

**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE
DEPARTMENT OF ANATOMY**



**EVALUATION OF THE ACUTE AND SUB-CHRONIC TOXICITY OF AQUEOUS
EXTRACTS OF LEAVES OF *MORINGA STENOPETALA* ON SOME BLOOD
PARAMETERS, AND HISTOPATHOLOGY OF THYROID GLAND, PANCREAS AND
ADRENAL GLANDS IN RATS**

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**DEPARTMENT OF ANATOMY, SCHOOL OF MEDICINE, COLLEGE OF
HEALTH SCIENCES**

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THESIS

By: Lemessa Debela Feyissa

**A thesis submitted to the school of Graduate Studies of Addis Ababa University in
partial fulfillment of the requirements for the Degree of Masters of Science in
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**ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES SCHOOL OF
GRADUATE STUDIES DEPARTMENT OF ANATOMY**

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List of Abbreviations

AAU:	Addis Ababa University
EPHI:	Ethiopian Public Health institute
ATM:	African traditional medicine
GIT:	Gastro intestinal tract
Mg:	Milligram
Kg:	Kilogram
0 _c :	Degree Celsius
CNS:	Central nervous system
SNNPR:	Southern Nation Nationalities and People Region
ANOVA:	Analysis of variance
SPSS:	Statistical package for social sciences
S.E:	Standard Error
TSH:	Testosterone stimulating hormone
T ₃ :	Triiodothyronine
T ₄ :	Thyroxine
Cm:	Centimeter
Ml:	milliliter
BW:	Body weight
EDTA:	Ethylenediaminetetraacetic acid
WHO:	World health organization
Km:	Kilometer
LD50:	Median Lethal dose
OECD:	Organization of Economic Co-operation and Development
WBC:	White blood cell count

RBC:	Red blood cell count
ALP:	Alkaline phosphatase
AST:	Aspartate aminotransferase
ALT:	Alanine aminotransferase
HC:	Haemoglobin concentration
MCHC:	Mean corpuscular haemoglobin concentration
MCH:	Mean corpuscular haemoglobin
MCV:	Mean corpuscular volume
PLC:	Platelet count
CHOL:	Cholesterol
HGB:	Haemoglobin
GGT:	Gamma-glutamyl transpeptidase
GLU:	Glucose
HDL:	High density fat level
LDL:	Low density fat level
TG:	Triglyceride
NBF:	Neutral buffered formalin
H and E:	Harris haematoxylin and eosin
SEM:	Standard error of mean
L.F:	Leaf
CHS:	College of Health Sciences
TMP:	Traditional Medicinal Plant

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Identification

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Abstract

Moringa stenopetala is a plant having vital traditional medicinal value used in human as anti malarial, antihypertensive, anti diabetic and antispasmodic herbal medicine and also has high nutritional value. This study was carried out to investigate the acute and sub-chronic toxicities of aqueous extracts of leaves of *M. stenopetala* in rats. Changes on body & organ weight, behavior, and gross pathology were investigated. Biochemical, blood parameters, lipid profiles and histopathology of the thyroid gland, adrenal gland and pancreas were also studied.

In acute toxicity study, the rats were randomly divided in to four groups (3 rats/group) and the experimental group received 500, 1000, 3000 and 6000mg/kg of the extracts, while the control group received distilled water orally by gavages. In subchronic toxicity study, the experimental rats were randomly divided into three groups 6 rats/group for female and 4 rats/group for males. The experimental group in both sexes received 500 and 1500mg/kg of the extract in group I and II respectively, whereas the control group received distilled water for 90 days once daily. After 90 days of administration of the extract, the rats were anesthetized by diethyl ether then the blood samples were collected and the rats were scarified and the thyroid gland, adrenal glands and pancreas were removed, fixed, processed and stained for histological examination.

In acute toxicity study, rats treated with up to dose of 6000mg/kg body weight showed no toxic signs on behavior, gross pathology and body weight. In the sub-chronic toxicity study treated rats showed no significant changes on behavior, gross pathology, hematological (except slight decrement in WBC & platelets, $p > 0.05$) and biochemical parameters (except organ & body weight increment $p > 0.05$), thyroid function test (except increment in TSH & T3, decrement in T4) as compared with control rats. Lipid profiles also showed changes but not significant statistically. There were no significant difference in the gross and histopathology of the thyroid gland, adrenal gland and pancreas of the experimental rats as compared to control group. These results show that the aqueous extraction of *M.stenopetala* did not produce adverse effects in experimental rats after acute and sub-chronic treatment. Increment in TSH level might be due to the effect of the plant extract on hypothalamus, but change in T3 & T4 is related to its effect on the gland.

Key words: *M.stenopetala*, Toxicity, aqueous extract of leaves of *M.stenopetala*, TMP

1. Introduction

1.1. Back ground of the study

1.1.1. Definition and role of traditional medicine

Traditional medicine as defined by WHO(2000) is the sum total of the knowledge, skill and practice based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health, as well as in prevention, diagnosis, improvement or treatment of physical and mental illnesses.

Medicinal herbalism is the study of herbs and their medicinal uses (Ameh et al., 2011).This definition can be extended to include cultivation, collection, or dispensing of aromatic plants, particularly those considered to have medicinal properties. The pharmacological treatment of disease began long ago with the use of herbs.

The use of traditional medicine is a worldwide reality in that it has been used since the existence of mankind in all nations. Almost 65% of the world's populations have incorporated traditional medicine (mainly herbs) into their primary modality of health care (Fabricant and Farnsworth, 2001). Many people in developing countries, particularly those in rural areas, have more access to traditional than modern medicines and use them more frequently for primary health care. African Traditional Medicine (ATM) is the mainstay of primary health care for the majority of those in the rural areas in Africa (CAMH2,2005) and up to 80% of the population uses traditional medicine for primary health care(WHO, 2003). For instance, an estimated 90% of the people in Ethiopia use traditional medicine to meet their primary health care needs, as do 70% of people in Benin, India, and Tanzania (Patwardhan, 2005). Traditional medicine has also been described by the WHO as one of the surest means to achieve total health care coverage of the world's population (CAMH, 2004).In Ethiopia more than 800 plant species have been employed as medicinal plants

(Tesema, 2002). Most of the traditional medicinal plant preparations are used in fresh form. Oral, dermal and nasal routes are the routes of application of remedies. Squeezing, grinding, boiling, chewing, crushing and tying are the methods of remedy application (Gidey, 2011). The advantage of traditional medicine includes its accessibility and relative cheapness (Patwardhan, 2005).

Traditional Medicine may have been used by communities and found to be effective through long experience. But their method of action may not be understood in modern scientific terms, and they often consist of mixtures of different active substances (Patwardhan, 2005).

1.1.2 Harmful Effects of Traditional Medicine

The safety of several commercially available herbs has recently come into question due to reports of adverse effects and potential interactions with prescribed drugs (Popatal *et al.*, 2001). Some plants used in traditional medicines, such as taenicides, are widely known to be toxic. For instance, blindness and changes in central nervous system function have repeatedly been reported in people who consumed *Hagenia abyssinica* (Rokos, 1969). A research conducted on histopathological and toxicological effects of crude saponin extract from *Phyllanthus niruri* which is antimicrobial agent, in rabbit, by Ajibade and Famurewa, (2012), has indicated multiple foci of tubular necrosis and haemorrhages in kidney as well as marked hyperplasia of the mucosal layer of the intestine. Additionally, a research done by Ajibade *et al.* (2011) on the effects of methanol extract of the seeds of *Moringa oleifera* in rats revealed that the plant causes portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver as well as cortical congestion and interstitial haemorrhages in the kidney. Moreover, there are numerous examples of potential side effects associated with the most commonly used herbal and other types of complementary and alternative

medicine(Rokos,1969, Popatal *et al.*, 2001, Kefale, 2005, Ajibade and Famurewa,2012).

By and large, real drawback in traditional medicine stems mostly from lack of precision in dosage and the imprecise nature of diagnosis, especially of chronic and complicated conditions (Dawit and Ahadu, 1993). However, significant achievement can be made in alleviating drug shortages through standardization of dosage and quality control of the total or partially purified extracts of widely used remedies that are proven safe and effective on the basis of *in vivo* and/or *in vitro* laboratory investigations.

Moreover, the concept of side effect is more probably elaborated in the traditional medicine (Gilani and Rahman, 2005). Inappropriate use of dosage results in chronic damage to blood composition and tissue of various organs. Likewise, the prolonged administration of ethanolic extract of *Khaya senegalensis* resulted in significant reduction in the alkaline phosphate activities of the kidney, and its body weight ratio. In contrast, the same prolonged administration of the extract resulted in significant increase in the serum sodium concentration (Adebayo *et al.*, 2003). According to khleifat *et al* (2002), chronic treatment with 50 mg/kg of *Teucrium polium*, induced marked cytoplasmic vacuolation of liver and kidney tubular cells. Some herbs are found to contain toxic constituents with various potential adverse effects. Dobb and Edis(1984) reported neuropathy and coma in a patient who took a herbal laxative to control weight. WHO(2008) also notes that, inappropriate use of traditional medicines or practices can have negative or dangerous effects and that further research is needed to ascertain the efficacy and safety of the several practices and medicinal plants used by traditional medicine system.

1.2 *Moringa stenopetala*

1.2.1 Botanical Distribution of *Moringa stenopetala* (Baker f) Cufodontis

Moringa stenopetala, a smooth barked deciduous tropical plant, is a traditional medicinal and nutritional plant in Ethiopia (Mekonnen, 1999). It is widely distributed in the southwestern part of Ethiopia at an altitude range of about 1100 to 1600 meters above sea level. The major growing areas are Arbamich, Negelle and Wellayta Sodo; it extends as far as Konso and the surrounding areas.

Moringa stenopetala, belongs to the family *Moringaceae* which comprises many species, most of which are used as traditional medicine in different countries of the world and is known for its folk remedies and it is often referred to as the African Moringa Tree because it is native only to Ethiopia and northern Kenya.

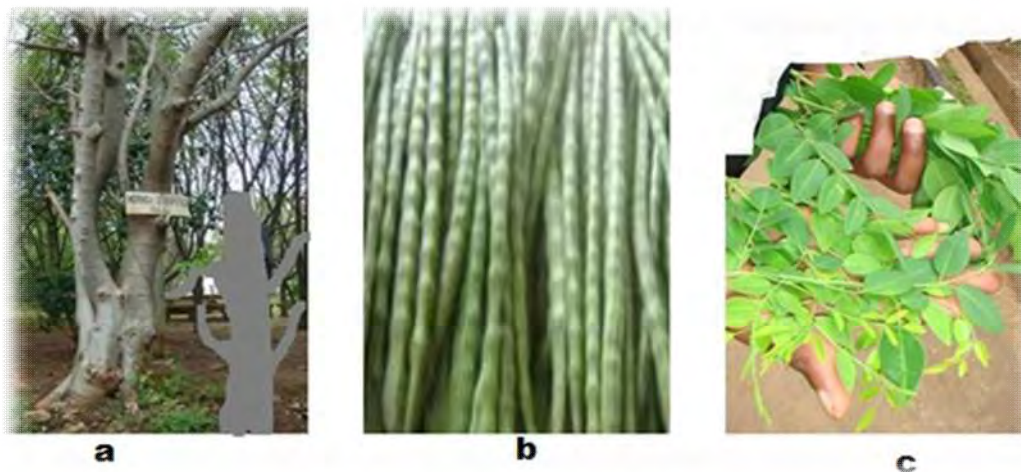


Figure 1: *Moringa stenopetala* tree (a), immature pods (b) and leaves(c) grown in Arbamich area, South Western part of Ethiopia.

1.2.2 Major Active Ingredients of *Moringa Stenopetala* Leaf

As indicated by the studies done on chemical composition of the leaves of *Moringa Stenopetala* (Baker F) Cufodontis, the leaves of this plant is reported to contain rutin, 4-(4'-O-acetyl-L-rhamnosyloxy)-benzylisothiocyanate and 4-(4'-O-acetyl-L-rhamnosyloxy)-benzaldehyde (Mekonnen and Gebrayesus, 2000) and 0-(rhamnopyranosyloxy) benzyl glucosinolate (Mekonnen and Drager, 2003). Compositional study of the leaves of *Moringa Stenopetala* (Baker F) Cufodontis by Abuye et al (2003) indicated that carbohydrates, crude fibers, vitamins (Vitamin C, β -carotene) and minerals such as Potassium, iron, Zinc, Phosphorous and Calcium are present in significant concentrations.

1.2.3 Medicinal and Nutritional use of *M.stenopetala*

M.stenopetala is reported to possess several medicinal values. *Moringa stenopetala* is one of the medicinal plants which are widely used for antidiabetic purpose in several areas. This has also been supported by a study done by Mussa et al (2008) for its hypoglycemic effect. Previous studies confirmed that the aqueous crude extract of the leaves of *Moringa stenopetala* plant and fractions isolated from these extracts have both hypoglycemic and antihyperglycemic effects (Mussa et al., 2008). Dried leaflet aqueous extract of *M. stenopetala* has shown a significant role in reduction of glucose and cholesterol level, this has also been supported by a study done by Ghebreselassie et al (2011). According to the study done by Mengistu et al (2012) on the activities of *Moringa stenopetala*, the dried leaflet aqueous extract of the plant has also a crucial role in reduction of blood pressure. The fresh root ethanol extract of *Moringa stenopetala* has antiparasitic (trypanosome brucei) activities by inhibition of parasitic growth (Mekonnen et al; 1999).

In addition to its medicinal value, *M.stenopetala* also has high nutritional use in many regions of the Ethiopia, especially Arbamich areas. The leaves of *M.stenopetala* are

taken as the best vegetable food that can be found in the areas where it is widely distributed. This is supported by the study conducted by Abuye *et al*(2003)by investigating the nutritional composition of the plant and indicate that leaves of *M. stenopetala* contains significant amount of protein, carbohydrate, crude fiber and calcium. Additionally, analytical results presented by Abuye *et al* (2003) indicate *M. stenopetala* as a source of additional nutrients like vitamin A, vitamin C and carotenoids such as α -carotene, β -cryptoxanthin, zeantin and lutein.

The flowers are used as main sources of nectar for honey bees; the seeds are used in clearing muddy water, the wet or dried root part chopped and mixed with water is also used to treat malaria as indicated on the study conducted by Mekonnen and Gessesse (1998). *M. stenopetala* leaves are cooked and eaten as vegetables, and the leaves and roots are used to treat malaria, hypertension and gastrointestinal problems (Mekonnen *et al*, 1998). The leaves of *M. stenopetala* contain high contents of essential amino acids and vitamins. It is considered as a priority crop to alleviate malnutrition and reduce poverty.

1.3. Toxicological Studies

To set up the safety and efficacy of new herbal drugs, toxicological studies are very essential experiments (Adedapo *et al.*, 2004; Kuei *et al.*, 2008; Etuk *et al.*, 2009). Toxicology is a multidisciplinary endeavor in the biological sciences that studies about the adverse effects of bioactive substance: drugs, chemicals, medical devices, vaccines, food additives, naturalproducts, pesticides and herbicides on living organisms (Kuei *et al.*, 2008).

Human data on toxicity of chemicals are noticeably more important to safety evaluation than those obtained from the exposure of experimental animals. However, controlled exposures of human to hazardous or potentially hazardous

substances are limited by ethical considerations (Thoolen *et al.*, 2010; Deora *et al.*, 2010). Where such information is not available, as in the case of all new synthetic chemicals, data must be obtained from test on experimental animals. Rats are one of the experimental animals most commonly used in clinical research, including drug toxicity tests. Rats have long been used as a model for toxicity testing of various agents. Along with mice, rats are the most common species used in studies to identify the toxic, carcinogenic, and teratologic potential of chemicals and drugs. In addition, they are frequently used to study the carcinogenic process, both tumor initiation and promotion. Rats are widely used for evaluating the safety and efficacy of new drugs. Rats are also useful in teratologic studies because of its short reproductive cycle, large litter size, and relatively few spontaneous congenital anomalies. As mammals, they are genetically related to humans, but unlike larger species they are relatively low maintenance, require much less space and food and they can breed many generations in a relatively short period of time. Another important behavior is that laboratory rats are generally docile and, if handled frequently and gently, will become tame and easily trained.

In toxicity studies, pathologic testing is an important part of safety assessment for drugs and chemicals. Pathological parameters routinely implemented during assessment of toxicity include, Clinical pathology (i.e., clinical chemistry, hematology and urinalysis); Morphologic Pathology (i.e., histopathology, gross pathology and organ weight); and live findings in animals (i.e., clinical observations of signs and symptoms of toxicity, evaluation of body weight and food consumption) (WHO, 2000; Jemal, 2005). The effect of aqueous extract of leaves of *M.stenopetala* on liver, kidneys and intestine were well studied on rats but the effect of this plant materials were not studied on endocrine glands such as thyroid gland, adrenal gland and pancreas. Therefore, effects of the plant extract on thyroid gland, adrenal gland and pancreas of rats were evaluated in this particular study.

1.4 Blood and Its role

Hematology is the science that deals with the nature, functions and diseases of blood and blood forming tissues. Blood is the fluid that circulates throughout the body via the arteries and the veins, providing vehicles through which different substances are transferred between the various organs and tissues. Blood is a sticky, opaque fluid with a metallic taste. It is a viscous fluid connective tissue, which is heavier and thicker than water. It does not connect any structure and has no role in providing mechanical support, but is taken as a special connective tissue because of its common origin from mesenchyme. It performs a number of functions dealing with substance distribution, regulation of blood levels of particular substances, protection, prevention of blood loss by activating plasma proteins and platelets for initiating clot formation when a vessel is broken. It is also involved in prevention of infection by synthesizing and utilizing antibodies, activating WBCs to defend the body against foreign invaders.

1.4.1 Composition of blood

Blood is composed of plasma that constitutes about 55% and formed elements which constitute about 45% of the total blood volume of the body (Taylor et al., 1997).

1.4.2 The formed elements of blood

The formed elements of the blood include erythrocytes or Red Blood Cells (RBC), White Blood Cells (WBC) and Platelets. Erythrocytes are the most abundant blood cells and have a simple structure, with no nucleus or common cytoplasmic organelles such as mitochondria at maturity. The absence of nucleus in mature erythrocyte is believed to increase surface area for gas exchange. The normal concentration of RBCs in the blood is approximately 3.9 – 5.5 million per μl in women and 4.1– 6 million per μl in men (Junqueira and Carneiro, 2005). RBCs are packed

with hemoglobin, the oxygen carrying protein pigment which gives red coloration to the blood and has red cell indices containing mean cell volume and mean cell hemoglobin concentration. The packed cell volume and the hemoglobin concentration may be reduced in number due to effect of administered herbal medicine indicating that the herbs may produce some anemic effect (Aniagu *et al.*, 2005).

White blood cells are capable of a crawling movement known as amoeboid movement which allows them to squeeze through pores in capillary wall to reach the tissue and site of infection and perform their function outside blood vascular system. They use the blood stream as mode of transportation to reach their destination and play an important role in body's defence mechanisms against infectious as well as introduced foreign substances, including biologically active ingredients of administered herbal medicine (Taylor *et al.*, 1997). In the latter case, dose dependent increase in number of WBC count indicates that the administered herbal medicine contains biologically active components that stimulate the immune system by increasing the number of defensive WBC (Taylor *et al.*, 1997).

There are five types of WBC which constitute different concentrations of the total WBC count. These include the neutrophils, eosinophils, basophils, monocyte and lymphocyte constituting about 40-75%, 1-6%, <1%, 2-10% and 20-40% respectively in humans (Taylor *et al.*, 1997) and responds differently towards foreign or non self substances that is introduced into the body.

Neutrophils are abundant WBCs and are commonly squeezed between the cells of the capillary wall and wander through the intercellular space where they are actively phagocytose, engulf, digest and eliminate disease causing microorganisms. According to Pass and Freeth (1993), in rats the total leukocyte count was $9(6-18) \times 10^3/\mu\text{l}$ of which neutrophils account 14-20% of the total circulating leukocytes and involved in defense against bacteria and fungi. They play an important role in host

defense mechanisms against all classes of infectious agents and they are also involved in the pathology of various inflammatory conditions by accumulations and liberation of active proteolytic enzymes (Ramprasath *et al.*, 2006).

Eosinophils are white blood cell that contains granules filled with chemicals that damage parasites and involved in allergic reactions. Eosinophils constitute small portion of WBC and increase in number during allergic conditions. They also increase in response to administered herbal medicine (Zhong Shi, 2004). In rats, eosinophils accounts for 1-4% of the total circulating leukocytes as indicated by Pass and Freeth (1993).

Basophiles are the least abundant components of WBC and produce heparin a chemical found in damaged tissues which are anticoagulants and histamine. Basophiles are type of white blood cell that involved in allergic reactions. The percentage composition of basophiles was rare in total circulating leukocytes in rats (Pass and Freeth, 1993).

Monocytes, the largest leukocyte, is found in the peripheral blood and are converted to tissue macrophages in organs, such as the spleen, lymph nodes, liver (Kupffer cells), the lung (alveolar macrophages), brain (microglial cells) and intestine (Zhu, 2002). White blood cell scavenge and destroy invading microorganisms. In rat tissues, monocytes develop into macrophages and the percentage composition of monocytes in total circulating leukocytes in rats is 1-6 % (Pass and Freeth (1993). Blood monocytes are not terminal cells; rather, they are precursor cells of the mononuclear phagocyte system. The mononuclear phagocyte system, formerly known as the reticuloendothelial system, consists of phagocytic cells whose main function is the elimination of foreign materials. Impaired function of the mononuclear phagocyte system is believed to predispose to infection. Its members include the monocyte of peripheral blood, the kupffer cell, the splenic macrophage, the various tissue macrophages (e.g. in alveoli, bone marrow, and serosal cavities), and their

precursors in the bone marrow. Macrophages, which are distributed throughout the body are present in most organs and constitute the mononuclear phagocyte system. They are long-living cells and may survive for months in the tissues. In certain regions, macrophages have special names, for instance Kupffer cells in the liver, microglial cells in the central nervous system, Langerhans cells of the skin, and osteoclasts in bone tissue.

The lymphocytes are components of WBC that respond against foreign substances that are introduced to the body. There are of two types of WBCs which are crucial to the adaptive immune response (B-cells and T-cells). In rats, the percentage composition of lymphocytes in total circulating leukocytes is 69-86 % (Pass and Freeth (1993). It was indicated that the number of lymphocyte may be reduced in blood due to the presence of some chemicals in certain medicinal plants which inhibits lymphopoiesis via interference with cytokines, which are responsible for lymphocytes differentiation from bone marrow stem cells indicating that the herbal medicine may contain biologically active ingredients that has anti-lymphatic activities (Aniagu *et al.*, 2005).

Platelets are also formed elements of blood which are small, disc shaped, anucleated fragment of cells developmentally originated from megacaryocytes in the bone marrow (Grantner and Hiatti, 2007). They are the smallest of circulating fragment of cells which form plugs on damaged areas and initiates blood clots. Pass and Freeth (1993) reported that, the normal range of platelets in a healthy adult rat blood lies between $500-1,000 \times 10^3/\mu\text{l}$.

1.4.3 Biochemical Constituents of Blood

Plasma is a pale straw-colored liquid consisting of 90% water and a variety of substances of low or high molecular weight that make up 10% of the plasma within solution and suspension (Taylor et al, 1997). It is an aqueous solution of organic and salts which are constantly exchanged with the extracellular fluid of body tissue. Plasma consists of plasma proteins, such as albumin, globulin and fibrinogen and other components, including biochemical substances.

The Biochemical compositions of blood such as blood urea, uric acid, creatinine, cholesterol, blood glucose level, alkaline phosphatase (ALP) aspartate aminotransfrase (AST), alanine aminotransferase (ALT) and electrolytes are used to evaluate the toxic effect of medicinal plants. It was indicated that some herbal medicines causes elevation of blood glucose level, blood urea nitrogen, cholesterol, AST, ALT and albumin in serum blood following the administration of herbal medicine indicates its toxic effect in animal model (Oyewole and massaquoi, 2008). From these points of views, change in these biochemical compositions of blood as well as enzymatic activities in the serum blood are used to indicate the earlier toxic effect of medicinal plants. Reports on blood composition revealed that, rat's serum is composed of similar components as human serum (Pass and Freeth (1993)).

Increment in AST and ALT concentrations in plasma indicates damage of liver and heart as was indicated by Wasan *et al.*, (2001). ALP is commonly found in the biliary tree and bile duct, a block in this system will cause an elevated ALP. Urea and Creatinine are generally markers of renal function. A high level (outside of the reference range) of urea is indicative of acute renal dysfunction whilst a high level of creatinine is indicative of chronic renal dysfunction.

1.5 Structure and Function of Thyroid Gland

The thyroid gland is the body's largest endocrine gland and has a relatively simple anatomy with its shape resembling that of a butterfly with open wing. This gland lies deep to the sternothyroid and sternohyoid muscles, located anteriorly in the neck at the level of C5-T1 vertebrae. It consists primarily of right and left lobes, anterolateral to the larynx and trachea. A relatively thin isthmus unites the lobes over the trachea, usually anterior to the second and third tracheal rings. This gland is surrounded by a thin fibrous capsule, which sends septae deeply into the gland. Dense connective tissue attaches the capsule to the cricoid cartilage and superior tracheal rings. External to the capsule is a loose sheath formed by the visceral portion of the pretracheal layer of deep cervical fascia (Moore, 2010). The gross structure of the thyroid gland in laboratory animals with two lobes connected by an isthmus is similar to that of humans. The structures of T3 and T4 are the same in laboratory animals and humans. This gland is taken as the only endocrine gland whose secretory product is stored in great quantity. This accumulation is also unusual in that it occurs in the extracellular colloid. In humans, there is sufficient hormone within the follicles to supply the organism for up to three months (Junqueira, 2005).

Developmentally, thyroid gland is originated from the endoderm of the floor of primitive pharynx. The pattern of thyroid development among rodents and humans is similar but rats are born relatively with immature thyroid.

Thyroid tissue is basically composed of 20–30 million microscopic spheres known as thyroid follicles and the follicles are lined by a simple epithelium with central cavity containing a gelatinous substance called colloid (Junqueira, 2005). The major constituent of colloid is the large glycoprotein thyroglobulin, which contains the thyroid hormones within its molecule (Guyton & Hall, 2006). As Compared to the follicles in human, which are large with abundant colloid and follicular cells that are relatively flattened (low cuboidal), rodent follicles are relatively small and often

surrounded by cuboidal epithelium (U.S. EPA, 1998). It is proposed that the morphological differences, in part, are due to differences in thyroid hormone turnover.

The function of thyroid gland is to synthesize the hormones thyroxine (T_4) and triiodothyronine (T_3), which are important for growth, for cell differentiation, and for the control of oxygen consumption and the basal metabolic rate in the body (Junqueira, 2005). Thyroid hormones are generated from tyrosine and iodine on thyroglobulin molecule (Arneson and Brickell, 2007). About 93% of the hormone produced by the thyroid follicles is thyroxine and the remaining 7% is Triiodothyronine (Guyton and Hall, 2006). About 80% of T_3 is formed in peripheral cells from enzymatic conversion of T_4 (Arneson and Brickell, 2007). In rats, the lobes of the thyroid gland are long, reddish-brown in color and connected by an isthmus. One lobe lay on either side of the larynx, half of its length extends over the trachea and the size of the thyroid gland depends on the weight of the experimental animals used for the study (Robert, 1937). Generally, thyroid gland produces thyroid hormones T_3 and T_4 , which control the rate of metabolism, and calcitonin, a hormone controlling calcium metabolism.

1.6 Structure and Function of Pancreas

The pancreas is an organ containing two distinct populations of cells, the exocrine cells that secrete enzymes into the digestive tract, and the endocrine cells that secrete hormones into the bloodstream. Therefore, pancreas can be taken as a compound gland consisting of exocrine and endocrine parts which are commonly found throughout the entire vertebrate series with various degrees of morphological, anatomical and cytological variations.

The name pancreas was derived from the Greek roots 'pan' meaning 'all' and 'creas' meaning 'flesh'. In human, it consists of an organ of 70-150 grams measuring 15-25

cm in length and It is connected to the duodenum by the ampulla of Vater, where the main pancreatic duct joins with the common bile duct (Slack,1995). In the human, the terms head, neck, body and tail are used to designate regions of the organ from proximal to distal, while in rats, the shape of the pancreas is rather less well defined (Slack,1995).This accessory digestive gland lies retroperitoneally and transversely across the posterior abdominal wall, posterior to the stomach between the duodenum on the right and the spleen on the left (Moore,2010).The root of the transverse mesocolon lies along its anterior margin. In higher vertebrates, the pancreas is a compact and lobulated gland, whereas in the lower ones, it is highly variable in nature.

Pancreas is derived from the endoderm and has embryological origin as two buds developing on the dorsal and the ventral side of the duodenum. Most of the pancreas is derived from the dorsal pancreatic bud. The larger dorsal pancreatic bud appears first and develops a slight distance cranial to the ventral bud. It grows rapidly between the layers of the dorsal mesentery. The ventral pancreatic bud develops near the entry of the bile duct into the duodenum and grows between the layers of the ventral mesentery. As the duodenum rotates to the right and becomes C-shaped, the ventral pancreatic bud is carried dorsally with the bile duct. It soon lies posterior to the dorsal pancreatic bud and later fuses with it.

Microscopically pancreas contains pancreatic islets and acinar cells constituting endocrine and exocrine parts of pancreas respectively. Concerning the functions of pancreas, its exocrine part secretes pancreatic juice from the acinar cells and endocrine parts secrete glucagon and insulin from the pancreatic islets.

1.7 Structure and Function of Adrenal Gland

The suprarenal (adrenal) glands are located between the superomedial aspects of the kidneys and the diaphragm, where they are surrounded by connective tissue containing considerable perinephric fat. The suprarenal glands are enclosed by renal fascia by which they are attached to the crura of the diaphragm (Moore, 2010). The adrenal gland is reported to be the most common endocrine organ associated with chemically induced injuries Ribelin(1984). It is especially important to understand the structure and function of the adrenal gland to correctly interpret the significance and mechanisms of drug-induced lesions. This gland has two main parts, as the outer cortex and the inner medulla; the adrenal cortex is composed of 3 distinct zones. The outer zone is the zona glomerulosa and is composed of a thin region of columnar cells arranged in an arched or arcuate pattern. This zone is also called the zona multiformis in animals because of its different patterns of arrangement of secretory cells (Thomas et al., 2001). The zona glomerulosa produces the steroid hormone aldosterone, which is responsible for increasing sodium reabsorption and stimulating potassium excretion by the kidneys and there by indirectly regulating extracellular fluid volume. Loss of this zone or the inability to secrete aldosterone may result in death due to retention of high levels of potassium with excess loss of sodium, chloride, and water (Thomas et al, 2001).

The zona fasciculata constitute the second cell layer of adrenal cortex which is located between zona glomerulosa and reticularis. It is relatively the thickest zone(>70% of the cortex) and is composed of columns of secretory cells separated by prominent capillaries(Thomas et al, 2001). The cells are polyhedral and have many intracellular lipid droplets. This zone produces glucocorticoids for controlling the uptake and release of free fatty acids and triglycerides.

The third and the inner most cell layer is zona reticularis which is composed of polyhedral cells, whose arrangement is less linear and more as round nests or

clumps of cells. The cells form anastomosing cords and often contain dark staining lipofuscin pigment. This zone is located between zona fasciculata of the cortex and the outer margin of medulla. It produces glucocorticoid and in some species small amounts of sex steroids, namely, androgens, estrogens, and progestins. This zone is more distinct in rats compared to mice. Adrenal cortex is required for life, particularly the secretion of aldosterone, but the functions of the medulla are not essential for life.

The medulla of adrenal gland is the inner most part of the gland. The cells of the adrenal medulla are derived from the neural crest in contrast to the mesodermal origin of the adrenal cortex. The secretory cells of the adrenal medulla are called chromaffin cells because of the formation of colored polymers of catecholamines after exposure to oxidizing agents, such as chromate. The cells secrete epinephrine and norepinephrine into the blood in response to acetylcholine or calcium ion. There are three types of adrenal medullary cells (Capen et al.,2001).The cells include, Epinephrine(66-75%),norepinephrine(25-35%) and small granule containing cells which is 1% in rats(Capen et al.,2001).The medullary cells produce other peptides in addition to Epinephrine and norepinephrine, like metenkephalin, substance P, neurotensin, neuropeptide Y,and chromogranin A. In addition, the adrenal medulla contains presynaptic sympathetic ganglion cells. In the rat,norepinephrine and epinephrine are stored in separate cell types that can be distinguished ultrastructurally. The hormone containing core of secretory granules in norepinephrine cell is electron dense and surrounded by a wide submembraneous space, whereas epinephrine containing granules are less dense with finely granular matrices (Thomas et al, 2001).In immature rat adrenals, granules of varying densities may be found in the same cell types. The normal adult Wistar rat adrenal gland contains an average of 29 nmol norepinephrine and 17 nmol epinephrine. Pheochromocytomas in rats contain sparse numbers of secretory granules and produce predominantly norepinephrine, while most normal chromaffin cells in rats produce epinephrine (Tischler et al, 1990).

1.8 Significance of the study

Studies on medicinal value of herbal medicines are important to enhance the healthcare of this country where an estimated 90% of people use traditional medicine to meet their primary health care needs. *M. stenopetala* has been used to treat several human diseases traditionally in some parts of Southern Nation, Nationalities and People Region (SNNPR) of Ethiopia. Although there are some studies carried out on medicinal and nutritional values of *M. stenopetala*, the possible effect of the plant on pancreas, thyroid gland and adrenal gland is not studied. Therefore, the present study evaluated the acute and subchronic effects of crude extract of leaves of *Moringa stenopetala* on thyroid gland, pancreas and adrenal gland on top of the blood parameters. This study could also help impart scientific justification for the effect of aqueous extract of leaves of *M.stenopetala* on thyroid gland, pancreas and adrenal gland as well as on hematological parameters. It can contribute towards promotion of quality of the country's traditional use of herbal medicine. *M. stenopetala* is drought resistant, nutritionally useful plant apart from its traditional medicinal values for a number of ailments. Farther enriching of its medicinal assessment is also the aim of the study.

2. Objectives of the study

This study was conducted with the following objectives:

2.1. General objective

- To evaluate the toxicological effect of subchronic treatment with *Moringa stenopetala* leaf extract on some blood parameters, histopathology of thyroid gland, pancreas and adrenal gland in laboratory-bred rats.

2.2. Specific objectives

- To investigate if there is any signs of toxicity in the experimental animals and to determine the oral LD50
- To observe the effect of the extract on general body weight and weight of thyroid gland, pancreas and adrenal gland in rats.
- To determine the goiterogenic effect of *Moringa stenopetala* leaf extract
- To evaluate the effects of aqueous extract of *M. stenopetala* on blood parameters, and histopathology of thyroid gland, pancreas and adrenal gland in experimental rat.
- To determine TSH, T3 and T4 as well as cholesterol, triglyceride, low density and high density fats levels following chronic toxicity study.

3. Materials and Methods

3.1 Study Design:

Laboratory based experiment

3.2 Study Setting:

AAU, School of Medicine, Histology and Pathology core laboratories College of Health Sciences (CHS) and Ethiopian Public Health Institute (EPHI), directorate of traditional and modern medicine research.

3.3 Study area

The study was conducted in Addis Ababa University, college of health science school of medicine, department of Anatomy (histology Laboratory) and EPHI.

3.4 Preparation of Plant Materials and Extraction

Leaves of *Moringa Stenopetala* were used to conduct this experiment. Fresh leaves of *M. stenopetala* were collected from Arbamich area, Southwest Ethiopia, 502 km away from Addis Ababa, in March 2014. The plants were identified by botanist and the plant samples were given to the National Herbarium of Science Faculty, Addis Ababa University for collection and preservation.

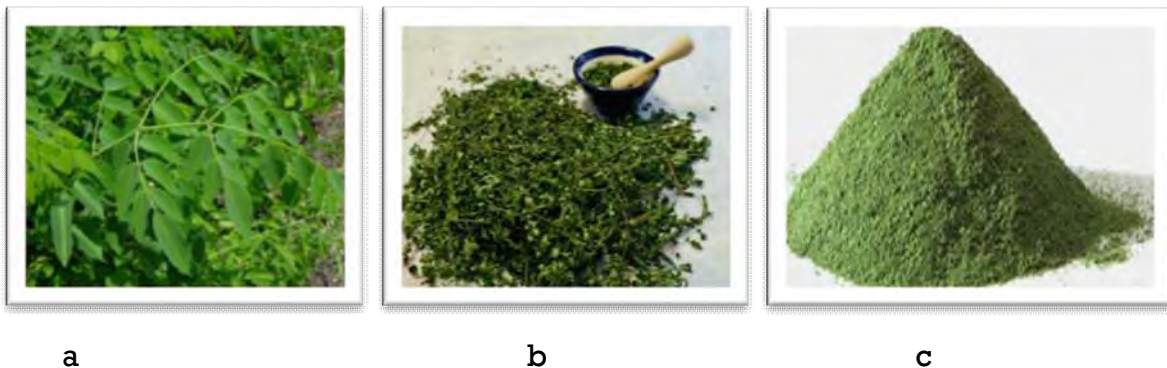


Figure 2: *M. stenopetala* fresh leaves (a), Dried leaves (b) and Powdered leaves(c)

3.5 Plant Material Extraction

The collected plant specimens were confirmed at the Herbarium of medicinal plants of the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. The leaves were cleaned from any extraneous materials, dried at room temperature and ground to powder. The powder was stored in plastic bag till extracted with water to obtain aqueous crude extract. The powder was mixed with water in Erlenmeyer flasks and placed in an orbital shaker at room temperature for 4 hours. The mixture was then filtered with cotton and Whatman filter paper (18.0 cm size). The filtrates were freeze-dried in a lyophilizer to yield a crude extract. From 500 g dry leaf, which was dissolved in a total of 5000ml distilled water(1:10), 100.167 g (20.03%) of crude extract was obtained. The crude extract was kept in a desicators at -20° C until used.

3.6 Experimental Animals

Three months-old laboratory-bred albino rats were used for this experiment, which were kept under uniform laboratory conditions. The experiments were conducted on a total of 42 rats including controls for sub-chronic toxicity study (18 female and 12 males) and 12 female rats for acute toxicity study. The animals were fed on pellets and water *ad libitum*. Light was set for 12 hours on and 12 hours off and the temperature was maintained at 25-30°C.

3.7 Grouping of Experimental Animals

The rats were randomly divided into four groups (one group of controls and three experimental) in acute toxicity study while in subchronic toxicity study the rats were divided in to three groups (one group of control and two experimental). In acute toxicity study, the rats were divided into four groups each group with three rats. Rats in control group (IV) received distilled water orally and the treated rats in group I, II and III received the plant extract at required dose of 1000, 3000 & 6000 mg/kg body

weight, respectively as a single dose using intragastric tube. In subchronic toxicity study, rats in the control group (group III) were orally given 0.5 ml of distilled water while group I and II were given the aqueous leaf extract of *M.stenopetala* using intragastric tube to achieve the required doses of 500 and 1500 mg/kg body weight, respectively once a day at 24 hours intervals for 90 days for subchronic toxicity study and then sacrificed. At the end of the study, blood samples were collected from each rat and examined for hematological and biochemical parameters. Thyroid gland, pancreas and adrenal gland were removed, preserved in 10% neutral formalin, stained and examined for histopathological profiles. The effects of treatment with aqueous extract of *M. stenopetala* on hematological, biochemical and histopathological features were compared with control group following standard procedures.

3.8 Ethical Consideration

All experiments were conducted following the approval by the responsible bodies of the school of medicine, AAU and EPHI in line with the highest standard for the humane and compassionate use of animals in biomedical research. Animals used in this study were not subjected to any unnecessary painful and terrifying situations (OECD, 2008). To keep the pain and suffering minimal during any surgical intervention all animals were given diethyl ether anesthetic and analgesic; and the procedure was carried out by a well-trained person. The animals were protected from pathogens; and placed in appropriate environment. The numbers of animals were reduced to the minimum consistent with achieving the scientific objectives of the study.

3.9. Cage Side Observations

In each cage, animals were carefully observed individually before and after dosing periodically for any changes in skin and fur, eyes, respiratory effects, autonomic effects such as salivation, diarrhea, urination and CNS effects such as tremors.

3.10 Acute Toxicity Study

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 425 (OECD 2001). The rats were starved of feed i.e., fasted overnight prior to extract administration but allowed free access to drinking water (OECD 2001). Following the period of fasting, the animals were weighed and the test substances were administered. After the extract has been administered, food was withheld for further 3-4 hours. The fasted body weight of each animal was determined and the doses were calculated according to the body weight. After the determination of the LD50 for the extract, the doses for oral administrations of each animal were calculated relative to their average body weight and administered orally to each rats in a group. As shown in Table 1, single oral doses, 1000, 3000 and 6000 mg (mg/kg body weight) of the extract were administered for treatment groups, while the control group was given the vehicle, 0.5ml/kg distilled water (0.5ml/ rats). The animals were kept under observation for 14 days post-treatment in order to check for any behavioral or clinical manifestations of oral acute toxicity test.

At the end of the experimental period, the rats were scarified and post-mortem gross observation was performed on their internal organs, including thyroid gland, pancreas and adrenal gland.

Table 1: Doses for acute treatment of the aqueous extracts of *M. stenopetala* leaf

Groups	Dose mg/kg/bwt	Number of rats per group
I	1000	3
II	3000	3
III	6000	3
IV	0.5ml dist.water/kg	3

3.11 Sub-chronic Toxicity Study

The experiment was conducted to assess the effect of aqueous extract of leaves of *M. stenopetala* on general body weight, weight of thyroid gland, pancreas and adrenal gland in addition to histopathology and hematological changes in each group. The actual dose of the plant extracts corresponding to each experimental group were calculated on the basis of body weight. After the administration of the extract, the animals in all groups were regularly and carefully monitored for any behavioral changes or any clinical symptoms of toxicity, such as vomiting, diarrhea, etc. and death.

Table 2: Doses for subchronic treatment of the aqueous extracts of *M. stenopetala* leaf (female rats)

Groups	Dose mg/kg/bw	Number of rats per group
I	500	6
II	1500	6
III	0.5ml dist.water/kg	6

Table 3: Doses for subchronic treatment of the aqueous extracts of *M. stenopetala* leaf (male rats)

Groups	Dose mg/kg/bw	Number of rats per group
I	500	4
II	1500	4
III	0.5ml dist.water/kg	4

3.11.1 Body Weight Measurement

Individual weight of the experimental and control groups of animals was recorded as an initial weight before the test substance was administered and then weighed weekly. At the end of the test, surviving animals were weighed and humanely sacrificed under anesthesia by diethyl ether. The pancreas, thyroid and adrenal gland were carefully removed and weighed on electronic balance (PA4102C, China).

The relative organ weight was calculated using the weight of animal and weight of pancreas, thyroid gland and adrenal gland using the formula indicated by Aniagu, *et.al.*, (2005), so that comparison was made between the control and the experimental groups.

3.11.2 Gross Pathology

All gross pathological changes were recorded for individual animals in each group. The change in this case is important to observe any physical changes that may exist between each group of animals. The parameters scrutinized during gross pathological study of the target organs were change in color & texture of the organs, any necrosis & spot on each organs and change in size of the target organs like any enlargement.

3.12. Blood Collection for Haematological and Biochemical Analyses

At the end of the experimental period, animals belonging to each group were weighed on a digital electronic balance and were anesthetized under diethyl ether anesthesia by placing each animal in an air tight dissector jar with cotton soaked in ether. Blood samples of 2-5ml were withdrawn quickly through cardiac puncture using a 5ml heparinized syringes and collected in a test tube with anti-coagulant EDTA and without anti-coagulant. Then, hematological parameters including

haemoglobin concentration(HC), red blood cell count(RBC), white blood cell count(WBC), mean corpuscular haemoglobin concentration(MCHC), mean corpuscular haemoglobin(MCH), mean corpuscular volume(MCV) and lymphocyte, platelet count(PLC) were analyzed using Auto-haematology analyzer system (Sysmex XT-1800i, Japan) from the blood in a test tube with EDTA . The blood samples within a test tube containing no EDTA were allowed to clot, then centrifuged at 3000 revolution per minute for about 20 minutes to obtain the serum so that biochemical parameters which included, urea, creatinine, AST, ALP, ALT,CHOL,GGT,GLU,HDL,LDL and TG were analyzed by using clinical chemistry analyzer(Bioteqnique, Italy), from the serum blood, there by comparison were made between the control and experimental groups for subchronic toxicity study. Effects of aqueous extract of *Moringa stenopetala* leaves on TSH,T3 and T4 levels among treated groups against the control rats were also analyzed using clinical chemistry analyzer(cobas e 411 analyzer,Germany).

3.13 Animal Dissection and Tissue Collection

At the end of the experimental period animals belonging to each group were sacrificed after weighing on a digital electronic balance under diethyl ether anesthesia. Immediately after anesthesia, the abdominal cavity was opened and the pancreas and adrenal glands were removed. Simultaneously the anterior aspect of neck of the rat was opened carefully and thyroid gland was removed. The removed organs were cleared of any surrounding tissues and put on clean paper and weighed quickly on electronic balance and the organ weight was then recorded.

Randomly the longitudinal section of the thyroid and adrenal glands and transverse section of the whole left lobe of the pancreas was removed and fixed for histological processing. The left lobe of the pancreas was selected because this lobe represents a major part of pancreatic tissue where the islets of langerhans are located as indicated by Urban & Fischer (2003).

3.14 Histological Processing

The thyroid gland, adrenal gland and pancreas sections taken randomly for tissue processing were fixed in 10% neutral buffered formalin (NBF) overnight at room temperature. After fixation, the tissue sections were washed with water to remove excess fixatives for about six hours and dehydrated with increased concentration of alcohol of 70% for two hours, 90% for two hours, absolute alcohol-I, II for one and half hours, and III overnight. The dehydrated tissues were cleared in two changes of xylene (I and II) for one and half hours and two and half hours, respectively. The tissues were then infiltrated with three changes of paraffin wax (I, II and III) for one and half hours, two and half hours and overnight, respectively. Finally the tissues were embedded in paraffin wax in square metal plates forming tissue blocks, where by each tissue block was labelled and stored at room temperature till sectioned.

The tissue blocks were sectioned in ribbons at a thickness of 5 μm with Leica microtome (Leica RM 2125RT Nussloch GmbH, Germany). The ribbons of the section were collected at every 5th sections and put onto the surface of a warm water bath of temperature of 40⁰c. The floating ribbons over the surface of warm water were mounted onto pre-cleaned slides spread with egg albumin. The slides containing paraffin wax were arranged within the slide holder and placed in an oven with temperature of 40⁰c for about 20 minutes so as to fix the tissue to the slides and allowed to cool at room temperature for 30 minutes and stained regressively with routine Harris haematoxylin for 6 minutes and eosin for 17-20 second (H and E).

For routine H and E staining, two series of coupling jars were prepared. One for paraffin removal and hydration and the other was for dehydration and clearing. So sections were placed in xylene- I for 5 minutes and xylene II for 2 minutes again to remove the paraffin from tissue and hydrated with decreasing concentrations of absolute I, II and 95% alcohol for two minutes each, 70% of alcohol for three minutes and 50% alcohol for five minutes. The tissue sections were washed with tap water for five minutes and stained regressively with Harris haematoxylin for 6 minutes, then

washed under running tap water for five minutes again. The slides were immersed in acidic alcohol for differentiation and controlling over stained haematoxylin for 1 second and then put in bluing solution (Sodium bicarbonate) until they became blue. After bluing, the slides were counter stained with eosin for 17-20 seconds and then washed in tap water for two minutes. The sections were dehydrated with increasing alcohol concentration of 50%, 70%, 95%, absolute I and II for two minutes each. The dehydrated sections were cleared with xylene I and II for three minutes each and permanently mounted on microscopic slides using DPX and cover slips and then observed by light microscope for the investigations of any histological change, thereby the histology of the treated groups were compared with histology of the control group.

3.14.1 Preparation of Tissue for Microscope

The slides of Thyroid gland, Adrenal gland and Pancreas were examined under light microscope fitted with different objectives like (10x, 25x, 40x and 100x) for histological investigations of photomicrographs of selected samples were taken using digital camera at magnification of x40 using MC 80 DX Auto microscope camera (Zeiss, Germany) to observe changes on histology of thyroid gland, adrenal gland and pancreas. Based on observations made, comparisons were made between control and experimental groups.

3.15 Communication of the result

Results of the study would be compiled in the form of thesis, and communicated to all concerned bodies and institutions including the Department of Anatomy (AAU), Traditional and Modern Medicine Research Directorate (EPHI) and to the advisors. The findings would be published in peer-reviewed reputable journal for the public.

3.16 Statistical Analysis

The results were analyzed statistically using one way analysis of variance (ANOVA) using the SPSS version 15 computer software to identify the possible difference between body weight , relative organ weight , haematological and biochemical values followed by student's t-test to compare the difference between control and treated groups. All data were expressed as mean $\pm$ standard error of the mean (SEM). Differences at $p < 0.05$ were considered to be significant (Khleifat *et al.*, 2002). The difference between groups with respect to variable under investigation were considered to be significant at significance level (α) = 5% or $p \leq 0.05$.

4. Results

4.1 Yield of the Crude Aqueous Extract of *M. stenopetala* Leaves

Following maceration, sedimentation, filtration and concentration processes of 500g powder of *M.stenopetala* leaves using water as solvent, 100.167 g of crude extract was obtained as shown in Table 4. The percentage yield of the extract was about 20.03%.

Table 4: Total powder, yield and percentage of yield obtained from aqueous extraction of leaves of *M.stenopetala*

Parts of the plant	Weight of powdered plant(g)	Weight of crude extract(g)	% yield of crude extract (%)
Leaves	500	100.167	20.03

4.2 Results of acute toxicity study

4.2.1 Determination of LD50 of Aqueous Extract of *Moringa Stenopetala*

The acute toxicity study was a single dose toxicity test carried out to determine the approximate lethal dose of the aqueous extract of *M. stenopetala* leaves in albino rats. Oral administration of a single dose of the extract at doses of 1000, 3000 and 6000 mg/kg body weight did not produce any mortality as compared to the control rats during the 14 days of observation period.

4.2.2 Effects on physical signs of toxicity

In acute toxicity studies, no rats showed signs and symptoms of toxicity at all doses (1000, 3000 and 6000mg/kg/body weight) as compared with the control rats. No death of rats was also observed at all dose levels. The parameters used to observe the physical signs of toxicity were pilo-erection, salivation, appetite, locomotion, food & water intake, lacrimation, diarrhea, urination, depression, breathing and excitement. No effects were observed in the given parameters in all groups at all dose levels as compared to control groups within 14 days observation period of acute treatment.

4.2.3 Effects of the extract on general body weight

The acute effect of aqueous extracts of leaves of *M.stenopetala* on body weight of rats during a single dose administration is shown in Table 5. There was a gradual increase in body weight of rats in both treated and control groups. However, there was no difference in body weight gain between the treated and the control rats. The weight of rats treated with the extract at dose of 3000mg/kg body weight were increased by 1.5%, while those which were treated at 6000mg/kg body weight of the extract showed increment by 5.3% at the end of 14 days observation. Changes in weight gain in rats treated at dose of 1000mg/kg body weight were very low. Though the increment in body weight was statistically insignificant, relatively the rats treated with higher dose of the extract (6000mg/kg body weight) showed higher weight gain as compared to the control rats at the end of the 14th day follow up.

Table 5: Body weights of rats after treatment with *M. stenopetala* extract for acute toxicity study

Group	Treatment (mg/kg/bw)	Initial body weight (g)	Final body weight (g) at 14 day
I	1000	179.69±6.96	201.8m ±5.04
II	3000	186.33±7.126	210.67±5.78
III	6000	189.0±2.309	223.0±6.43
IV	Control(dist.water)	179.67±10.17	200±5.044

4.3 Sub-chronic toxicity study

4.3.1 Effects of the extract on gross pathology, behavior, organ and body weight

During long-term administration period, both treated and control groups did not show any severe physical changes in their behavior. No physical signs of toxicity were also recorded. In addition, no sign of toxicities were observed on target organs (thyroid gland, adrenal gland and pancreas). The parameters used for gross pathological observation in subchronic toxicity study were color, texture, necrosis, spot and size for both treated and control groups. As a result, no negative signs of toxicities were observed.

The subchronic effects of aqueous extracts of the plant on general body and organ weight of male rats are summarized in Table 6. The result showed gradual increment in body weight in both treated and control groups. The mean body weight gain in male rats treated with the extract were, respectively increased by 3.6 and 8.2% at doses of 500 and 1500mg/kg body weight at the end of 90 days follow up as compared to control rats. However, these changes observed in 500 and 1500 mg/kg body weight were statistically insignificant ($p>0.05$) as compared with the control rats ($P=0.008$).

All the way through 90 days observation period, no death was recorded in rats treated at dose levels of 500 & 1500mg/kg body weight. Gross examination of thyroid gland, adrenal gland and pancreas of treated rats showed no signs of toxicity. The mean absolute weights of target organs (thyroid gland, adrenal gland and pancreas) showed increment as compared to control rats. However the increments in organs weight were statistically insignificant.

Table 6: Body and organ weights of male rats after treatment with *M. stenopetala* extract for subchronic toxicity study

Group	Treatment (mg/kg/bw)	Initial body weight (g)	Final body weight (g)	Relative organ weight(g/100g bw)		
				Thyroid G.	Pancreas	Adrenal G
I	500	347.0±19.17	347.5±12.366	0.81±0.037	1.77±0.085	0.074±0.0048
II	1500	362.0±13.204	365.0±46.49	0.827±0.037	1.97±0.033	0.073±0.0058
III	Control (dist. water)	327.75±48.668	335.0±6.570	0.75±0.23	1.52±0.16	0.07±0.0048

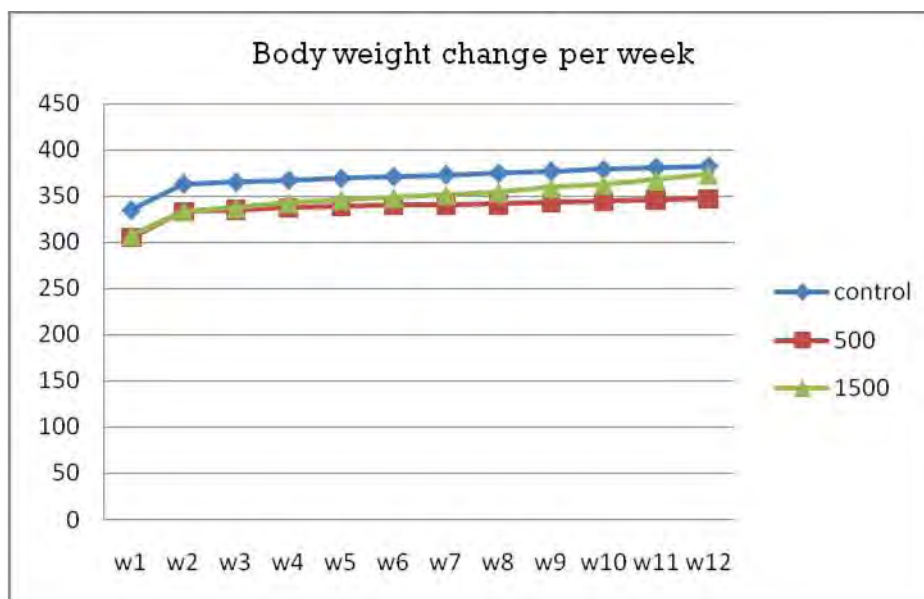


Figure 3: Comparison of body weight among aqueous extract of *M. stenopetala* leave treated groups at medium and high dose respectively for 500 and 1500mg/kg body weight against control male rats during 12 weeks of observation period.

The subchronic effect of aqueous extracts of the plant on general body and organ weight of female rats were illustrated in Table 7. The result showed gradual increment in body weight in both treated and control groups. The mean body weight increment in female rats treated with dose level of 500 mg/kg was by 2.7% and those treated at 1500mg/kg was increased by 1.9% as compared to the control rats. The change in 500 and 1500 mg/kg body weight was statistically insignificant ($p>0.05$) as compared with the control rats.

Regarding target organs of female rats, gross examination of thyroid gland, adrenal gland and pancreas of treated rats showed no signs of toxicity as compared to control group. The mean absolute weights of thyroid gland, adrenal gland and pancreas showed increment as compared to control rats, but the increment in organs weight were still insignificant statistically.

Table 7: Body and organ weights of female rats after treatment with *M. stenopetala* extract for subchronic toxicity study

Group	Treatment (mg/kg/bw)	Initial body weight (g)	Final body weight (g)	Relative organ weight(g/100g bw)		
				Thyroid G.	Pancreas	Adrenal G
I	500	251.33±6.29	256.67±3.612	0.73±0.053	1.492±0.194	0.072±0.0039
II	1500	253.83±3.156	254.50±4.0063	0.70±0.021	1.41±0.163	0.07±0.0026
III	Control(dist. water)	250.5±8.377	249.67±10.99	0.68±0.061	1.01±0.041	0.068±0.004

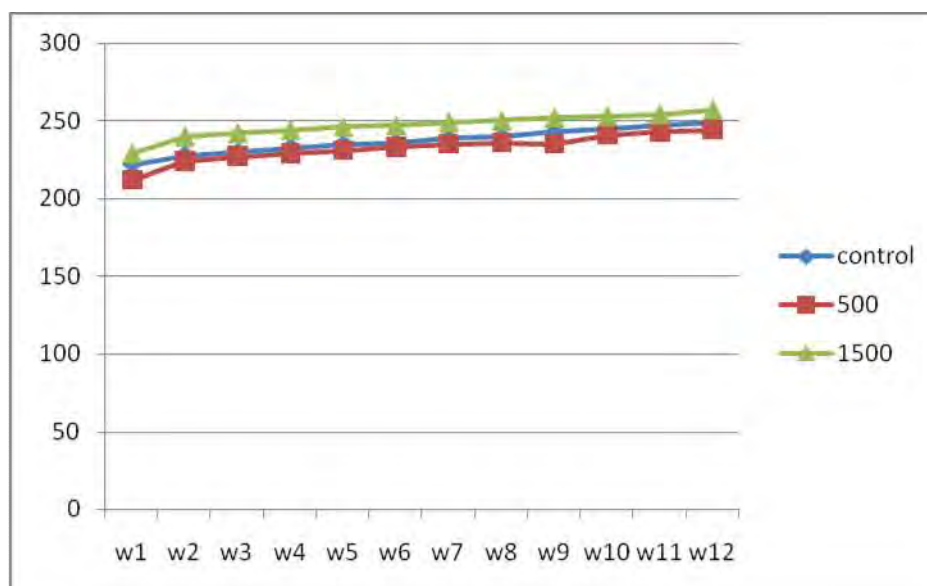


Figure 4: Comparison of body weight among aqueous extract of *M. stenopetala* leave treated groups at medium and high dose respectively for 500 and 1500mg/kg body weight against control female rats during 12 weeks of observation period.

4.3.2 Effects of the extract on Hematological parameters

Studies on the subchronic effects of aqueous extract of *M. stenopetala* on hematological parameters of blood showed that, there were insignificant difference in hematological composition of blood between control rats and those rats treated with the extract at both doses ($P>0.05$). Although there were decrease in the total WBC count by 23.98 and 1.73% in the rats treated with the extract, respectively at 500 & 1500mg/kg body weight, these were found to be statistically insignificant. Similarly, there were insignificant decrements in the platelets count by 16.6 and 17.6% in the rats treated with the extract respectively, at 500 & 1500mg/kg body weight. The change observed in MCH, MCHC, HCT, HGB and MCV were very low as shown in Table 8 and all were found to be statistically insignificant.

Table 8: Combined hematological analysis of both male and female rats after treatment with *M. stenopetala* extract

Hematological parameters	Control(dist. water)	Treatment groups (mg/kg bw)	
		500	1500
WBC x 10³/ µl	8.84±1.101	7.13±1.27	8.69±1.21
RBC x 10⁶/ µl	10.13±0.11	10.15±0.228	10.21±0.26
Platelets x 10³/ µl	1050.25±17.18	876 ±27.98	865.67±117.65
HCT (%)	52.025±.449	53.78±0.77	52.87±1.25
HGB (g/dl)	18.13±0.125	18.73±0.30	18.2±0.35
MCV (fl)	51.30±0.48	53.03±0.52	51.83±0.69
MCH (pg)	17.90±0.082	18.48±0.118	17.83±0.32
MCHC (g/dl)	34.93±0.18	34.83±0.166	34.43±0.22

4.3.3 Effects of the extract on thyroid function test

Serum T3, T4 and TSH levels were determined on day 90, and the results are summarized in Table 9 for male rats. The mean value of T4 was slightly decreased in male rats treated at dose of 1500mg/kg body weight although the change was found to be statistically insignificant ($p>0.05$). On the other hand, T3 and TSH levels showed slight increment in rats treated at dose of 1500 mg/kg of body weight though the difference is within the reference range as compared to the control group. The percentage increment of T3 were 17.1 and 9.4% respectively, at 500 and 1500mg/kg body weight. At the same time there were insignificant increment in the TSH level by 37.5 and 16.67% in the rats treated with the extract respectively, at 500 and 1500mg/kg body weight while T4 level was decreased by 1.44 and 7.5% respectively, at 500 and 1500mg/kg body weight as compared with control male rats.

Table: 9. Analysis of TSH, T3 and T4 levels in male rats after treatment with *M. stenopetala* aqueous extract

Groups	T ₃	T ₄	TSH
I	1.75±0.102	6.24±0.129	0.008±0.00082
II	1.60±0.057	5.89±0.77	0.006±0.0004
II(control)	1.45±0.08	6.33±0.15	0.005±0.00

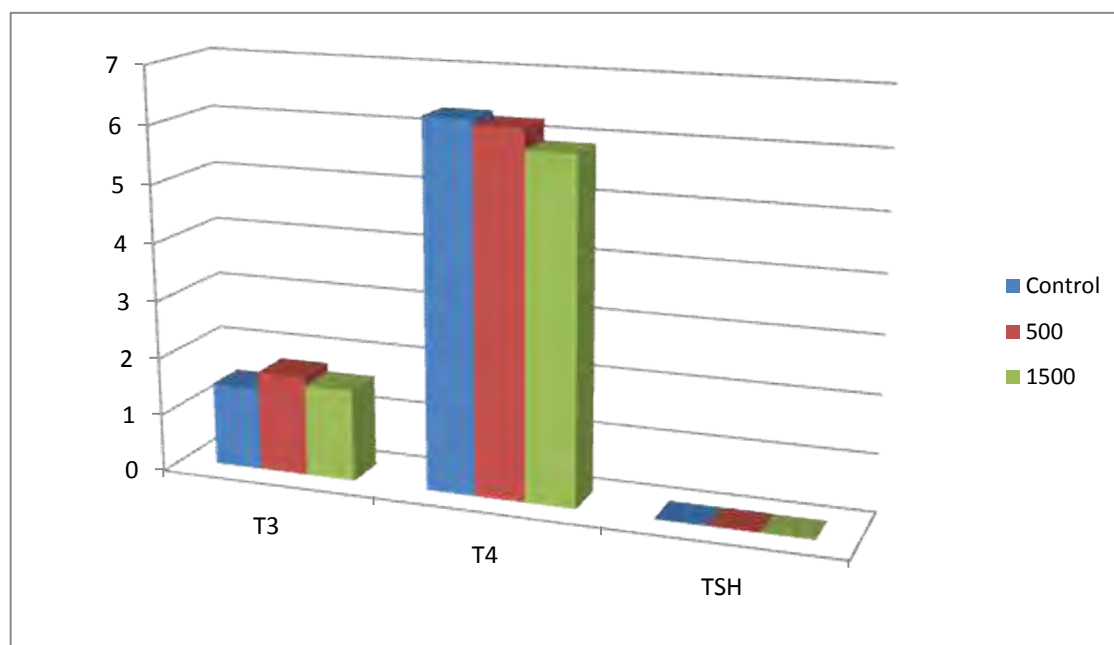


Figure 5: Bar chart showing Serum T3, T4, and TSH levels in control & treated male rats

In this study TSH, T4 and T3 levels were also tested for female rats in association to goiter and found to be statistically insignificant ($P>0.05$). Although there were decrease in the level of T3 by 0.63% and increase by 11.6% respectively, at 500 and 1500mg/kg body weight, there were found to be statistically insignificant. Though the difference were statistically insignificant, TSH showed increment by 25.4 and 28.6% in the rats treated with the extract respectively, at 500 and 1500mg/kg body weight and finally, T4 level were decreased by 40.13 and 11.4% respectively, at 500 and 1500mg/kg body weight in female rats Table 10.

Table: 10. Analysis of TSH, T3 and T4 levels in female rats after treatment with *M. stenopetala* aqueous extract

Groups	T ₃	T ₄	TSH
I	1.59±0.047	4.66±0.34	0.0067±0.00085
II	1.81±0.056	5.86±0.28	0.0073±0.0018
III(control)	1.60±0.069	6.53±0.48	0.005±0.00

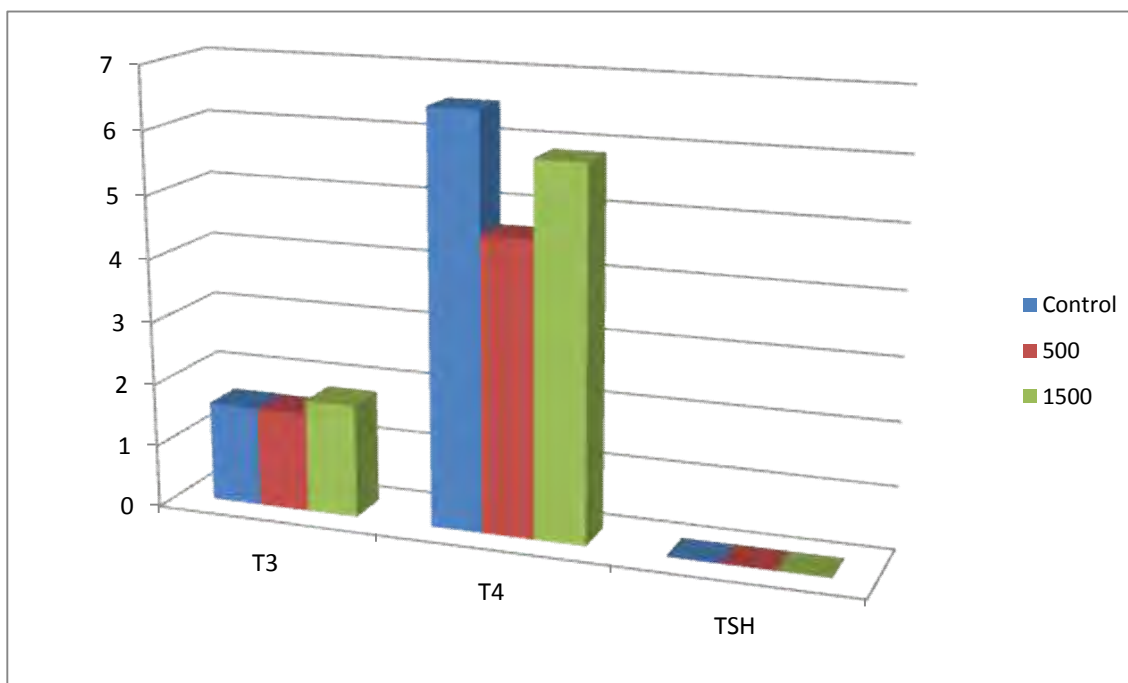


Figure 6: Bar chart indicating Serum T3, T4, and TSH levels in control & treated female rats

4.3.4 Effects of the extract on Triglyceride, Cholesterol, HDL & LDL levels

Analysis of the levels of triglyceride, cholesterol, HDL and LDL among the *M.stenopetala* treated rats, at both doses, and control rats showed no statistical difference. However, there were trends of increment or decrement in the values as compared with those of the controls as described below.

The levels of serum triglycerides and cholesterols of treated and control male rats were determined in this study. Male rats treated with aqueous extract of leaves of

Moringa stenopetala, showed insignificant increase in serum levels of total cholesterol, HDL and LDL at dose level of 1500mg/kg body weight with slight decrease in cholesterol & HDL at dose level of 500mg/kg body weight (Table 11). The serum cholesterol level were decreased by 11.50, while increased by 4.95% in rats treated with the extract respectively, at 500 and 1500mg/kg body weight. Similarly, there were decrement in serum HDL level by 7.43 but, increased by 5.6% in male rats treated with the extract respectively, at doses of 500 and 1500mg/kg body weight. Finally, TG was decreased by 39.07 and 29.2% in male rats treated with the extract respectively, at both doses(500 and 1500mg/kg). The changes in the level of serum cholesterol, HDL, LDL and TG were found to be statistically insignificant ($p>0.05$) at both doses.

Table: 11. Lipid profile for 90 days administration of aqueous extract of *Moringa stenopetala* leaves on cholesterol, HDL, LDL & TG among treated groups against the control values in male rats.

Groups	cholestol	HDL	LDL	Triglyceride
I	54.275±.933	54.85±.718	9.8275±.464	58.03±3.43
II	63.65±3.895	62.45±4.723	12.2875±2.679	62.45±9.59
III(control)	60.50±.949	58.925±1.123	9.185±.741	80.7±17.05

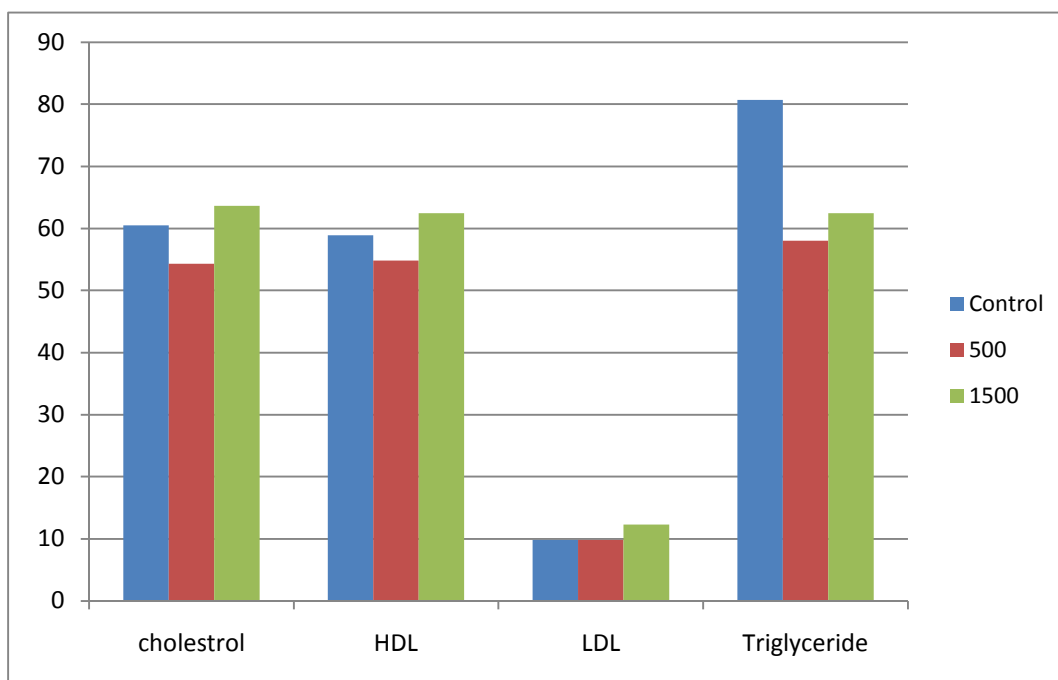


Figure 7: Bar chart representing Serum Cholesterol, HDL, LDL & Triglyceride levels in control & treated male rats

Female rats treated with aqueous extract of leaves of *Moringa stenopetala*, showed an increase in serum levels of triglyceride, total cholesterol, and LDL at dose level of 500 & 1500mg/kg body weight with decrease in HDL at dose level of 500 & 1500mg/kg body weight as compared to control rats as summarized in table 12. But the changes did not show significant variation statistically ($p>0.05$) as compared to control rats. Total cholesterol levels were increased by 8.07 and 24.12% in female rats treated with the extract respectively, at 500 and 1500mg/kg body weight.

Correspondingly, there was increase in LDL by 27.7 and 31.54% respectively, at 500 and 1500mg/kg body weight. Consistently, TG were increased by 17.85 and 6.6% respectively, at 500 and 1500mg/kg body weight of the extract treated groups as compared with the control female rats. But, serum HDL level were decreased by 232.33 and 160.16% respectively, at 500 & 1500mg/kg body weight and found to be statistically significant ($P<0.05$).

Table: 12. Lipid profile for 90 days administration of aqueous extract of *Moringa stenopetala* leaves on cholesterol, HDL LDL among treated groups against the control values in female rats.

Groups	Cholestol	HDL	LDL	Triglyceride
I	87.075±22.165	79.325±14.887*	13.93±5.036	79.85±6.32
II	105.50±11.413	101.33±6.121*	14.712±2.350	70.22±7.64
III(control)	80.05±9.46	263.617±187.352	10.072±2.118	65.6±2.74

*P<0.05 at doses of 500 and 1500mg/kg body weight for only HDL

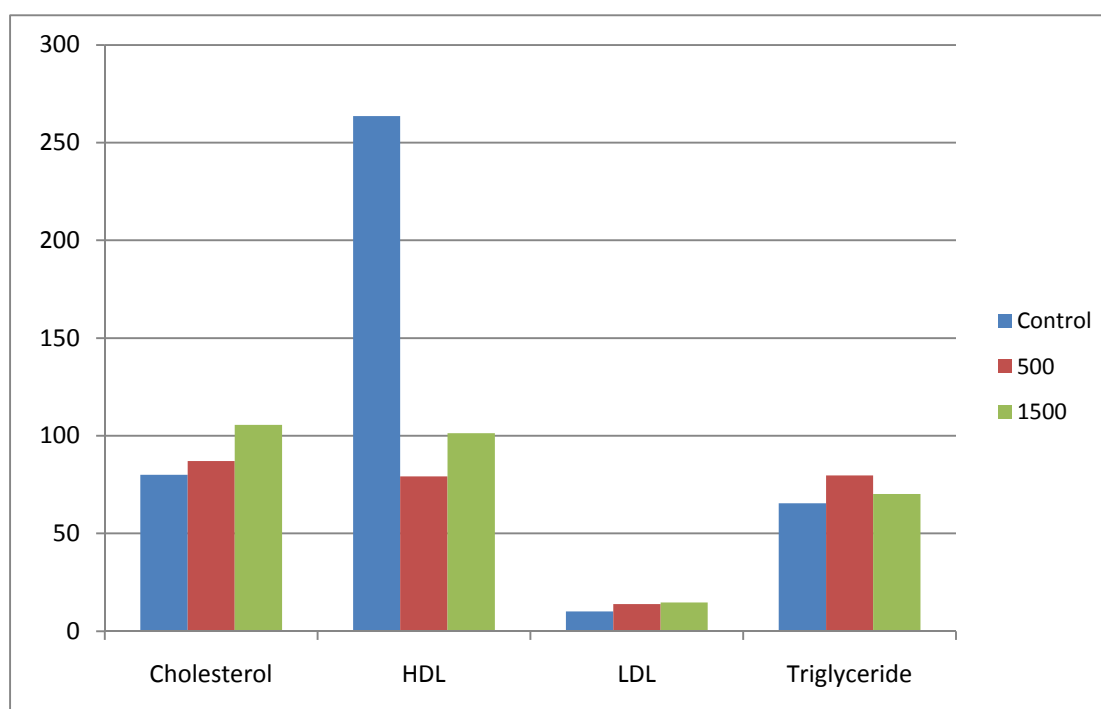


Figure 8: Bar chart representing Serum Cholesterol, HDL, LDL & Triglyceride levels in control & treated female rats

4.3.5 Light Microscopic Examination

4.3.5.1 Effects of the extract on histology of the thyroid gland

Histopathological studies of the thyroid gland section in the control and treated groups of male rats are shown in Figure 5. Histopathological analysis of thyroid glands from the experimental group of animals (Figure 5 B & C) showed normal architecture of thyroid colloids, thyroid follicles surrounded by follicular cells with normal appearance as compared to control rats (Figure 5A). The parafollicular cells also exist having their normal morphological appearance. The result indicates normal cells with no necrotic follicles, inflammatory reaction, and no peelings of necrotic cells in the follicles were present. There were no differences between the thyroid glands of rats receiving different doses of the plant extract (500 & 1500mg/kg of body weight).

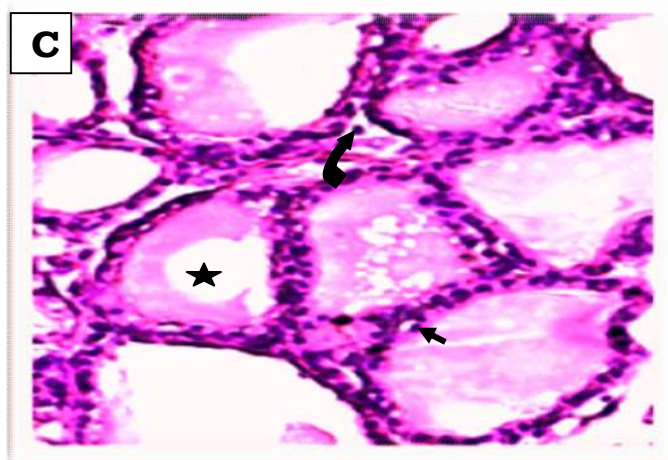
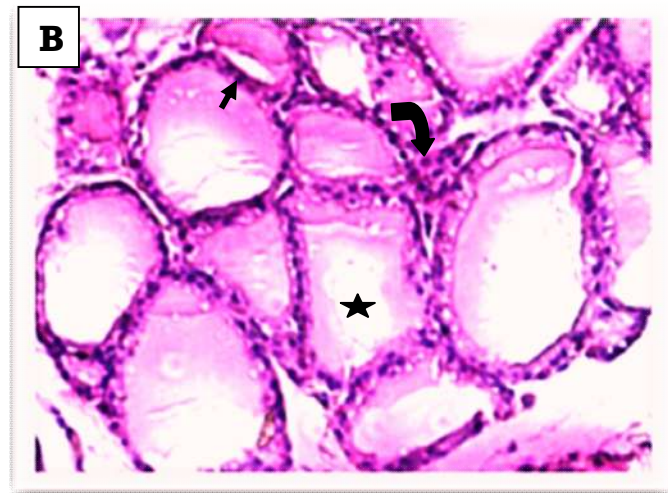
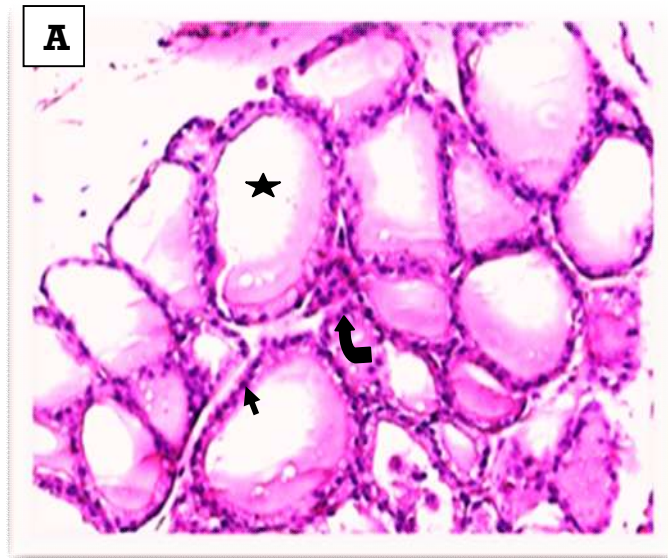


Figure 9: Photomicrographs of thyroid glands of male untreated (control) rats (A), and rats treated with *M.stenopetala* leaf extract at doses of 500 mg/kg body weight (B) and 1500mg/kg body weight(C).(H and E, staining;x300).The straight arrow (→) indicate follicular cells, curved arrow parafollicular cells (↪) and follicles with colloid(★)for treated and control groups.

4.3.5.2 Effects of the extract on histology of the Adrenal glands

Histopathological analysis of adrenal glands from the experimental group of animals showed normal architecture of capsule, cortical area and medulla of adrenal gland along with the normal arrangement of cells at each zones of cortex of both treated and control rats (Figure 6). No histological difference between adrenal glands of treated and control rats were observed. The capsule was well defined with elongated spindle shaped connective tissue cells having fine granular cytoplasm, oval nuclei and abundant connective tissue fibers (Figure 6, A-C). The blood vessels, usually arteries were present in the capsule but, venous channels were also seen. On the basis of difference in arrangement of the parenchymal cells, the cortex was divided into three zones, named as zona glomerulosa, fasciculata and reticularis as illustrated in Figure 6, A-C. The cells in all these regions of cortex indicate their normal arrangement in both treated and control groups. In addition, the cells in the medulla of adrenal gland also illustrated their normal architecture in all groups. The cytoplasm was lightly stained showing many vacuoles and fine granules. The cells were rounded to polygonal and arranged in groups or columns. Numerous sinusoids of variable size were found between the cells columns.

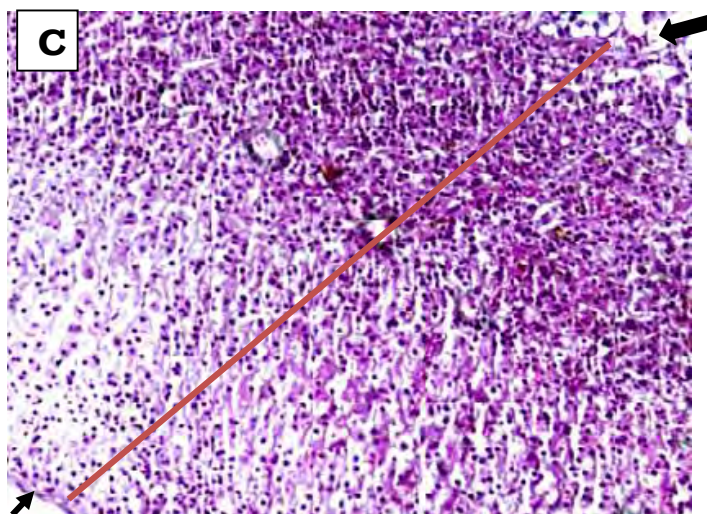
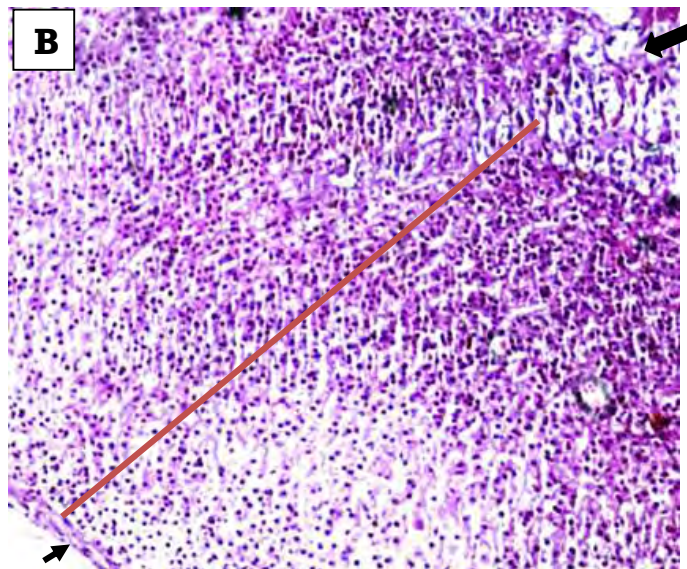
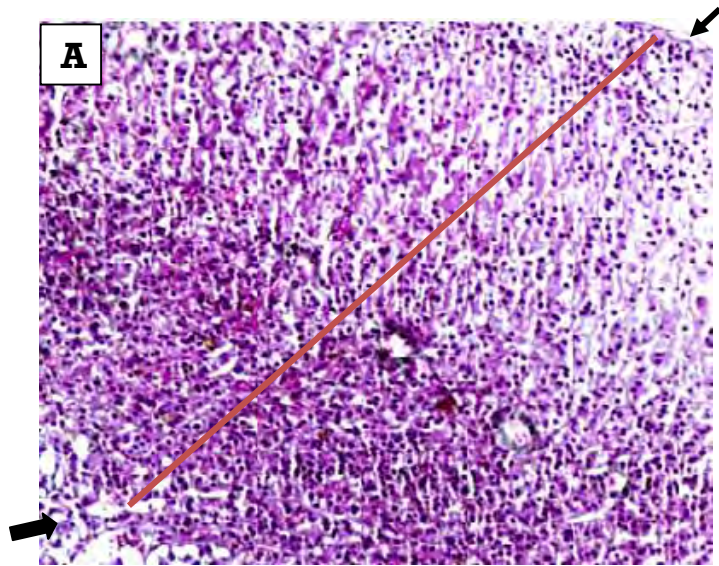


Figure 10: Photomicrographs of Adrenal glands of male untreated (control) rats (A), and rats treated with *M.stenopetala* leaf extract at doses of 500 mg/kg body weight (B) and 1500mg/kg body weight(C). (H and E, staining; x300).The thin arrow (→) indicates the capsule of the gland, cortical zones of the gland is shown by straight line (—) and Medullar portion is shown by thick arrow (➡) for both treated and control rats.

4.3.5.3 Effects of the extract on histology of the pancreas

The results on histopathological analysis of pancreas from the experimental group of animals showed normal architecture of endocrine and exocrine parts of pancreas along with the normal arrangement of cells in both treated and control rats. No histological difference between treated and control rat's pancreas were observed. The endocrine cells of pancreas (islet of langerhan cells) were found arranged in cords or clumps, between which are connective tissue fibers and capillary networks as indicated in Figure 7 .In comparison with control groups no histological difference was examined in treated rats at dose level of 500 & 1500mg/kg body weight of animals treated with aqueous extract of leaves of *M.stenopetala*. The exocrine cells (acinar cells) of pancreas of treated group at both dose levels were seen arranged in normal architecture as compared to control rats having their pyramidal shaped cells around small lumina in the center of which were lightly stained centroacinar cells. The exocrine region of pancreas also contained intercalated ducts as indicated in Figure 7, C. Endocrine cells and exocrine cells of pancreas were separated from each other by a thin connective tissue capsule.

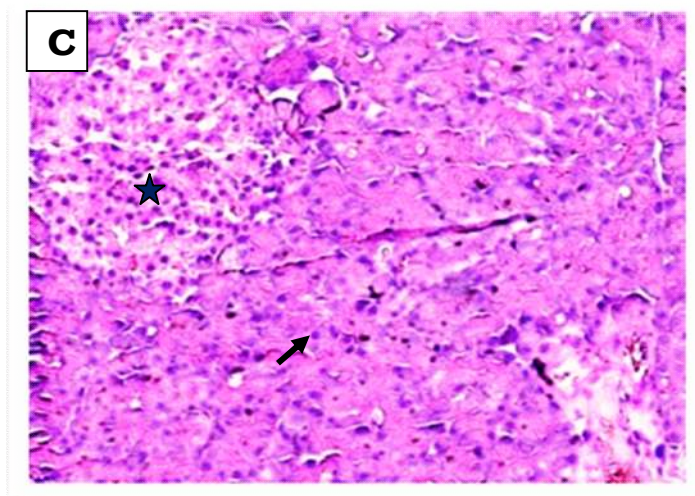
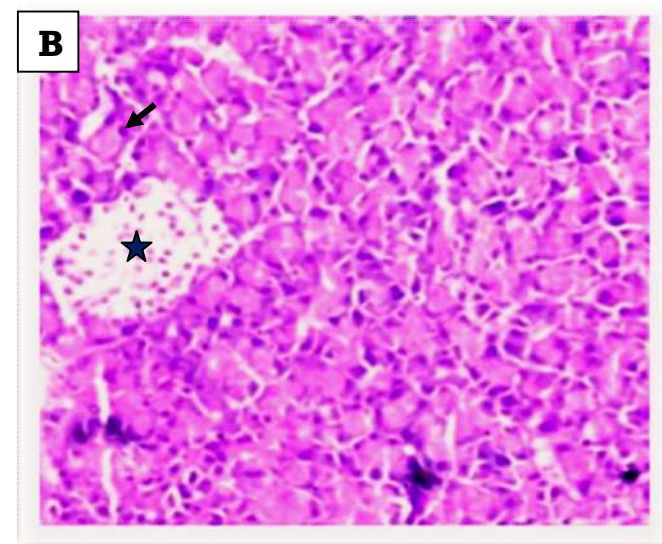
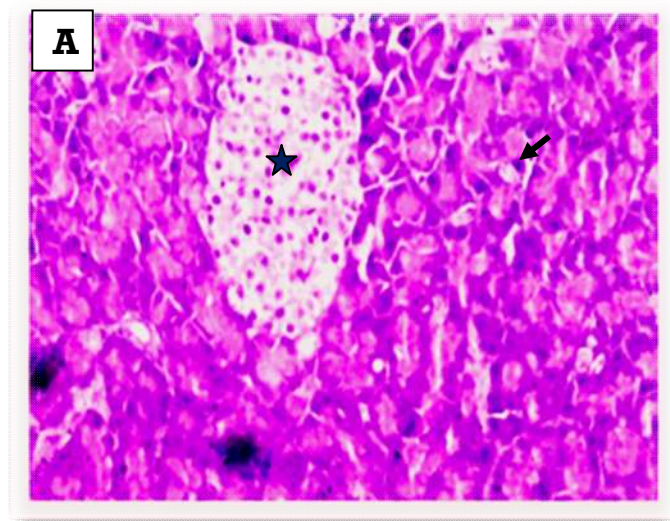


Figure 11: Photomicrographs of pancreas of male untreated (control) rats (A), and rats treated with *M.stenopetala* leaf extract at doses of 500 mg/kg body weight (B) and 1500mg/kg body weight(C).(H and E, staining; x300). The arrow (➡) indicates acinar cells, islets of langerhans cells (★) for treated and control groups.

5. DISCUSSION

For a long period of time, significance of traditional medicines has increased considerably in many parts of the world, especially in the line of medicine and pharmacology (Farhat *et al.*, 2011). In many developing countries including Ethiopia, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet their primary health care needs. The use of medicinal plants and associated knowledge for the treatment and management of several diseases has been gaining acceptance, worldwide, especially in the developing countries, such as Ethiopia where 80% of the population rely on traditional medicines as primary health care (Sujon *et al.*, 2008; Tesfaye *et al.*, 2009; WHO, 2011). To use traditional medicinal plants as a primary health care, the medicinal benefits and toxic properties should be investigated to determine their safety and efficacy for the benefits of mankind (Ezekwesili *et al.*, 2010, Farhat *et al.*, 2011). However, such scientific studies have not so far involved the investigation of the effect of most traditional medicinal plants on most organs of experimental animals which are related to human in many aspects. *Moringa stenopetala* is one of such traditional medicinal plants with multipurpose. Despite the widespread use of the extracts of leaf parts of *Moringa stenopetala* for treatment of various human disease like malaria, hypertension, gastrointestinal problems in different regions of Ethiopia, no toxicity investigation has been undertaken on thyroid gland, adrenal glands and pancreas as well as hematological parameters of laboratory animals and hence the present study is the first of its kind. Therefore, the acute and subchronic studies have been investigated in this study, to assess the effects of the plant extract on hematological profiles, lipid profiles as well as histopathology of thyroid gland, adrenal glands and pancreas in rat model. In addition to this, the percentage yield of the aqueous extract of leaves of *M.stenopetala* were also analyzed and computed.

According to OECD (2001) and Saganuwan (2011), determination of the acute toxicity is the primary step in the toxicological investigation of an unknown substance. The index of acute toxicity is the median lethal dose (LD50) (Deora *et al.*, 2010, Oduola *et al.*, 2010, Saganuwan, 2011). In this investigation, no signs and symptoms of toxicity were observed with any of the tested doses during 14 days of observation period. There was no change in behavioral and neurologic profiles as compared to the control group. Furthermore, no death was also recorded in any of the rats treated with the the extract at 1000, 3000 and 6000mg/kg body weight in acute toxicity study. 6000mg/kg is the highest dose level where no adverse treatment related findings were observed. Therefore, 6000mg/kg body weight dose was the no observed adverse effect level (NOAEL) or the threshold level in this test. This result reveals that the LD50 of the plant extract is greater than 6000mg/kg body weight. Any compound or drug with the oral LD50 estimate that exceeds 1000mg/kg body weight is taken as low toxic and relatively safe (Clarke and Clarke. 1977). The present result, therefore, indicates that, aqueous leaf extract of *M.stenopetala* is comparatively safe on acute exposure. This result was coincided with the study conducted by Nardos *et al* (2011) and Toma, *et al* (2012),who reported that following administration of the extract of *M.stenopetala* at the dose of 5000mg/kg , no death and behavioral change were observed in mice model.

The result showed that rats treated with the extract at 1000, 3000 and 6000 mg/kg body weight were increased in body weight compared to the controls, although these were not statistically significant ($p>0.05$). Such increase in body weight might be due to the rich nutritional value of the plant as was stated by Teshome *et al* (2001). This is also in agreement with the study conducted by Ghebereselassie *et al* (2011) which states that mice treated with aqueous extract of *M. stenopetala* showed increment in body weight.

In this study, the effects of long-term treatment with *M. stenopetala* aqueous leaf extract on some hematological and biochemical parameters of the blood, and histopathology of thyroid gland, adrenal gland and pancreas were scrutinized.

Treatment with doses of 500 and 1500mg/kg of body weight of the extract was well tolerated by all the rats, as there were no toxic effects observed by direct visual observation of the animals in the subchronic toxicity study throughout the 90 days of the experiment. There was no death and noticeable behavioral changes recorded during the course of the experiment in all treatment groups at both dose levels as compared to the control group. The same findings and conditions were also described by Faizis *et al* (1998).

As indicated by Vahalia *et al* (2011), decrease in body weight is an essential guide for determination of toxicity. There were increments of mean body weight gain in the treated rats as was observed for acute toxicity study. Though, increase in the mean body weight were observed in subchronic and acute toxicity studies insignificantly, there were more weight gain in rats treated at both doses in subchronic study as compared to control groups. The reason may be because of the prolonged incremental effect in the subchronic. The increase in body weight of the rats was supported by previous studies done by Ghebereselassie *et al.*, (2011), Toma *et al.*, (2012) with the leaf extract in mice model. The reason for the increment in the body weight following treatment with the extract could be related to high nutritional value of the plant as stated in section 1.2.3.

Thyroid gland, adrenal glands and pancreas were the target organs used for toxicity tests in this particular study. As a result, the absolute weights of thyroid gland, adrenal glands and pancreas of the rats treated with dose levels of 500 and 1500mg/kg of the extract in both male and female rats showed slight increment as compared to the control groups, respectively. But, the increment was statistically not significant ($p>0.05$). The relative increment in organ weight of the rats as compared

to the control group might be due to the fact that, leaves of *Moringa stenopetala* contains glucosinolate which might bring slight increment in organ weight as indicated by Mekonnen and Draeger,(2003), Bennet et al.,(2003) and Vermorel et al.,(2006) . The variation of organ weight may also be caused by age, body size and rate of growth of rats. This is supported by findings of Webster et al., (1947),Dunns,(1967).

Regarding gross pathological examination, no signs of toxicity like spot, necrosis, and bleeding were observed on thyroid gland, adrenal gland and pancreas. In addition, the organs did not show any marked change in texture, size and color in both sex groups at all dose levels in acute and subchronic toxicity studies.

Blood parameters play a critical role in diagnosis, assessing progression, and in the characterization of disease and phenotypes in clinical and research situations. Blood is an important indicator to determine the physiological and pathological status of animals. It also acts as pathological reflector of the whole body; hence hematological parameters are important in diagnosing the functional status of exposed animals to toxicant (Joshi et al., 2002). According to Yakubu et al (2008), evaluation of blood parameters conveys an important idea to determine the harmful effect of foreign compounds including plant materials. The normal ranges of blood parameters can be altered by the ingestion of some toxic plants (Ajagbonna *et al.*, 1999).

In the present study, the effect of prolong oral administration of the aqueous leaf extract at graded doses of 500 and 1500mg/kg for 90 days were examined on hematological parameters. Rats treated with aqueous leaf extract of *M. stenopetala* at both doses showed non-significant changes ($p>0.05$) in their hematological parameters as compared with control rats. The parameters studied and found within normal reference range were WBC, RBC, PLT, HCT, MCV, MCH, MCHC and HGB as indicated in (Table 8). However, although not statistically significant there was slight decrement in the mean WBC counts in rats treated with the extract at dose levels of

500 & 1500mg/kg(Table 8).This work is in agreement with results reported on non-toxic effect of the plant on the WBC count as indicated by Ghebreselassie et al(2011).The platelet count was also slightly decreased in rats treated with the extract at both dose levels of 500 & 1500mg/kg, but the change was again statistically insignificant($p>0.05$). The result was similar with the work of Getachew (2012) who reported the insignificant change of *G. stenophylla* extract on the normal value of platelets. The absence of negative effects of aqueous extract of the plant may indicate that the plant contains no toxic compounds. This is in agreement with the study conducted by Ghebreselassie et al.(2011) on *M. stenopetala* leaf extract administration in mice model and *Ginidia stenophylla* root extracts administration in mice model(Getachew,2012).

Goiter is associated with change in thyroid physiology, which may be caused due to iodine deficiency in the diet. Deficient iodine means that thyroid hormones cannot be synthesized with a resultant rise in TSH levels. TSH causes thyroid enlargement by stimulating follicle growth and the development of new blood vessels. Apart from iodine deficiency, xenobiotics may also affect thyroid function by altering biosynthesis, secretion, absorption, or metabolism of the thyroid hormones (Crisp et al, 1998).

In addition to iodine deficiency, enlargement of thyroid gland can be associated with consumption of some plant foods containing goiterogenic substances (Wolde Gebriel et al 1993). In the present study, the T3, T4 and TSH level were analyzed and the change was compared among treated groups and control rats in both male and female. With regard to consumption of *M. stenopetala* leaves, it was observed that the mean values of TSH was slightly increased in both male and female groups treated with 500 and 1500 mg/kg of the plant extract as compared to control group though the increment is statistically insignificant. TSH levels are regulated by the hypothalamus, and also by other regulatory mechanisms, producing a feedback loop so that TSH increases as thyroid hormones decrease and TSH decreases when

thyroid hormones increase. Furthermore there are reports where TSH level was inversely correlated with T4 levels but the levels of T3 were variable (Mokshagundam and Barzel, 1993; Chuang *et al.*, 1998; Mansoor *et al.*, 2011). The better co-relation of TSH with T4 may be due to the reason that T4 is mainly produced from pituitary gland, while only 7% of T3 is secreted (Sinha,2013). The rest of the T3 production is dependent on the peripheral conversion of T4 to T3 which in turn depend on many factors, including bioavailability of enzyme deiodonase, drugs, disease in which inactive T3 form instead of T3. Elevated TSH level directly reflects impaired thyroid hormone production (Saha *et al.*, 2007).The increment of TSH level may possibly be due to the effect of the plant extract on hypothalamus. In this study, the mean T4 value of rats in both male and female groups which took the aqueous extract of *M. stenopetala* at all dose levels decreased as compared to control groups, but still the change is statistically insignificant. This is also supported by Alemayehu (2013). This result suggests that the *Moringa stenopetala* leaves may have effect in the control of hyperthyroidism and in lowering of the level of T4 in the blood. Same study is also in line with the study conducted by Agsam *et al* (2011), which states that, rats treated with the extract of leaves of *Moringa olifera* resulted in marked decrement in the serum levels of T4; hence it can be taken as the most effective in treatment of hyperthyroidism. Nagi *et al* (2000) mention that T3 and T4 levels were positively correlated but not strongly, this may be due to the fact that biosynthesis of T4 is confined to the thyroid gland. While more than 80% of T3 is synthesized out of the thyroid gland e.g. in (liver) and each of the pathways has its own regulatory mechanism. Therefore, in this particular study changes in T4 and T3 level may be due to the fact that *M. stenopetala* extract may have some effects on thyroid gland but not affect other organ. However, the effect of the extract is not enough to cause goiter as the values are within the reference range. This is also supported by Cherinet *et al* (2003) which states that, *M. stenopetala* contains cyanogenic glucosides although the reported content was less than that expected to cause goiter. T3 is generally accepted to be the active form of the various thyroid hormones and some data seem to indicate that T3 may exert both

long and short-term effects on the metabolism of most tissues. In this study, T3 shows increment in male rats treated with 500 and 1500mg/kg and also increased in female rats treated with 1500mg/kg, but the change is not statistically significant.

The total cholesterol, HDL and LDL showed increased in male rats treated with 1500mg/kg of the plant extract in comparison to control rats. But the increment in cholesterol, HDL & LDL was statistically insignificant ($p>0.05$). However, a slight decrement of cholesterol, HDL as well as TG was seen in male rats treated with 500 mg/kg of aqueous leaf extract of *Moringa stenopetala*, but still the decrement is statistically insignificant. However, the total LDL level did not show any significant difference among male rats treated at dose level of 500mg/kg as compared to control rats. Serum triglyceride level increased in male rats treated with 1500 mg/kg of the extract but showed decrement in rats treated with 500 mg/kg of the extract. Though the change is statistically insignificant, the level of cholesterol, HDL and LDL relatively show some increment at high dose in male rats but, this result may not indicate the negative impact of the extract on the tested parameters as they are within the reference range as compared to control rats.

As shown in table 12, the total cholesterol and LDL levels showed increased in female rats treated with 500 & 1500mg/kg of the plant extract in comparison to control rats. But the increment in cholesterol, & LDL was statistically insignificant ($p>0.05$). However, marked decrement of HDL was seen in female rats treated with the extract at 500 & 1500 mg/kg of aqueous leaf extract of *Moringa stenopetala*, respectively as compared to control rats and the decrement is statistically significant ($p<0.05$). The decrement in HDL may be due to the effect of the extract as serum lipid lowering activity in female rats. This is in agreement with the findings of (Mahendra et al., 2010) which states that aqueous extract of *M.oliefera* have effect in lipid lowering activity. Serum triglyceride level increased in female rats treated with the extract at doses of 500 and 1500 mg/kg body weight.

Light microscopic observation at the doses of 500 mg/kg and 1500 mg/kg of aqueous extract of *M. stenopetala* showed no marked histopathological changes on the thyroid gland, pancreas and adrenal gland of rats as compared to the control group. The microscopic structure of the thyroid gland in control rats showed characteristic features indicating the normal morphology of follicular cells in spheres surrounding colloids and parafollicular cells nestled in space between the follicles. The treated groups also show similar characteristics as compared to control rats.

Regarding the microscopic examination, the plant extract did not produce any adverse effect. This suggests that the plant material did not contain chemicals which disturb the normal morphological architecture of cells in thyroid gland, adrenal gland as well as pancreas of treated rats as compared to controls.

6. CONCLUSION

The present study showed that the leaf aqueous extract of *Moringa stenopetala* does not produce significant toxicity on behavior, hematological and biochemical parameters as well as histology of pancreas, thyroid and adrenal glands at the doses tested.

7. RECOMMENDATION

Detailed chronic toxicity studies of the extract should be carried out on hematological, biomedical and histopathology of thyroid gland, adrenal gland and pancreas on volunteer humans following standard procedures.

The goiterogenic effect of the plant extract should be done on other non-rodent animals using other solvents as medium of extraction like ethanol and butanol.

The plant extract contains high nutritional value and may contribute for overweight so its consumption should be dose dependent for users.

Investigation of the plant extract should be carried out on hypothalamus as change in T4 and T3 level depends on hypothalamus.

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8.1 APPENDIX-I:

PREPARATION OF WORKING CHEMICALS OR SOLUTIONS

1. 10% Neutral Buffered formalin

40%formalin.....	100ml
Distilled water.....	900ml
Sodium dihydrogen phosphate, monohydrate.....	4gm
Disodium hydrogen phosphate anhydrous.....	6.5gm

2. Egg albumin solution

Fresh egg white.....	50ml
Glycerol.....	50ml
Sodium salicylate.....	1ml
Distilled water.....	5ml

3. Harris Hematoxylin (H)

Hematoxylin crystals.....	2.5gm
Potassium alum.....	50gm
Glacial acetic acid.....	20ml
Absolute alcohol.....	25ml
Distilled water.....	500ml

4. 1% Acid alcohol

70% Alcohol.....	500ml
Hydrochloric acid, concentrated.....	0.5ml

5. Preparation of bluing solution or Sodium bicarbonate

Sodium bicarbonate.....	1gm
Distilled water.....	100ml

6. 1 % Aqueous Eosin (E)

Distilled water.....	100ml
Eosin, both water alcohols soluble.....	1gm
Glacial acetic acid.....	0.5ml

8.2 APPENDIX- II:

TISSUE FIXATION PROTOCOLS

1. 10 % buffered formalinovernight
2. Running tap water.....overnight
3. 70% alcohol.....2hrs
4. 90% alcohols2hrs
5. Absolute alcohol: I1½ hrs
6. Absolute alcohol: II1½ hrs
7. Absolute alcohol: III.....1½ hrs
8. Absolute alcohol: IV.....overnight
9. Xylene: I.....1½ hrs
10. Xylene: II.....1½ hrs
11. Wax: I1½ hrs
12. Wax: II2½ hrs
13. Wax: IIIovernight

8.3. APPENDIX- III:

TISSUE STAINING PROTOCOL (H & E STAINING PROCEDURE)

1. Xylene-I.....	10min
2. Xylene-II.....	10 min
3. Absolute alcohol- I.....	5 min
4. Absolute alcohol- II.....	5 min
5. 95 % alcohol.....	3 min
6. 70 % alcohol.....	3 min
7. 50% alcohol.....	3 min
8. Distilled water.....	5min
9. Harris Hamatoxylin.....	10min
10. Running tape water.....	5 min
11. 1% acid alcohol.....	30 sec
12. Running tape water.....	5 min
13. Sodium carbonate solution.....	30 sec
14. Running tape water.....	5 min
15. Eosin.....	1-2min
16. Running tape water.....	5 min
17. 70 % alcohol.....	3 min
18. 95 % alcohol.....	3 min
19. Absolute alcohol- I.....	1½ hrs
20. Absolute alcohol- II.....	1½ hrs
21. Xylene – I.....	5 min
22. Xylene-II.....	5 min