance.

3.2.5 Alanine aminotransferase (ALT)

Measurement was based on the kinetic UV test method developed by Wróblewski and LaDue.

Principle

Glutamic-pyruvic transaminase (GPT-ALT) catalyses the reaction between alpha-ketoglutaric acid and alanine giving L-glutamic acid and pyruvic acid. Pyruvic acid, in the presence of lactate dehydrogenase (LDH) reacts with NADH giving lactic acid and NAD. The rate of NADH consumption is determined photometrically and is directly proportional to the GPT activity in the sample.

Protocol

To test tubes the following was performed:

Sample	100 µL
Reagent	1000 µL

The reaction mixture was mixed well and allowed to stand for 1 minute at 37^{o}C. Decreasing absorbance was measured at 340 nm per minute for 3 minutes and the change per minute was calculated and multiplied by the factor 1746.

3.2.6 Aspartate aminotransferase (AST)

Measurement was based on the kinetic UV test method developed by Karmen.

Principle

Aspartate transaminase (GOT-AST) catalyses the reaction between alpha-ketoglutaric acid and L-aspartate giving glutamate and oxaloacetate. Oxaloacetate, in the presence of malate dehydrogenase (MDH) react with NADH giving malate and NAD. The rate of NADH decrease is determined photometrically and is directly proportional to the GOT activity in the sample.

Protocol

To test tubes the following was performed:

Sample	100 µL
Reagent	1000 µL

The reaction mixture was mixed well and allowed to stand for 1 minute at 37^{o}C. Decreasing absorbance was measured at 340 nm per minute for 3 minutes and the change per minute was calculated and multiplied by the factor 1746.

3.2.7 Alkaline phosphatase (ALP)

Measurement was based on the kinetic UV test method developed by Bowers and McComb.

Principle

p-Nitrophenyl phosphate is converted to p-nitrophenol and phosphate by alkaline phosphatase. The increase of absorption at 405 nm is proportional to the alkaline phosphatase activity in the sample.

Protocol

To test tubes the following was performed:

Sample	100 µL
Reagent	1000 µL

The reaction mixture was mixed well and incubated at 37^{o}C for 60 seconds. The increase in absorbance was measured every 30 seconds at 405 nm for 2 minutes and the change in absorbance per minute calculated and multiplied by the factor 2720.

3.2.8 Albumin

Measurement was based on the colorimetric end point test method developed by Rodkey.

Principle

The measurement of serum albumin is based on quantitative binding to the indicator 3,3',5,5'-tetrabromo-m-cresolsulphophthalein (bromocresol green, BCG). The albumin-BCG complex absorbs maximally at 578 nm.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	5 µL
Standard	-	5 µL	-
BCG reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at room temperature. The absorbance of the sample and standard was measured against

reagent blank at 620 nm. The concentration of the standard was then multiplied by the product of sample absorbance/standard absorbance.

3.2.9 Bilirubin

Measurement was based on the colorimetric end point test method developed by Molley and Evelyn.

Principle

Total bilirubin in the sample reacts with diazotised sulphalinic acid in the presence of accelerator. Direct bilirubin (conjugated) reacts in acid environment with diazotized sulphalinic acid. The formed coloured azobilirubin is measured photometrically at 546 nm.

Protocol

To test tubes the following was performed:

Total bilirubin

	Sample	Blank
Total bilirubin reagent	1000 µL	1000 µL
Nitrite reagent	20 µL	-
Sample	50 µL	50 µL

Direct bilirubin

	Sample	Blank
Direct bilirubin reagent	1000 µL	1000 µL
Nitrite reagent	20 µL	-
Sample	50 µL	50 µL

The reaction mixture was mixed well and incubated in the dark at room temperature for 5 minutes. The absorbance of the sample was measured against respective sample blank within 8 minutes at 546 nm. The concentration was calculated by subtracting the blank absorbance from the sample absorbance and multiplied by the factor 20.2.

3.2.10 Creatinine

Measurement was based on the colorimetric kinetic test method developed by Jaffe.

Principle

Creatinine in alkaline solution reacts with picrate to form a coloured complex which absorbs at 500-520 nm. The amount of complex formed is directly proportional to the creatinine concentration.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	100 µL
Standard	-	100 µL	-
Picrate and buffer reagents	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and after 30 seconds at room temperature the initial absorbance was read and read again after 1 minute. The change in absorbance in a minute of the standard and sample were measured against the reagent blank at 500 nm. The concentration of the standard was then multiplied by the product of sample absorbance/standard absorbance.

3.2.11 Urea

Measurement was based on the colorimetric end point test method.

Principle

The Berthelot reaction has long been used for the measurement of urea and ammonia. The present method is a modified Berthelot method. The urea colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonia by the use of urease. Ammonium ions then react with a mixture of salicylate, sodium nitroprusside and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Urea buffer	1000 µL	1000 µL	1000 µL
Urea enzyme reagent	100 µL	100 µL	100 µL
Standard	-	10 µL	-
Sample	-	-	10 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			
Urea colour developer	1000 µL	1000 µL	1000 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			

The absorbance of sample and standard was measured against reagent blank at 578 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.2.12 Cholesterol

Measurement was based on the enzymatic colorimetric test method.

Principle

Cholesterol esters are hydrolysed to produce cholesterol. Hydrogen peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantypyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.2.13 High density lipoprotein (HDL)

Measurement was based on the CHOD-PAP tests method

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated by the precipitating reagent. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction remains in the supernatant in this phase and is determined by an enzymatic (CHOD-PAP) method.

Protocol

Precipitation step

To test tubes the following was performed:

Sample	Precipitating reagent
500 µL	500 µL

Mix and allow standing for 5 minutes. Centrifuge for 10 minutes at 3000 rpm and determining the cholesterol content by the CHOD-PAP method. Only clear supernatant must be used.

Assay step

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	50 µL
Standard	-	50 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 520 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance and a serum dilution factor of 2.

3.2.14 Low density lipoprotein (LDL)

Low density lipoprotein was calculated using the Friedewalds' equation; when all measurements are in millimole per liter (mmol/L):

LDL cholesterol = Total Cholesterol – HDL cholesterol – (Triglyceride/2.22)

3.2.15 Triglyceride

Measurement was based on the enzymatic colorimetric test method

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinoneimine indicator if formed from hydrogen peroxide, 4-aminophenazone and 4-chorophenol under the catalytic influence of peroxidase.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.2.16 Serum ascorbate (vitamin c)

Method

Vitamin C was determined using the method of (Benderitter *et al.*, 1998). Proteins were precipitated with 10% trichloroacetic acid (TCA) and centrifuged at 1500xg for 5 minutes. Then 75 µl of dinitrophenyl hydrazine (2 g DNPH, 230 mg thiourea and 270 mg $CuSO_4$. $5H_2O$ in 100 ml 5 M H_2SO_4) was added to 500 µl reaction mixture. The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5 ml

sulfuric acid 65% (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the Vitamin C content of the sample was subsequently calculated, using a vitamin C standard.

3.2.17 Malondialdehyde (MDA)

Method

The malondialdehyde level in serum was estimated spectrophotometrically according to (Buege and Aust, 1978). About 0.2ml aliquot of serum was added to 1.0 ml of 0.375% thiobarbituric acid solution in 0.25 M HCl and mixed with 4.0 ml of 15% trichloroacetic acid. After incubation at 100°C (15 min), the samples were cooled, centrifuged and the supernatants were evaluated spectrophotometrically at 535 nm against a reference blank. The MDA was determined using the molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and results were expressed in µmol/L.

3.2.18 Haematology

The procedures for RBC, WBC, platelets and their respective differentials were performed with automated analyzer following manufacturers' protocol.

3.3 Statistical analyses

The antioxidant indices, biochemical, haematological and weight changes results were analyzed using one-way analysis of variance (ANOVA) and are presented as mean and the standard error of the mean (SEM). Dunnett's multiple comparison tests was used to establish any statistical significance between control and experimental groups. Statistical significance was set at $p < 0.05$. The statistical software used was

GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

CHAPTER FOUR

RESULTS

The results of the experiments conducted using MO in diet are presented below. The baseline measurements are shown in the appendix.

4.1 Experiment 1

4.1.1 Rats growth curves

Figures 4.1, 4.2 and 4.3 present the growth curves for rats during the experiment at days 30, 60 and 90. The means of body weight were plotted and there were no significant weight variations among the percentage MOD groups compared to the control.

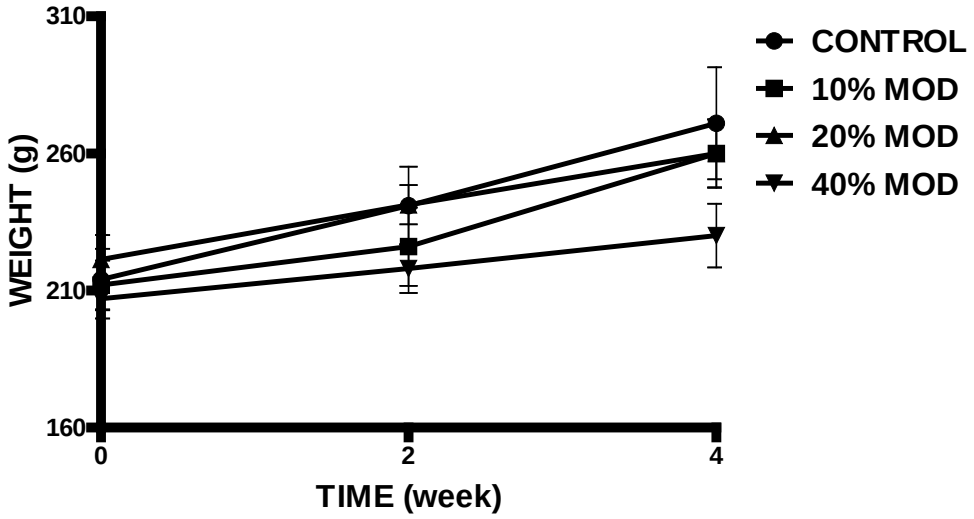


Figure 4.1 Weight changes observed at two week intervals for 30 days of feeding in rats.

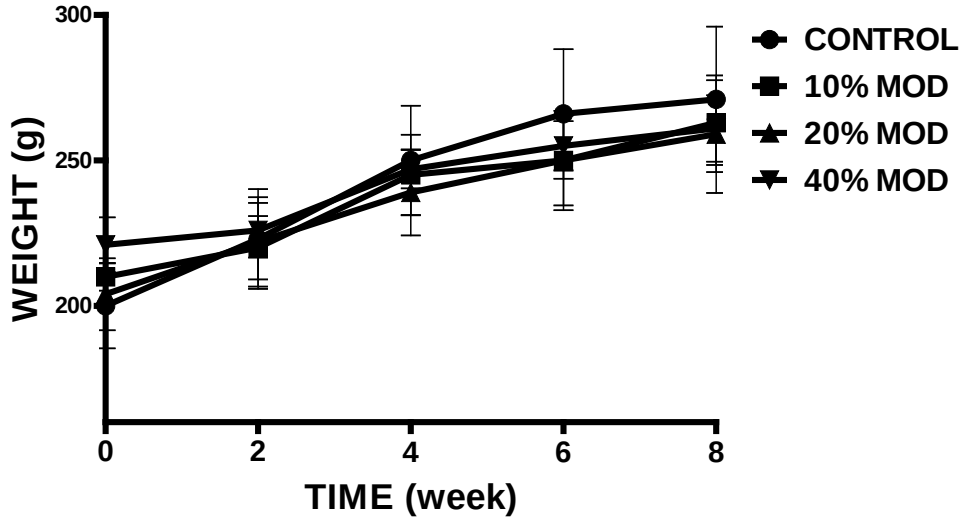


Figure 4.2 Weight changes observed at two week intervals for 60 days of feeding in rats.

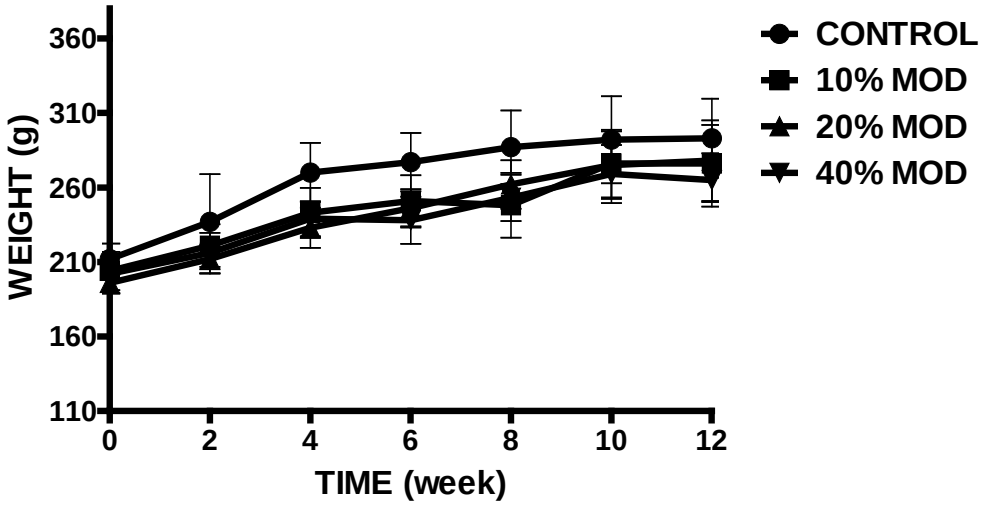


Figure 4.3 Weight changes observed at two week intervals for 90 days of feeding in rats.

4.1.2 Biochemistry

4.1.2.1 Fasting blood glucose (FBS)

Generally, the MOD exerts normo-glycaemic tendency compared to the controls. The fasting blood glucose levels as seen in Table 4.1.2.1(a&b) for a 30-day and 60-day feeding period were all insignificant. In relation to the control, the FBS levels were lowered as percentage MOD increased. This was readily evident for 40% MOD ($p < 0.001$) as shown in Table 4.1.2.1c.

Table 4.1.2.1a Fasting blood sugar at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
FBS (mmol/L)	4.7±0.2	5.5±0.2	5.5±0.2	5.2±0.3

The results are presented as mean ± SEM, n = 5.

Table 4.1.2.1b Fasting blood sugar at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
FBS (mmol/L)	5.0±0.1	5.5±0.1	4.8±0.2	5.5±0.3

The results are presented as mean ± SEM, n = 5.

Table 4.1.2.1c Fasting blood sugar at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
FBS (mmol/L)	4.7±0.2	4.7±0.3	3.8±0.1	3.2±0.3[b]

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

4.1.2.2 Liver function tests (LFTs)

Tables 4.1.2.2(a, b & c) show the measurements of serum enzymes ALT, AST and ALP, albumin and bilirubin portions for the groups at 30, 60 and 90 days respectively. It can be seen from Tables 4.1.2.2(a-c) that there were some variations in most of the parameters but with no significance when compared to the controls. However, in Table 4.1.2.2a ALP recorded significant increases in 10% MOD ($p < 0.001$) and 40% MOD ($p < 0.001$). Similarly, in Table 4.1.2.2c, AST increased significantly for 40% MOD ($p < 0.05$).

Table 4.1.2.2a Liver function tests at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
ALT (U/L)	45.2±7.9	32.8±7.7	55.4±5.5	49.4±7.8
AST (U/L)	220.7±37.3	170.8±14.7	237.0±17.2	202.0±10.4
ALP (U/L)	61.3±7.8	129.8±6.6[b]	101.3±14.2	130.6±15.3[b]
ALB (g/L)	38.0±3.7	42.6±2.0	42.6±2.0	39.8±4.3
T. BIL (µmol/L)	9.5±1.1	9.2±0.1	9.2±0.5	10.1±1.0
D. BIL (µmol/L)	4.9±0.3	5.2±0.1	5.2±0.1	5.4±0.2
I. BIL (µmol/L)	4.8±0.7	4.0±0.5	4.0±0.5	5.4±0.5

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.2.2b Liver function tests at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
ALT (U/L)	49.6±3.3	48.0±6.3	61.3±6.7	43.8±7.4
AST (U/L)	204.3±20.2	193.0±9.4	206.0±19.1	181.0±13.9
ALP (U/L)	151.8±25.5	182.4±21.4	179.8±29.4	247.7±36.7
ALB (g/L)	46.8±0.7	41.2±2.0	43.5±1.5	44.1±2.2
T. BIL (µmol/L)	7.7±1.2	5.7±0.8	8.4±1.0	8.5±0.9
D. BIL (µmol/L)	4.2±0.2	3.8±0.2	4.0±0.5	4.6±0.5
I. BIL (µmol/L)	4.9±0.9	2.4±0.9	6.1±0.8	3.2±0.5

The results are presented as mean ± SEM, n = 5.

Table 4.1.2.2c Liver function tests at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
ALT (U/L)	52.6±3.3	49.6±6.8	91.6±48.6	100.2±22.9
AST (U/L)	214.5±34.9	240.0±13.1	214.3±24.1	303.8±7.8[a]
ALP (U/L)	189.8±30.7	126.4±5.9	158.8±12.1	160.0±18.6
ALB (g/L)	37.3±1.5	36.6±1.8	35.6±1.0	37.4±1.3
T. BIL (µmol/L)	7.3±1.1	10.3±1.5	8.5±1.0	11.8±1.0
D. BIL (µmol/L)	2.8±0.1	3.2±0.2	2.8±0.3	3.7±0.4
I. BIL (µmol/L)	4.5±1.1	7.1±1.3	5.8±0.7	8.2±0.9

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

4.1.2.3 Renal function tests (RFTs)

Tables 4.1.2.3(a, b & c) show the measurements of serum creatinine and urea for the groups at 30, 60 and 90 days respectively. In Table 4.1.2.3b significant increases were observed for 20% MOD ($p < 0.001$) and 40% MOD ($p < 0.0001$) in urea levels with respect to the control. All the other variations in the analytes were insignificant.

Table 4.1.2.3a Renal function tests at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
CREAT (µmol/L)	87.0±5.5	99.4±7.6	97.5±6.3	103.8±7.2
UREA (mmol/L)	8.2±0.3	7.2±0.7	9.6±0.4	9.5±0.3

The results are presented as mean ± SEM, n = 5.

Table 4.1.2.3b Renal function tests at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
CREAT (µmol/L)	108.3±5.4	105.0±4.3	110.2±4.3	103.8±7.2
UREA (mmol/L)	9.1±0.5	10.1±0.9	13.8±0.9[b]	15.8±1.2[c]

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.2.3c Renal function tests at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
CREAT (µmol/L)	59.0±3.1	68.7±6.6	58.0±6.1	58.5±3.8
UREA (mmol/L)	5.2±0.3	5.7±0.5	5.7±0.4	5.5±0.1

The results are presented as mean ± SEM, n = 5.

4.1.2.4 Lipid profile

Tables 4.1.2.4(a, b & c) show the measurements of total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglyceride for the groups at 30, 60 and 90 days respectively. The lipid profile reveal very little variation occurring and thus appeared to be more regulated among the groups as seen in Table

4.1.2.4(a-c). The only changes considered significant was seen in Table 4.1.2.4a with significant drops in the triglyceride levels 10% MOD ($p < 0.05$), 20% MOD ($p < 0.05$) and 40% MOD ($p < 0.05$).

Table 4.1.2.4a Lipid profile at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
T. CHOL (mmol/L)	1.7±0.1	1.8±0.1	1.8±0.1	1.5±0.1
HDL-C (mmol/L)	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1
LDL-C (mmol/L)	0.2±0.1	0.5±0.1	0.4±0.2	0.2±0.1
TRIG (mmol/L)	0.6±0.1	0.4±0.1[a]	0.4±0.1[a]	0.4[a]

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.2.4b Lipid profile at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
T. CHOL (mmol/L)	2.4±0.2	2.0±0.1	2.2±0.1	2.1±0.1
HDL-C (mmol/L)	1.1±0.1	1.0±0.1	1.0±1	1.0±0.1
LDL-C (mmol/L)	0.9±0.2	0.8±0.1	0.8±0.1	0.7±0.1
TRIG (mmol/L)	1.1±0.1	0.8±0.2	0.8±0.2	0.9±0.1

The results are presented as mean ± SEM, n = 5.

Table 4.1.2.4c Lipid profile at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
T. CHOL (mmol/L)	2.1±0.2	2.2±0.2	2.1±0.2	2.5±0.1
HDL-C (mmol/L)	0.9	0.9	1.0±0.1	1.0±0.1
LDL-C (mmol/L)	1.0±0.2	1.1±0.2	0.9±0.2	1.2±0.1
TRIG (mmol/L)	0.5±0.1	0.5±0.1	0.5	0.5±0.1

The results are presented as mean ± SEM, n = 5.

4.1.3 Haematology

4.1.3.1 WBC count and WBC differentials

Tables 4.1.3.1 (a, b & c) present the data for WBC, neutrophils, lymphocytes, monocytes, eosinophils and basophils. Apart from a few changes that occurred in a few groups there were not many significant changes noted. Monocyte levels were significantly lowered as seen in Table 4.1.3.1a for 20% MOD ($p < 0.05$) and 40% MOD ($p < 0.05$), WBC in Table 4.1.3.1b was increased for 20% MOD ($p < 0.05$) and an increase in monocyte levels is shown in Table 4.1.3.1c for 10% MOD ($p < 0.05$) and 20% MOD ($p < 0.05$) all with regard to the respective controls.

Table 4.1.3.1a WBC count and differentials at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
WBC (K/µL)	6.0±1.0	5.1±1.4	5.6±0.6	3.8±1.3
NEUTROS (%)	17.3±1.1	17.9±5.3	16.3±2.8	17.4±2.6
LYMPHS (%)	63.1±4.4.	56.6±8.2	66.8±5.9	54.1±9.4
MONOS (%)	10.8±1.9	6.0±1.7	4.7±0.5[a]	5.3±1.2[a]
EOSINOS (%)	3.8±0.4	2.6±0.7	1.3±0.3	2.8±0.7
BASOS (%)	6.6±1.3	3.1±0.9	3.2±0.6	4.3±1.1

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.3.1b WBC count and differentials at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
WBC (K/µL)	1.7±0.2	2.4±0.3	3.1±0.3[a]	2.5±0.3
NEUTROS (%)	25.5±8.7	39.7±4.7	41.0±8.3	22.7±5.0
LYMPHS (%)	52.5±5.0	34.3±5.0	38.3±5.2	52.1±5.0
MONOS (%)	11.9±2.6	7.5±2.5	9.3±2.2	12.7±3.0
EOSINOS (%)	2.2±0.6	2.6±0.8	1.2±0.6	4.7±1.0
BASOS (%)	10.5±1.1	14.1±2.5	7.8±1.2	8.6±1.3

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.3.1c WBC count and differentials at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
WBC (K/µL)	8.5±1.1	7.6±0.5	8.2±1.1	8.8±1.7
NEUTROS (%)	17.7±2.7	24.2±3.7	18.1±2.3	10.1±1.8
LYMPHS (%)	71.9±2.8	63.0±4.4	69.4±2.1	77.5±2.5
MONOS (%)	4.5±0.7	7.6±0.1[a]	7.3±0.5[a]	5.9±1.0
EOSINOS (%)	1.7±0.2	1.9±0.3	2.0±0.3	1.8±0.2
BASOS (%)	1.9±0.1	2.1±0.4	2.2±0.4	2.5±0.5

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

4.1.3.2 RBC count and RBC indices

Tables 4.1.3.2 (a, b & c) show the results for RBC, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and red cell size distribution width. Unlike the WBC count and differentials, any significant changes attributable to MO that occurred were recorded in Table 4.1.3.2a. RBC was significantly raised in all three MOD groups ($p < 0.0001$), similarly the haemoglobin was also raised for 20% MOD ($p < 0.05$) and 40% MOD ($p < 0.05$) and this was the same in heamatocrit levels for 10% MOD ($p < 0.001$), 20% MOD ($p < 0.001$) and 40% MOD ($p < 0.0001$) if compared to their controls. However, for 10% MOD, the MCV ($p < 0.05$) and MCH ($p < 0.0001$) were lower in values than their controls.

Table 4.1.3.2a RBC count and differentials at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
RBC (M/µL)	6.5±0.2	7.9±0.2[c]	7.8±0.1[c]	7.9±0.2[c]
HB (g/dL)	13.3±0.5	14.5±0.7	15.2±0.1[a]	15.6±0.5[a]
HCT (%)	62.1±1.7	72.7±2.2[b]	72.4±0.7[b]	74.1±1.8[c]
MCV(fL)	97.7±0.5	92.3±1.2[a]	93.9±0.6	94.1±1.2
MCH (pg)	20.6±0.2	18.4±0.5[c]	19.6±0.2	19.8±0.4
MCHC (g/dL)	21.3±0.3	19.9±0.5	20.9±0.2	21.0±0.3
RDW (%)	15.7±0.5	16.0±0.4	16.0±0.3	17.3±0.5

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

Table 4.1.3.2b RBC count and differentials at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
RBC (M/µL)	7.5±0.3	7.6±0.2	7.4±0.3	8.1±0.1
HB(g/dL)	12.7±0.3	12.9±0.3	12.6±0.4	13.9±0.4
HCT (%)	65.5±2.0	66.9±0.8	64.0±2.1	71.2±1.6
MCV (fL)	88.6±1.0	88.0±1.1	86.1±1.0	87.7±1.2
MCH (pg)	17.2±0.3	17.0±0.2	16.9±0.1	17.1±0.3
MCHC (g/dL)	19.4±0.2	19.3±0.1	19.7±0.1	19.5±0.1
RDW (%)	16.1±0.4	15.5±0.4	15.2±0.2	15.7±0.3

The results are presented as mean ± SEM, n = 5.

Table 4.1.3.2c: RBC count and differentials at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
RBC (M/µL)	6.7±0.3	6.9±0.2	6.7±0.2	7.1±0.1
HB (g/dL)	13.3±0.4	13.8±0.3	13.6±0.5	13.9±0.3
HCT (%)	57.3±1.2	59.6±1.1	58.4±1.5	60.4±0.8
MCV (fL)	85.3±1.6	86.4±1.8	86.5±0.7	85.3±0.8
MCH (pg)	19.8±0.3	19.9±0.4	20.1±0.3	19.7±0.1
MCHC (g/dL)	23.3±0.3	23.1±0.1	23.3±0.3	23.1±0.2
RDW (%)	15.0±0.7	14.3±0.7	15.4±0.4	15.7±0.3

The results are presented as mean ± SEM, n = 5.

4.1.3.3 Platelets

Tables 4.1.2.3 (a, b & c) show the results for platelets and mean platelets volume. The platelet count shows decreasing patterns in the experimental groups; as the percentage of MO increased. In Table 4.1.3.3c platelet level declined significantly for 40% MOD ($p < 0.05$) compared to the control. There were no significant changes in values of MPV between the controls and the experimental groups.

Table 4.1.3.3a: Platelets at day 30 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
PLATS. (K/µL)	764.3±103.8	757.2±43.6	693.4±31.1	616.0±67.0
MPV (fL)	7.6±0.2	7.2±0.3	7.0±0.2	7.4±0.3

The results are presented as mean ± SEM, n = 5.

Table 4.1.3.3b: Platelets at day 60 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
PLATS. (K/µL)	636.8±85.0	743.4±72.7	570.6±75.1	563.4±57.7
MPV (fL)	8.7±0.4	7.5±0.1	7.6±0.5	8.4±0.4

The results are presented as mean ± SEM, n = 5.

Table 4.1.3.3c: Platelets at day 90 of feeding on MOD

Parameter	Group			
	Control	10% MOD	20% MOD	40% MOD
PLATS (K/µL)	733.0±60.5	691.8±31.3	569.0±72.3	499.6±42.7[a]
MPV (fL)	7.2±0.1	7.0±0.1	6.9±0.1	6.7±0.1

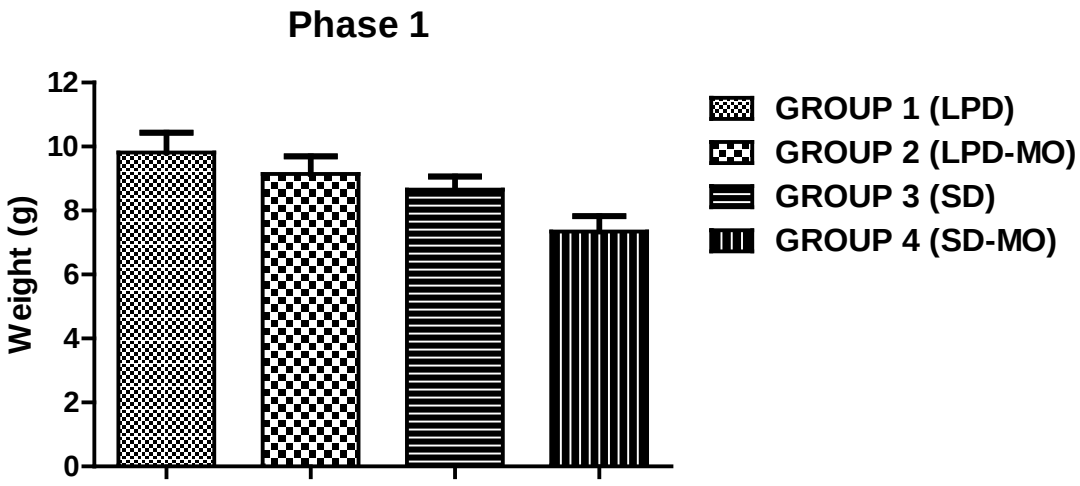
The results are presented as mean ± SEM, n = 5. P-values significant from control; a = $p<0.05$, b = $p<0.001$ and c = $p<0.0001$.

4.2 Experiment 2

4.2.1 Mice growth charts

Figure 4.4 displays the graph of weight changes with time during the experiment with mice. The growth charts did not show any significant changes in the recorded body weights for the different diet types. However, at the end of the experiment the LPD-MO group had the lowest weight followed by SD-MO in phase 2.

a.



b.

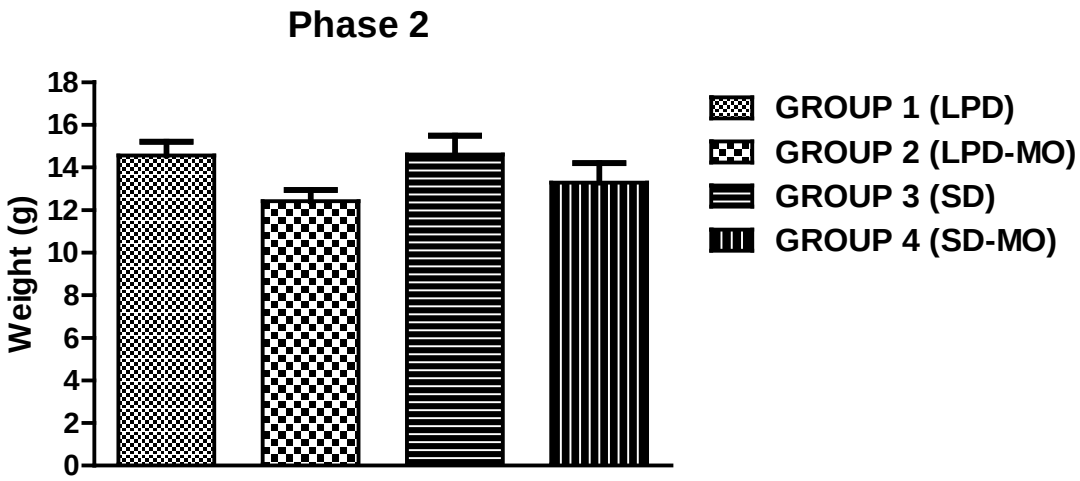


Figure 4.4 Weight changes observed when mice (a) were all fed the same diet for 14 days (phase 1), (b) were fed different diets for 21 days (phase 2).

4.2.2 Oxidative stress indices

Figures 4.5 and 4.6, show the concentrations of serum ascorbic acid and malondialdehyde respectively; in the mice after 21 days of feeding on the specified diet. Ascorbic acid concentrations in Fig 4.5 were significantly higher in animals fed

on diets containing MO as compared to those not on MO diet. Similarly, MDA activities in Fig 4.6 were significantly low in mice on the MO containing diet as compared to those not on MO diet.

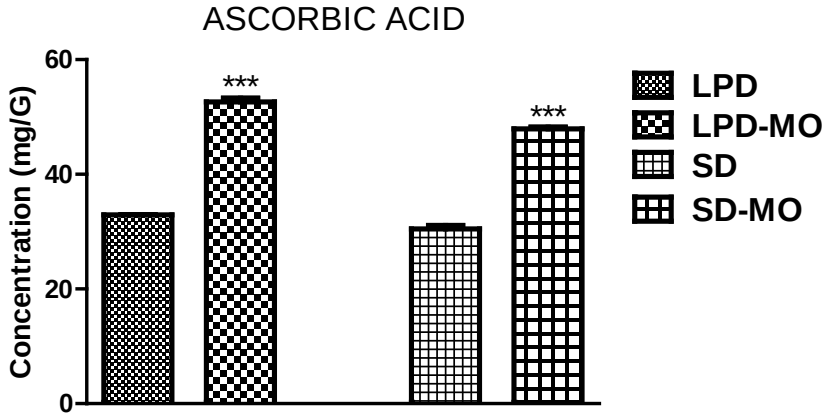


Figure 4.5 A graph showing the concentrations of ascorbic acid in the serum of mice fed on the different diets.

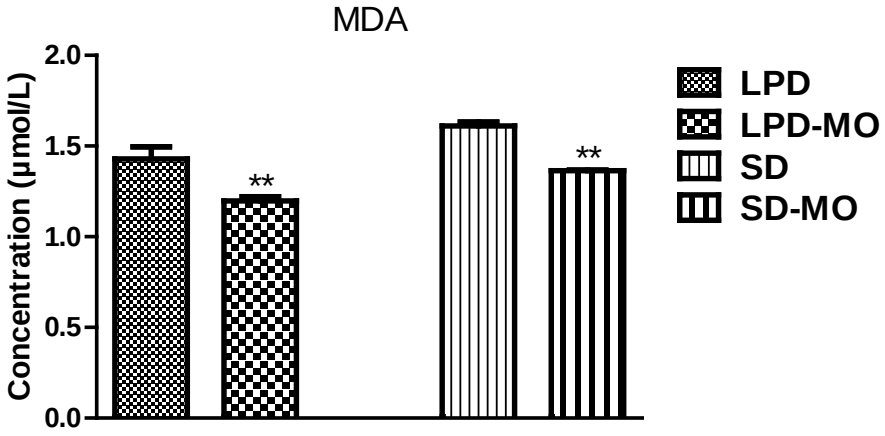


Figure 4.6 A graph showing the concentrations of malondialdehyde (MDA) in the serum of mice fed on the different diets.

Table 4.2.1 shows the results of the correlation coefficient and p-values between vitamin c and MDA for the groups. There was a significant positive correlation for the LPD while the rest of the groups had insignificant negative correlations.

Table 4.2.1 Correlation between plasma Vitamin C and Malondialdehyde

Statistic	LPD	LPD-MO	SD	SD-MO
Correlation Coefficient r	0.9972	-0.2876	-0.9897	-0.05246
P-value	0.0481	0.8143	0.0912	0.9666

n = 3

CHAPTER FIVE

DISCUSSION AND RECOMMENDATION

The effects of the leaf powder of *Moringa oleifera* as a nutritional supplement was assessed for its toxicity and protection against oxidative stress. The results are discussed below.

5.1 Growth Curves/Charts

The growth curves and charts for both rats and mice did not show any stunted or retarded growth meaning that all the animals were growing normally. In Figure 4.1-4 (48-49, 61pp), it was realized that all the weight changes at weekly or daily interval were not significant. However, it was observed in the 90 days that the experimental (with MO diet) groups had low body weights with respect to the controls (non-MO diet). It is possible that the MO was responsible for this weight effect. Adadepo *et al.*, (2009), observed similar results using aqueous extract of the MO leaf. However, in their 21 days study the weight gain reduced with graded doses which are contrary to the 30 days of this study. Another reason that can explain the insignificance in weight changes is the fact that the animals used had lean body mass. This was evident in the lipid profile (Table 4.1.2.4a-c; 54-55pp) measurements; where the change in total cholesterol was insignificant. In the obese state the figures are likely to be otherwise.

5.2 Fasting blood sugar (FBS)

Fasting blood sugar levels are used to help diagnose diabetes mellitus and hypoglycemia. A randomly timed test for glucose is usually performed for routine screening and nonspecific evaluation of carbohydrate metabolism. The only

significant change for an overnight fast was in Table 4.1.2.1c (p50), where a decrease occurred for 40% MOD after 90 days feeding. This is an indication that the MO may be a hypoglycaemia agent when used for a long period of time. This suggests that while providing nutritional benefits it can lower blood sugar too for some people.

5.3 Hepatic/liver function and assessment

The liver function was aimed at specific roles associated with the liver in the body in maintaining homeostasis. These are the synthetic and secretory or excretory activities. Enzymatic assessment is used to determine liver cell integrity.

5.3.1 Enzymatic activity

Certain enzymes found in the liver cell may be found in the blood as part of normal processes such as death of cells or as a result of injury/damage or disease. In the normal enzyme release process, the concentration or activity of the enzyme in question are negligible contrary to those of injured/damaged or diseased cells. There are several enzymes (see section 2.10.1; p28) whose activity may be measured to determine the integrity of the liver cell and thus diagnose the condition present. In this work the most common enzymes (ALT, AST and ALP) whose activities were measured as part of routine liver assessment were employed in assessing the effect of MOD on the liver.

Alanine aminotransferase (ALT)

Alanine aminotransferase though may be produced by other tissues such as the heart, skeletal muscle and kidney, is more specific to the liver and thus used as a reliable marker for liver specific condition (Fischbach, 2003; Chernecky and Berger, 2008).

Some herbal or natural remedies are known to be hepatotoxic and may cause increased levels of the enzyme, nonetheless coffee is known to cause a falsely decreased result (Chernecky and Berger, 2008). Through the 90 days of study there was no significant reduction or elevation of the ALT concentration in the blood that can be attributed to the MO content of the diet. This is in contrast to the results of Adadepo *et al.*, (2009), in which they showed significant increases in the enzyme activity. The change that was readily observed is a slight rise in the MOD groups as the days were increased and this may be a result of the normal cell leakages and deaths occurring in the liver. The MO did not cause cell damage.

Aspartate aminotransferase (AST)

Aspartate aminotransferase is an enzyme used in liver assessment though it is less specific to the liver. It is predominant in the heart tissue, it also occurs in the skeletal and renal tissues. Notwithstanding, it is used together with ALT in hepatotoxic evaluations. Like ALT the same herbal or natural remedies are also reported to cause increased levels in the blood. In the first two months the results were not significant but for the third month the increase in AST for the 40% group was significant. In the work of Adadepo *et al*., (2009), they reported a general significant increase in AST levels after 21 days. Aspartate aminotransferase activity is normally elevated in haemolysed blood samples (Fischbach, 2003). Moringa oliefera leaves contains saponins (Makkar and Becker, 1997) which can dissolve cell membranes thereby resulting in haemolysis. All of the other results did not point to possible saponins related haemolysis. The MO content may not be enough to cause haemolysis. There might have been some haemolysis during blood collection from the jugular vein.

Alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme found in bone, liver, intestine, and placenta and will increase its activity in blood when there is any underlying conditions in any of these tissues. Other factors will also increase the concentrations in the blood for example any types of biliary tree obstruction increases synthesis of ALP by the hepatocytes adjacent to the biliary canaliculi which then leaks into plasma. As a result plasma ALP level rises (Rao, 2006; Burtis *et al.*, 2008). From the results, in the first month ALP activity was increased significantly for two MOD groups (Table 4.1.2.2a-c; 51-52pp) but for the following two months no significant increases were reported. Again, Adadepo *et al.*, (2009) observed similar results with the highest dose administration in their 21 days study. The significant increases in the ALP, though, were caused by MO may not be an indication of liver cell damage (Fischbach, 2003). It may be that MO was interacting with other tissues such as bone or biliary tract and this contributed to the increased changes.

Presumably, the result of ALT, AST and ALP points to the fact that the use of MO in the diet has no undesirable consequence on the liver enzymatic status. The duration of use in the diet or the quantity has no adverse effects as shown by the results in Table 4.1.2.2a-c (51-52pp). The differences in results between Adadepo *et al.*, (2009) and this work could be due to the different states of the MO used and the treatment it had undergone.

5.3.2 Synthetic activity

Albumin is a major serum protein in the blood aside the globulins and it is responsible for maintaining oncotic pressure and transport of insoluble substances in the blood.

Albumin is synthesized by the liver and is used as an index of nutritional status evaluation (Fischbach, 2003). Malnutrition and inflammation of the liver may result in low serum albumin concentration but increased serum albumin is likely to be implicated in water loss or dehydration. The result shows a relatively stable serum albumin concentration thus the synthetic ability of the liver was not compromised by the MO leaf powder but rather the liver was able to produce albumin needed for vital systemic functions.

5.3.3 Secretory or excretory activity

Bilirubin is a product of mainly haemoglobin breakdown and it is transported by albumin in the blood to the liver and other cells for catabolism. Bilirubin is detectable in two forms; conjugated (direct) bilirubin and unconjugated (indirect) bilirubin. The two forms put together are known as total bilirubin. Jaundice is the condition associated with increased bilirubin concentration in the blood and may arise from haemolysis, hepatitis or hepatic obstruction (Fischbach, 2003; Burtis *et al.*, 2008). The result from Table 4.1.2.2a-c (51-52pp) shows no significant decreases or increases with respect to the matched controls. The MO did not induce either haemolysis or liver injury that could have raised the serum bilirubin fractions as will occur in diseased states. Thus bilirubin metabolism was normal irrespective of MO in the diet.

5.4 Renal function

This part assessed the integrity of the kidneys in eliminating creatinine and urea. These are just indices of glomerular function. When kidney function is compromised, the concentrations of these substances are elevated in the blood and so are they in the

serum. This is the reason why serum levels of these metabolites can be employed as a diagnostic tool for renal function.

5.4.1 Glomerular function

Creatinine is a byproduct of creatine phosphate catabolism in the skeletal muscles resulting from energy production and it is readily excreted by the kidney. It is an index of renal function and therefore when its level builds-up it implies that the glomerular filtration rate is slowed or reduced (Chernecky and Berger, 2008; Wilson, 2008). From Table 4.1.2.3a-c (p53) it can be seen that the serum creatinine concentrations were not significantly changed. Certain natural herbal remedies are known to decrease or increase the blood creatinine levels (Chernecky and Berger, 2008). However, MO did not create any significant change. The kidneys' clearing rate was not affected in any way by the MO administration.

Urea is produced in the liver as a result of protein metabolism. Urea nitrogen is the nitrogen portion of urea and it is transported in the blood to the kidneys, where it is excreted. The result pointed out significant increases in Table 4.1.2.3b (p53) for 20% MOD and 40% MOD. When the kidneys removal cannot match up with the rate of production of this product, high concentrations are measured. Other factors such as high protein intake, increased protein catabolism, stress and dehydration can be responsible for such increases (Chernecky and Berger, 2008). The most probable explanation for this occurrence is the fact that MO as a rich source of protein led to more production of the product. Also, MO as already stated has diuretic property which might have caused dehydration thereby resulting in the high urea levels. This may not be a problem of kidney function as serum creatinine was normal.

5.5 Lipid profile

The lipid profile when performed gives an idea of an individual's risk of developing cardiovascular diseases. The total cholesterol, HDL-C, LDL-C and triglyceride are used to assess this risk.

Total cholesterol

Cholesterol is a sterol compound synthesized exogenously in the liver from dietary fats and endogenously within the cells. It is present in all body tissues and is a major component of bile salts, steroid hormones, low-density lipoproteins (LDLs), brain and nerve cells, cell membranes, and some gallstones. Cholesterol is transported in the blood by the low density lipoproteins to the peripheral blood and high density lipoproteins from the peripheral blood to the liver. When the levels of cholesterol in the blood is high (hypercholesterolemia), especially in combination with low levels of HDL, it is known to increase a person's risk of atherosclerosis and heart disease. In the results (Table 4.1.2.4a-c; 54-55pp), it can be seen that no significant changes were recorded for total cholesterol concentrations. The MO was able to maintain the relative total cholesterol concentrations and such effect will be desirable for preventing unhealthy increases. High total cholesterol is a risk factor for developing cardiovascular diseases.

High-density lipoprotein cholesterol (HDL-C)

High-density lipoprotein is a type of cholesterol carried by alpha-lipoprotein. HDL is believed to help protect against the risk of coronary artery disease (CAD) and has been shown to be inversely related to the risk of coronary heart disease. The result did not show any significant changes in HDL-C for duration of feeding. The MO appears

not to have any direct influence on HDL-C metabolism. This probably suggests that it may not increase the HDL-C production and its protective effect against CAD will be negligible if the subject does not have adequate HDL-C.

Low-density lipoprotein (LDL-C)

When very low-density lipoproteins (VLDL) are degraded, intermediate density-lipoproteins (IDL) are produced which are taken up by the liver and converted to low-density lipoproteins (LDLs). LDLs can be oxidized and are atherogenic and thus associated with an increased risk of arteriosclerotic heart and peripheral vascular disease (Chernecky and Berger, 2008). This parameter did not record any significant changes, especially, decrease which promotes good cardiovascular health.

Triglyceride

Dietary triglycerides are carried as part of chylomicrons through the lymphatic system and bloodstream to adipose tissue, where they are released for storage. Triglycerides are also synthesized in the liver from fatty acids and from protein and glucose when these substrates are above the body's current needs and then stored in adipose tissue. They may be later retrieved and formed into glucose through gluconeogenesis when needed by the body. From the Table 4.1.2.4a (p54) there were significant decreases in its concentration for 10% MOD 20% MOD and 40% MOD with respect to the control. Some studies state that increased fasting triglyceride in the blood is a risk factor for coronary heart diseases, diabetes and pancreatitis (Wildman, 2009; Longe, 2008). The decreases therefore suggest that MO in this regard may prevent the elevation of triglycerides to dangerous levels thus reducing any such likely conditions noted above.

The role of MO in lipid (especially total cholesterol, HDL-C and LDL-C) metabolism was not observed though the growth chart (Figure 1-3; 48-49pp) could suggest weight control ability of the experimental compared to the controls. The ratio of HDL-C/LDL-C was over twice in Table 4.1.2.4a (p54) than those in Tables 4.1.2.4b&c (54-55pp). Nonetheless, its ability to significantly reduce triglyceride level in the short-term makes it a good ingredient as part of the diet when cardiovascular health is concerned.

5.6 Oxidative stress and antioxidant activity

In the body, lipid peroxides are formed. Free radicals mediate lipid peroxide formation. The process of lipid peroxidation is initiated by the attack of a free radical on unsaturated lipids, and the resulting chain reaction is terminated by the production of lipid breakdown products: lipid alcohols, aldehydes, or smaller fragments such as malondialdehyde. Thus, there is a cascade of peroxidative reactions, which ultimately leads to the destruction of the lipid and possibly the structure/membrane in which it is located. Diseases like cancer, diabetes and atherosclerosis are consequence of the formation of lipid peroxides in the body (Timbrell, 2009). Usually MDA estimation is used to assess extent of lipid peroxidation. There are free radical scavenging systems in the body (enzymatic and non-enzymatic); vitamin C is one of the non-enzymatic agents.

Vitamin C

Vitamin C is a water-soluble vitamin found in citrus fruits and leafy vegetables and tomatoes. It is absorbed from the diet through the small intestine and stored in the adrenal glands, kidney, spleen, liver, and leukocytes. Excess amounts of the vitamin

are excreted in the urine. Vitamin C is important in cellular structure, collagen synthesis, capillary integrity, wound healing, intestinal iron absorption, and resistance to infection. The serum ascorbic acid content of the animals fed on MO diet were significantly higher compared to those animals not on MO diet as seen in Figure 4.5 (p62). The *Moringa* species was shown to be higher in antioxidants such as ascorbic acid even in the dried state, when compared to fruits and vegetables known to have high antioxidant content such as strawberries (Yang *et al.*, 2006). Therefore, the addition of MO in the diet will provide substantial antioxidant benefits from ascorbic acid to the body.

Malondialdehyde (MDA)

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is malondialdehyde. Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation (www.enzolifesciences.com, 2008). In Figure 4.6 (p62), the mice fed on MO diet had significantly low serum MDA concentrations as compared to the controls. This means that the presence of MO is able to protect the cells and tissues against lipid peroxidation which ensures their integrity and normal functioning.

The high plasma Vitamin C negatively correlates with the low MDA concentrations though these were not significant statistically in the LPD-MO and SD-MO groups (Table 4.2.1, p63). This means that vitamin C as antioxidant was able to neutralize

free radicals (lipid peroxides) in the body; thus, the low MDA concentrations measured. Any oxidative stress likes to result, which may lead to undesirable effects on the body systems was prevented. However, Vitamin C as pro-oxidant may be expected to initiate the generation of free radicals leading to high MDA levels which was absent as shown in the results (Figure 4.5 and Figure 4.6, p62).

5.7 Haematology

Full blood count or complete blood count is normally conducted to be able to diagnose blood diseases such as different/forms of anaemia, lukaemia and thrombosis and it involves measurement of the concentrations of white blood cells (WBCs) and WBC differential, red blood cells (RBCs), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets and mean platelet volume (MPV) (Chernecky and Berger, 2008).

White blood cell count and WBC differential

WBC or leucocytes play a number of roles in the body's defense; thus they protect the body from pathogens (Saladin, 2003). The WBC differential test counts 100 WBCs and then gives percentages of the various forms of leucocyte present. There are five forms of the leucocyte namely eosinophils, basophils, lymphocytes, monocytes and neutrophils. Basophils, eosinophils and neutrophils are referred to as granulocytes because of their granular appearance while lymphocytes and monocytes are referred to agranulocytes.

Specifically, basophils function by releasing the chemicals histamine and heparin that are important in the inflammatory process; thus aiding the mobility and action of other leukocytes. Eosinophils function in parasitic infection and phagocytose cell debris in the later part of inflammation. Neutrophils are the most abundant part of the leukocyte and are the first cells that show up during an inflammation and remove cell debris by phagocytosis. Lymphocytes play an integral part in antibody response to antigen. Monocytes mature to function as phagocytes (Wilson, 2008). There were no significant changes in the WBC count though 40% MOD recorded a drop. For the differential counts in Table 4.1.3.1a (p56), the 20% MOD and 40% MOD indicated significant reductions in the monocytes percentages again in contrast to Adadepo *et al.*; (2009). The rest of the variations for the other leucocyte components were insignificant. Interestingly, in Table 4.1.3.1c (p57) the percentage monocytes were increased for both 10% MOD and 20% MOD and these were significant. The decrease suggests reduced phagocytic activity possibly induced by MO in the short term use. However, with time the levels of phagocytic activity were raised with respect to increases in monocytes in the other tables (56-57pp). Monocytes are also reported to be involved with removal of LDL-C and some reactive oxygen species (Wildman, 2009). In Table 4.1.3.1b (p56), the WBC count increased significantly for 20% MOD; this means the response was necessitated by the presence of MO as a foreign substance. Generally, MO does not produce any undesired effect in the long-term when defensive activities in body are concerned.

Red blood cell count and RBC indices

The red blood cells are the principal transporters of oxygen from lungs to tissues and carbondioxide from tissue to lungs; the gases are bound to the haemoglobin (Hb)

molecules. The results (Table 4.1.3.2b; p58) show significant increases in the RBC count of all the MODs with respect to the control. This point to an increase uptake or transport of oxygen to the tissues and removal of carbondioxide as well. Comparing to the control, all three MODs had their Hb levels increased and this was significant for the last two groups. Apart from carrying oxygen, the Hb molecules also contain iron which is the component that really binds up the oxygen (Fischbach, 2003). Increased or decreased numbers of Hb is associated with certain conditions. Anaemia is the condition where Hb is reduced while haemorrhage is the condition where Hb is elevated (Wilson, 2008). Iron deficiency anaemia is common in most parts of the world (Provan, 2007) and may also result from malnutrition. MO being rich in nutrients contains proteins, vitamins and minerals which are needed for Hb production and that have contributed to the increase in Hb and RBC. Phytates though present in the leaves does not interact so much as to making minerals unavailable for use in the body. Therefore MO can be used in boosting haemoglobin concentrations and treating iron deficiency anaemia especially in malnourished persons. Haematocrit is the percentage of red blood cells in a volume of whole blood. It is an important measurement in the determination of anemia or polycythaemia (Fischbach, 2003). The results indicated that haematocrit increased in MOD groups significantly. This conforms to the increased values of RBC and Hb too. Mean corpuscular volume (MCV) measures the average RBC size thereby helping to determine the form of anaemia present such as macrocytic or microcytic (Wilson, 2008). Mean corpuscular haemoglobin (MCH) is the average weight of the hemoglobin of each RBC and this index is valuable in diagnosing severe anemia (Fischbach, 2003; Chernecky and Berger, 2008); an increase MCH is associated with macrocytic anaemia while a decrease is associated with microcytic anaemia. Surprisingly, the result showed a

significant drop in MCV and MCH of 10% MOD. This does not suggest the presence of microcytic anaemia since most clinical measurements have reference ranges and these values were in the normal range. The other indices mean corpuscular haemoglobin concentration (MCHC) and red cell size distribution width (RDW) in the other hand did not have any significant changes.

Tables 4.1.3.2b–c (58-59pp) as well did not show any significant changes among the parameters assessed. This suggests that continuous use of the MO as a nutritional supplement will not result in any RBC disorders. It suggests that short term use as seen in Table 4.1.3.2a (p58) will be useful in the treatment of RBC related conditions such as iron deficiency anaemia.

Platelet count and mean platelet volume (MPV)

Platelets are important in the formation of platelet plugs during normal haemostasis, clot retraction, and coagulation factor activation (Fischbach, 2003; Provan, 2007). The platelet count appears to decrease from 10% MOD through 40% MOD in all of the days of measurement similar to findings of Adadepo *et al.*; (2009). The most significant of the declines was for 40% MOD as shown in Table 4.1.3.3c (p60). Low platelet count (thrombocytopaenia) is associated with spontaneous bleeding. However, MPV levels were not significantly reduced to rule out bone marrow involvement in the decline platelet count. The diet with highest percentage of MO presents the least platelet count, thus, the inclusion of MO in the diet can cause decreased production or dysfunction of the platelets in the long-term and will be necessary to use it in moderation. On the other hand, this condition will be useful in preventing thrombotic events.

5.8 Conclusion

Generally, the use of MO in the diet as a nutritional supplement has shown to be relatively safe, non toxic to the liver and the kidneys. It is able to reduce blood glucose levels over a long-term of use in normal rats, which implies it should be used cautiously since it may be able to cause hypoglycaemia. Long-term use improves defensive response to pathogens; it increases the RBC part of the blood which is good when treating anaemias. As an anti-oxidant it provides high amounts of ascorbic acid and relieves oxidative stress through lowering lipid peroxidation products MDA in the body.

5.9 Recommendation

The use of *Moringa oleifera* leaf powder in the diet for acute and chronic iron deficiency anaemia should be investigated.

Work should be done in obese subjects to assess its involvement in weight management.

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APPENDIX

BASELINE RESULTS

Liver function tests (LFT)

ALT (U/L)	69.00 ± 5.416
AST (U/L)	266.30 ± 36.55
ALP (U/L)	152.70 ± 25.11
ALB (g/L)	39.37 ± 1.213
TOT. BIL (µmol/L)	9.88 ± 0.611
DIR. BIL (µmol/L)	3.98 ± 0.368
INDIR. BIL (µmol/L)	5.89 ± 0.770

n=6. Results are presented as mean ± SEM.

Renal function tests (RFT)

CREAT (µmol/L)	83.62 ± 8.245
UREA (mmol/L)	6.78 ± 0.451

n=6. Results are presented as mean ± SEM.

Lipid profile

CHOL (mmol/L)	1.87 ± 0.080
HDL-C (mmol/L)	1.18 ± 0.087
LDL-C (mmol/L)	0.38 ± 0.091
TRIG (mmol/L)	0.68 ± 0.070

n=6. Results are presented as mean ± SEM.

Full blood count (FBC)

WBC (K/µL)	7.72 ± 1.544
Neutrophils (%)	14.18 ± 1.542
Lymphocyte (%)	67.86 ± 4.965
Monocyte (%)	9.65 ± 1.850
Eosinophils (%)	4.10 ± 1.532
Basophils (%)	4.21 ± 0.989
RBC (M/µL)	7.00 ± 0.360
Haemoglobin (g/dL)	13.34 ± 0.572
Haematocrit (%)	65.24 ± 3.179
MCV (fL)	93.26 ± 0.481
MCH (pg)	19.06 ± 0.240
MCHC (g/dL)	20.42 ± 0.183
RDW (%)	15.54 ± 0.539
Platelet (K/µL)	542.8 ± 110.7
MPV (fL)	7.32 ± 0.423

n=6. Results are presented as mean ± SEM.