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**Evaluation of the effects upon chronic administration of aqueous leaves
extract of *Moringa stenopetala* on blood parameters and histology of
liver and kidney of Wistar rats**

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List of Abbreviations and Acronyms

AAU:	Addis Ababa University
EPHI:	Ethiopian Public Health institute
TM:	Traditional medicine
mg:	Milligram
kg:	Kilogram
⁰ C:	Degree Celsius
ANOVA:	Analysis of variance
SPSS:	Statistical package for social sciences
gm:	gram
ml/dl/ μ l:	milliliter/deciliter/microliter
EDTA:	Ethylenediaminetetraacetic acid
FL:	Femto Liter
WHO:	World health organization
Km:	Kilometer
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
MCHC:	Mean corpuscular haemoglobin concentration
MCH:	Mean corpuscular haemoglobin
MCV:	Mean corpuscular volume
PLC:	Platelet count
Pg:	Pico liter
HGB:	Haemoglobin
GGT:	Gammaglutamyl transpeptidase
H and E:	Haematoxlin and Eosin
SEM:	Standard Error of Mean
IU:	International unit

Abstract

The world people use plants for the purpose of disease control and prevention as well as for nutritional purpose since prehistoric times. One of the plants used for such purpose is *Moringa* species. Traditionally, *Moringa stenopetala* and related species are used as antihypertensives, antidiabetics, anticancers, antioxidants, antimicrobials, antimalarials and edible material. Among the various species of *Moringa*, *Moringa stenopetala* locally known as “Shiferaw/Haleko” is indigenous to the Southwest region of Ethiopia.

Despite its important functions and widespread uses, there are very limited studies carried out on the hematological and histological effects of *Moringa stenopetala* to investigate its safety. In previous acute and subchronic studies, no effects were observed on body weight, gross pathology as well as histology of kidney and liver (except a mixed inflammatory cells infiltration and slight activation of Kupffer cells). Similarly, there was no effect on hematological and biochemical parameters. However, it is not known if it is same in a prolonged administration of the extract. Hence, this study is aimed to find out if there is any sign of toxicity upon chronic (six months) oral administration of aqueous leaves extract of *Moringa stenopetala* on blood parameters and histology of liver and kidney of Wistar rats.

The study was carried out at Ethiopian Public Health Institute and Addis Ababa University. The plant was collected from Arba Minch and aqueous extract was prepared. The experiment was conducted on 24 rats. They were grouped randomly into four; one control group administered distilled water and three experimental groups were administered aqueous extract of the leaves of *Moringa stenopetala* at the doses of 500, 1000, and 2000 mg/kg body weight orally for six months of chronic toxicity study by using OECD guidelines.

There was no significant change ($p > 0.05$) in body and organ weights, except a transitory decrease in body weight at the 2nd and 3rd weeks as compared to the controls, respectively in the female and male rats that received the extract at the dose of 2000 mg/kg body weight. Chronic treatment with 500, 1000 and 2000 mg/kg body weight of the leaf of aqueous extract did not significantly affect ($p > 0.05$) most of the investigated hematological parameters. However, it induced significant ($p < 0.05$) elevation in MCV of female rats at all doses when compared with

the control. There is also decrement of MCH at doses of 1000 mg/kg and 2000 mg/kg significantly, ($p < 0.05$) as compared to the control in male rats. There was no significant ($p > 0.05$) difference in ALP, AST, ALT and total bilirubin in female rats administered at all doses as compared to the controls. However, the ALP of male rats that received 2000 mg/kg body weight, AST and ALT of male rats that received 1000 and 2000 mg/kg body weight were found significantly ($p < 0.05$) increased as compared to the controls. Chronic treatment with the extract did not significantly affect the urea and creatinine levels, except in the female rats that received the extract at 2000 mg/kg where a significant ($p < 0.05$) decrease in urea and increase in creatinine levels as compared to the controls were observed. Histological evaluation showed some mononuclear leukocytic infiltration around the portal traid and central vein of the liver as well as cytoplasmic vacuolization of hepatocytes in male rats treated the extract at 1000 and 2000 mg/kg body weight; Furthermore, there were mononuclear leukocytic infiltrations around the glomeruli and medullary regions, as well as widened urinary space of the kidneys at 2000 mg/kg body weight in female rats.

Findings from the present study suggest that prolonged administration of the aqueous leaf extract of Moringa stenopetala at therapeutic dose is safe; however, it was toxic as doses accumulate.

Key words: Wistar rats, *Moringa stenopetala*, chronic toxicity, histology of liver and kidney, hematological and biochemical parameters.

1. Introduction

1.1. Background of the Study

1.1.1. Traditional and Herbal medicine

Traditional medicine (TM) is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (Berkes *et al.*, 2003). Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine (WHO, 2004). Traditional preparation comprises medicinal plants, minerals and organic matters. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy (Agrawal, 2009). Traditional healers in the world, where ethno medical treatment is frequently used to treat all kinds of ailments, provide herbal medicines they prepare from different plants without considering their toxicity. They keep no records and the information is mainly passed on verbally from generation to generation with great secrecy (Etana, 2010). Such secrecy makes indigenous knowledge or ethno medicinal knowledge vulnerable to distortion and in most cases, some of the lore is lost at each point of transfer; hence, there is a need for systematic documentation of such useful knowledge through ethno botanical research.

Most of the traditional medicinal plant preparations are used in fresh form. Oral, dermal and nasal are the most commonly employed routes of application of remedies. Squeezing, grinding, boiling, crushing and tying are the most frequently used methods of remedy application (Gidey, 2010). The use of traditional medicine has increased significantly over the past few years. However, as recent reports have shown, in addition to the many benefits there are also risks associated with the different types of TM. Although consumers today have widespread access to various TM treatments and therapies, they often do not have enough information on what to check when using TM in order to avoid unnecessary harm (Abebe, 1996).

People in different parts of the world depend on plant resources for their basic needs and are aware of many useful species occurring in their ecosystem (Ellen *et al.*, 2000). Plants have played a vital role in the prevention and treatment of diseases since prehistoric times. More than

35,000 plant species are being used around the world for medicinal purposes (Lewington, 1993) and, in Ethiopia there are 800 or more plant species employed as medicinal agents (Tesema *et al.*, 2002); which according to the data base of the National Herbarium has grown to 1000 and more will be added to the list as new studies bring new medicinal plants from various cultures. About 80% of Ethiopian people rely on traditional medicine to meet their health care needs (Bekele, 2007).

For many people in sub-Saharan countries, particularly those living in rural areas, herbal medicine is the only available, accessible and affordable source of health care. However, herbal medicine in Ethiopia has attracted very little attention in modern medical research and development. Less effort has also been made to upgrade the role of herbal medicine practice and identify side effects that may be associated to the use of plant medicines (Etana, 2010). As a result, it has become imperative to conduct research on plants to find out the toxicity and effectiveness of drugs for the benefit of man and animals and discard the ineffective, toxic and worthless drugs. One of such widely used plant medicines is *Moringa* species. It is traditionally used for treatment of diabetics, hypertension, malaria and other related diseases (Toma *et al.*, 2012).

In the utilization of indigenous/traditional systems of medicine in the modern day medicine, the drugs are primarily dispensed as water decoction or ethanol extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination (Agrawal, 2009). The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model(s). The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints.

The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals (Agrawal, 2009).

1.1.2. Uses of *Moringa stenopetala*

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. A recent report by Mengistu *et al.* (2012) indicated that *M. stenopetala* has blood pressure lowering effects. These researchers showed that crude aqueous leaf extract of *M. stenopetala* caused a significant drop in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure in normotensive anaesthetized guinea pigs. Leaf extracts of *M. stenopetala* are used to lower blood glucose and cholesterol levels. Ghebreselassie *et al.* (2011) reported that aqueous leaf extract of *M. stenopetala* increases body weight and reduce serum glucose and cholesterol levels in mice. Serum glucose and cholesterol levels were decreased significantly after six weeks of treatment.

Moringa has a potential as a food source in the tropics because the tree will have full leaves at the end of the dry season when other foods are typically scarce. A large number of reports on the nutritional values of *Moringa* exist in both the scientific and the popular literature. *Moringa* leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk and eggs (Ghosh, 2014). The several uses of *Moringa* species are shown in figure 1.



Figure 1: Uses of *Moringa* species (Ghosh, 2014)

1.1.3. Phytochemistry of *Moringa*

Phytochemicals are chemicals produced by plants, commonly, though, the word refers to only those chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients (Charlette *et al.*, 2013). An examination of the phytochemicals of *Moringa* species affords the opportunity to examine a range of fairly unique compounds. In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Mirsha *et al.*, 2011) For example, specific components of *Moringa* preparations that have been reported to have hypotensive, anticancer, and antibacterial activity listed by (Sreelatha *et al.*, 2011) include 4-(4'-O-acetyl- α -L-rhamnopyranosyloxy)benzylisothiocyanate, 4-(α -L rhamnopyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(L-rhamnopyranosyloxy) benzyl glucosinolate. Along with these compounds the *Moringa* family is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β -carotene or pro-vitamin A).

Moringa is a rich source of antioxidant. Its leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenol and carotenoids (Anwar *et al.*, 2007). *Moringa* is said to have approximately 46 antioxidants and is one of the most powerful sources of natural antioxidants. Antioxidants supply the free atoms needed by the human body and mitigate the effect of free radicals. *Moringa* leaves are rich in flavonoids, a class of antioxidants. The beta carotene present in *Moringa* leaves also acts as antioxidants (Mishra *et al.*, 2011). The antioxidants will have the maximum impact on the damage causing free radicals, only when it is ingested in combination with nutrients and a group of antioxidants. A combination of antioxidants is more effective than a single antioxidant on an equal weight basis due to antioxidant cascade mechanism. This is why *Moringa* tea acts as an effective source of antioxidants than any other herbal tea or even a Green tea (Mishra *et al.*, 2011; Tejas *et al.*, 2012).

1.1.4. *Moringa stenopetala* (Baker F) Cufodontis

Moringa stenopetala belongs to the family *Moringaceae*. It is a branched tree that grows 6 to 10 m tall, thick at base bark with white to pale gray or silvery coloration (Abuye *et al.*, 2003). It is endemic to East Africa mainly present in northern Kenya and southern part of Ethiopia (Orwa *et al.*, 2009; Padayachee and Baijnath, 2012). It grows abundantly in Southwest Ethiopia where the leaves are eaten as vegetable. The species is known by different vernacular names such as "Shiferaw" in Amharic, "Haleko" in Wollaytegn and "Cabbage tree" in English. It grows widely at an altitude range of 1000 to 1800 meter above sea level. The leaves are one of the best vegetable foods that can be found in the locality (Abuye *et al.*, 2003). The flowers are good nectar sources for honey; 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 (Mekonnen and Gessesse, 1998; Padayachee and Baijnath, 2012).

M. stenopetala is well adapted to semi-arid areas of 500 mm annual rainfall and continues to grow during the exceptionally long dry season. The study conducted by Melesse and Berihun, (2013) indicated that, the leaves of *M. stenopetala* are rich in protein (28.2%) and contain reasonable amounts of essential amino acids of which some are comparable with those found in soybean meal. It is a multipurpose tree that is cultivated both for human food and animal feed in Southern Ethiopia (Melesse and Berihun, 2013).

The genus *Moringa* includes about 14 species worldwide and is distributed widely in tropic and sub tropic zones. Many species of *Moringa* yield the commercially important *Moringa* seed oil biogas production, and waste water treatment which exhibits highly antimicrobial effect (Sahilu, 2010). Among the various species, *M. stenopetala* is indigenous to Ethiopia locally known as "Shiferaw/Aleko" (Mekete, 2008 and Sahilu, 2010). The leaves of *Moringa* are used in Ethiopia as spices to flavor a wide range of food products including tea as well as medicines (Abuye *et al.*, 2003). The medicinal uses are numerous and have been long recognized in the Ayurvedic and Unani systems of Medicine (Kumar *et al.*, 2011). *M. stenopetala* and related species *M. oleifera* are commonly used in folk medicines as hepatoprotective, antihypertensives, antidiabetics, anticholesterol, antispasmodic, anticancer, antiasthmatic, antimicrobial, antioxidant, in ocular diseases, and to treat many other ailments.



Figure 2: Photographs of *Moringa stenopetala* (Sahilu, 2010)

1.2. Blood Composition and Functions

Blood, the fluid that circulates in the cardiovascular system, has occupied a prominent place throughout history as an almost mystical fluid. Humans undoubtedly had made the association between blood and life by the time they began to fashion tools and hunt animals. A wounded animal that lost blood would weaken and die if the blood loss was severe enough. The logical conclusion was that blood was necessary for existence. This observation eventually led to the term lifeblood, meaning anything essential for existence (Everds, 2008).

The blood volume in adult rat is estimated to account 7.4% of its body weight; usually the younger rat has a large blood volume relative to their body weight than older rats (Brown et al., 1997).

Blood is a specialized connective tissue in which cells are suspended in fluid extracellular material called plasma. About five liters of blood in an average adult human is propelled mainly by rhythmic contractions of the heart; and moves unidirectionally within the closed circulatory system. The so-called formed elements circulating in the plasma are erythrocytes (red blood cells), leukocytes (white blood cells) and platelets (Ross, 2011; Mescher, 2010; Tortora and Derrickson, 2009).

The yellowish translucent, slightly viscous supernatant comprising 55% at the top half of the centrifugation tube is the plasma. It is an aqueous solution, pH 7.4, containing substances of low or high molecular weight that make up 8–10% of its volume. Plasma proteins account for approximately 7% of the dissolved components, with the remainder including nutrients, nitrogenous waste products, hormones, and many inorganic ions collectively called electrolytes. Through the capillary walls, the low-molecular-weight components of plasma are in equilibrium with the interstitial fluid of the tissues. The composition of plasma is usually an indicator of the mean composition of the extracellular fluid in tissues (Ross, 2011).

The rat red blood cells (also called erythrocytes) are the most numerous cells in the blood. Each liter of normal blood contains $135\text{--}250 \times 10^3$ μl red blood cells and their life span is 45–50 days (Everds, 2008). Their chief function is to transport the respiratory gases oxygen and carbon dioxide around the body. The red blood cells are small circular biconcave disks of 7–8 μm in diameter and they do not possess a nucleus. They are very thin and flexible and can squeeze through the narrow lumen of the capillaries, which have internal diameters of only 5–8 μm . Their shape gives red cells a large surface area to volume ratio which promotes efficient gas exchange. In a mature erythrocyte, the principal protein constituent of the cytoplasm is hemoglobin, an oxygen-binding protein, which is synthesized by the red cell precursors in the bone marrow (Pocock *et al.*, 2006).

Leukocytes are larger than the red blood cells, possess a nucleus, and are present in smaller numbers. Normal blood of adult rat contains around $7 \text{ --- } 10^9$ white cells per liter. These cells have a vital role in the protection of the body against disease (Everds, 2008). They are the mobile units of the body's protective system, being transported rapidly to specific areas of inflammation to give powerful defense against invading organisms. They possess several characteristics that enhance their efficacy as part of the body's defense system. They are able to pass through the walls of capillaries and to enter the tissue spaces in accordance with the local needs. This process is known as diapedesis. Once within the tissue spaces, leukocytes have the ability to move through the tissues by an amoeboid motion at speeds of up to $40 \mu\text{m min}^{-1}$ (Tortora and Derrickson, 2009)

The monocytes and lymphocytes are sometimes also referred to collectively as agranulocytes or mononuclear leukocytes. On the other hand, neutrophils, eosinophils, and basophils, named according to their staining reactions, are grouped under granulocytes or polymorphonuclear leukocytes. Although all the white blood cells are concerned with defending the tissues against disease-producing agents, each class of cell has a slightly different role to play. Granulocytes of adult human account around 70% of the total number of white cells in the blood (Silverthorn, 2013).

Neutrophils in adult human are the most numerous accounting 60-70% of the granulocytes with life span of 2 days (Everds, 2008). They are so named because their cytoplasm does not stain with eosin or with basophilic dyes (Ross, 2011). They are phagocytes, which are able to enter the intercellular spaces by diapedesis to engulf and destroy disease-producing bacteria. Enzymes within the cytoplasmic granules then digest the phagocytized particles. This action of the neutrophils forms the first line of defense against infection.

Eosinophils are so-called because their granules stain red in the presence of the dye eosin. Normally they represent about 2% of the total number of white blood cells but in people with allergic conditions such as asthma or hay fever, their population greatly increases. These cells have antihistamine properties and they congregate around sites of inflammation. Their lifespan is very short about 12-20 hours in rats (Mescher, 2010).

Basophils possess granules that stain blue in the presence of basic dyes such as methylene blue. They represent only about 0.5% of the white cell population. They produce heparin and histamine and are responsible for some of the phenomena associated with local immunological reactions such as local vasodilatation and increased permeability of blood vessels, resulting in local edema. They are stimulated by certain antigen complexes bound to immunoglobulin E (Tortora and Derrickson, 2009).

Monocytes represent around 5% and are larger than the other classes of white blood cells, having a diameter of 15-20 μm . Their nuclei are kidney shaped. They are formed in the bone marrow where they mature before being released into the circulation. Within 2 days, they have migrated to tissues such as the spleen, liver, lungs, and lymph nodes (Silverthorn, 2013). These cells are macrophages and act in much the same way as the neutrophils, ingesting bacteria and other large particles.

Lymphocytes represent around 25% of the total white cell population in adults (although in children they are much more numerous) and vary from 6 to 20 μm in diameter. However, lymphocytes of rat are greater than neutrophil which account 50-60% and their life span ranges from hours to years (Everds, 2008). They are of two types, the B lymphocytes, which mature in the lymphoid tissue such as the lymph nodes, tonsils, and spleen, and to a lesser extent in the bone marrow, and the T lymphocytes, which mature in the thymus. B cells have a very short life in the circulation (a few hours) but T cells can live for 200 days or more. Each has a very important part to play in the protection of the body against infection either by producing antibodies (B cells) or by participating in cell-mediated immune responses (T cells) (Silverthorn, 2013).

Strictly speaking, platelets are not cells at all. They are irregularly shaped membrane-bound cell fragments which are formed in the bone marrow by budding off from the cytoplasm of large polyploid cells called megakaryocytes. Megakaryocytes are derived from primitive hematopoietic stem cells. Platelets are 2-4 μm in diameter, and have a lifespan in the blood of around 10 days. Normal blood contains $(150-400) \times 10^9$ platelets. Platelets have an important role in the control of bleeding and in the maintenance of integrity of the vascular endothelium (Fox, 2011).

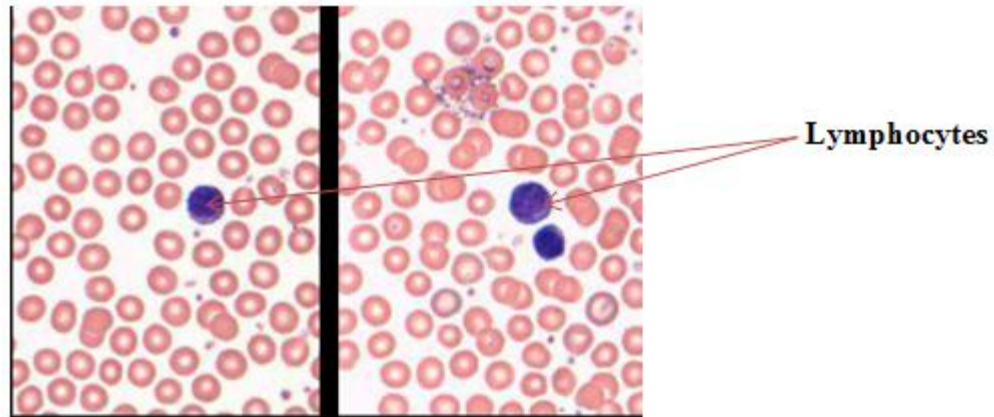


Figure 3: Formed elements of erythrocytes, leukocytes and platelets of rat (Everds, 2008)

Since blood has a key role for transportation of nutrients, hormones, metabolic wastes and immunological processes as well as homeostasis balance; its serum is preferred for several specific enzyme tests (Ross, 2011). Hematological parameters are still most highly accurate and reliable. These have made it to be utilized by most investigators for the purposes of diagnosis and treatment of diseases.

1.3. Liver Histology and Functions

The rat liver consists of 4 distinct lobes known as left lateral lobe, median lobe, right lobe and caudate lobe of different sizes (Figure 4). The left lateral lobe represents about 30-35% of the total liver weight and is located in the left lateral position dorsal to the median and cranio-ventral to caudate lobes and the stomach. It has a narrow pedicle containing portal vein, hepatic artery and bile duct. The pedicle is covered by the Glisson sheath and a narrow base, both located close to each other. The pedicle is attached to the infrahepatic caval vein and the base of the left portion of the median lobe. The base contains the left lateral liver vein. Interlobular ligaments connect the left lateral lobe with the upper caudate lobe. The median lobe represents about 35-40% of total liver weight and consists of two portions. The left portion and the right portion are separated by a deep fissure (MacSween, 2002).

The median lobe is located under the diaphragm and is fixed with the falciform ligament, which spans from xiphoid and diaphragm to the liver beginning at the interlobular fissure. The left portion is smaller and represents about one third of the whole median lobe.

The other two third are formed by the right portion. The median lobe has a wide base, surrounding almost half of the circumference of the vena cava (Madrakhimov, 2005).

The right liver lobe is located on the right side of the vena cava and consists also of two distinct portions, the right superior and the right inferior lobes. Together they represent about 15% of the liver volume. The upper right lobe (10-15%) is shaped like an egg sitting on the intrahepatic cava with the wide base extending to the paracaval part of the liver. Dorsal fixation consists of a wide hepatodiaphragmal ligament. The pyramidal shaped right inferior lobe with the tip pointing to the vena cava comprises 5-7% of the total liver mass and is dorsally attached to the diaphragm and on the ventral side to the cava (MacSween, 2002).

The caudate lobe, also called Spiegel lobe, is located on the left side of the cava below the left lateral lobe and represents 8-10% of the total liver mass. The lobe is divided into two portions – superior or upper lobe and inferior or lower caudate lobe. Both are thin and flat and show an oval shape. The upper caudate lobe is connected to the left lateral lobe via a thin interlobular ligament and is fully covered by the minor omentum. The lower portion of the caudate lobe is located behind the stomach in close neighborhood to the pancreas and spleen and is also covered by a fibrous capsule, attached to the dorsal wall of minor omentum (Madrakhimov, 2005).

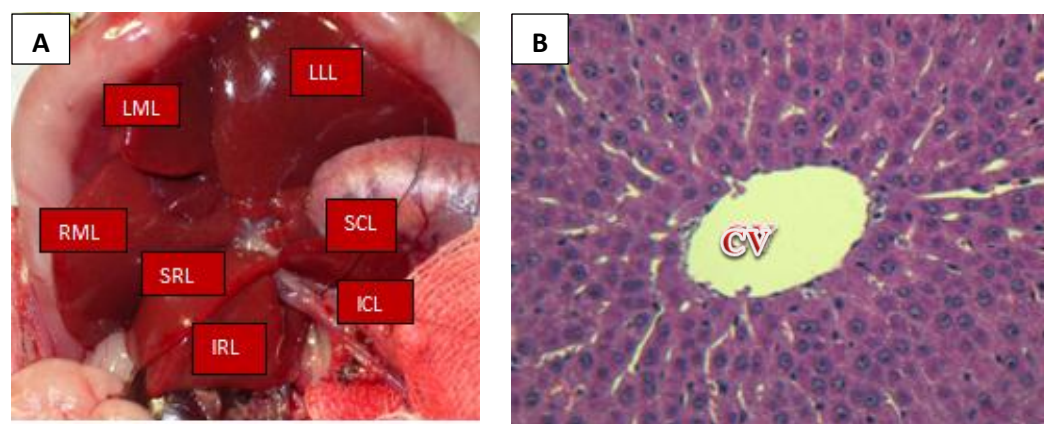


Figure 4: A gross anatomical lobes of rat liver in situ (A), LLL-Left lateral lobe, LML-Left median lobe, RML-Right median lobe, SRL-Superior right lobe, IRL-Inferior right lobe, SCL-Superior caudate lobe, ICL-Inferior caudate lobe and its histology (B), CV- Central vein taken from (Madrakhimov, 2005).

Despite the gross differences between liver of human and rat, their microscopic features are more or less similar. The liver is the largest gland in the body and it has both an endocrine and exocrine function. It is organized into lobes surrounded by a thick connective tissue capsule. Each lobe is subdivided into lobules by looser connective tissue (Glisson's capsule). In humans, this connective tissue does not completely outline the lobule. Blood enters the liver via the hepatic artery and portal veins, which send branches to the hepatic lobules (Ross, 2011).

The axis of histological lobule is the central vein, which is the beginning of hepatic vein. In this case, each lobule consists of plates of hepatic parenchymal cells that radiate out from the central vein. Separating the radial plates of the cells are the hepatic sinusoids, which are lined by endothelial cells. Kupffer cells (macrophages) span the sinusoids and attach themselves to the endothelial lining. The other cell type found in the persinusoidal space is the hepatic stellate cell (Ito cell), which is the primary storage site for hepatic vitamin A (Mescher, 2010).

Bile produced by the hepatic cells, is collected first in the small bile canaliculi and then in small bile ductules. It is carried away from the hepatic lobules in larger branches of the bile duct. The removal of bile from the liver via a duct system represents the exocrine function of the liver. A portal canal occurs in the connective tissue at the marginal angles of the lobules. Its three components, collectively known as portal triad, are branches of 1) hepatic artery 2) portal vein 3) bile duct. Lymph is collected in lymphatic vessels, which accompany the hepatic triad in the portal canal (Ross, 2011).

The important roles performed by the liver not only in the storage and release of nutrients, but also in the neutralization and elimination of a variety of toxic substances, have prompted investigations of its cellular constituents and organization. If toxins accumulate in the body faster than what liver could process them, then liver damage will result (Effendy, 2006)

1.4. Kidneys Histology and Functions

Kidneys are paired retroperitoneal organs situated in the posterior part of the abdomen on each side of the vertebral column. In the human, the upper pole of each kidney lies opposite the twelfth thoracic vertebra, and the lower pole lies opposite the third lumbar vertebra. The right kidney is usually slightly more caudal in position. The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness. Located on the medial or concave surface of each kidney is a slit, called the hilum, through which the renal pelvis, the renal artery and vein, the lymphatic, and a nerve plexus pass into the sinus of the kidney. The organ is surrounded by a tough fibrous capsule, which is smooth and easily removable under normal conditions (Boron and Boulpaep, 2012).

The kidney of the adult rat is bean – shaped, smooth, reddish-brown color and is covered by a thin connective tissue capsule that is adherent to sub-capsular connective tissue containing occasional fibroblasts. The rat kidneys lay alongside the vertebral column in the abdominal cavity, and the right kidney is situated more cranially than the left one (Al-Samawy, 2012)..

The mean weight of the adult rat right kidney is 1.1gm while, the left kidney is 0.96 gm. The mean length, width and thickness of right kidney is 1.28 cm, 0.88 and 0.81cm respectively while those of the left one is 1.23cm, 0.85 and 0.79cm respectively . Both the human and rat kidney consists of two regions, the outer cortex and the inner medulla. The basic unit of the kidney is the nephron. Each nephron can be subdivided into number of distinct parts in the cortex and medulla. Renal corpuscle is the first part of nephron and consists of glomerulus and Bowman's capsule, this is followed by the proximal convoluted tubules, loop of Henle which consists of ascending and then the descending limb of loop of Henle and distal convoluted tubules which is connected to the collecting tubules. The collecting tubules are not part of the nephron (Al-Samawy, 2012).

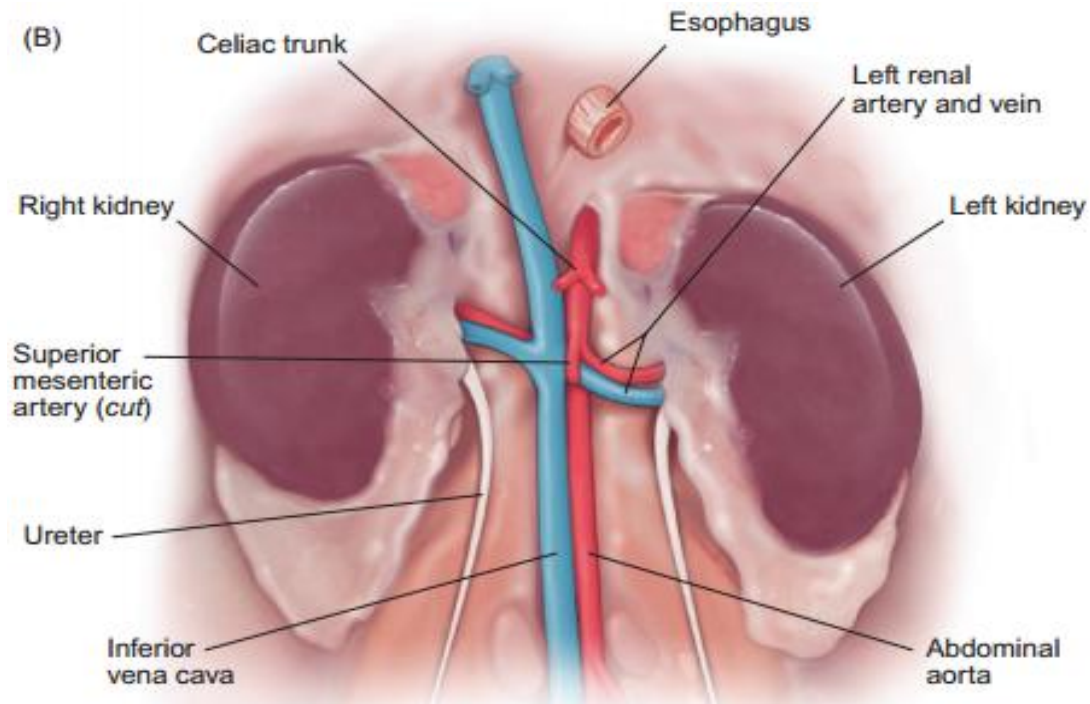


Figure 5: Rat kidneys *in situ* (Piper *et al.*, 2012)

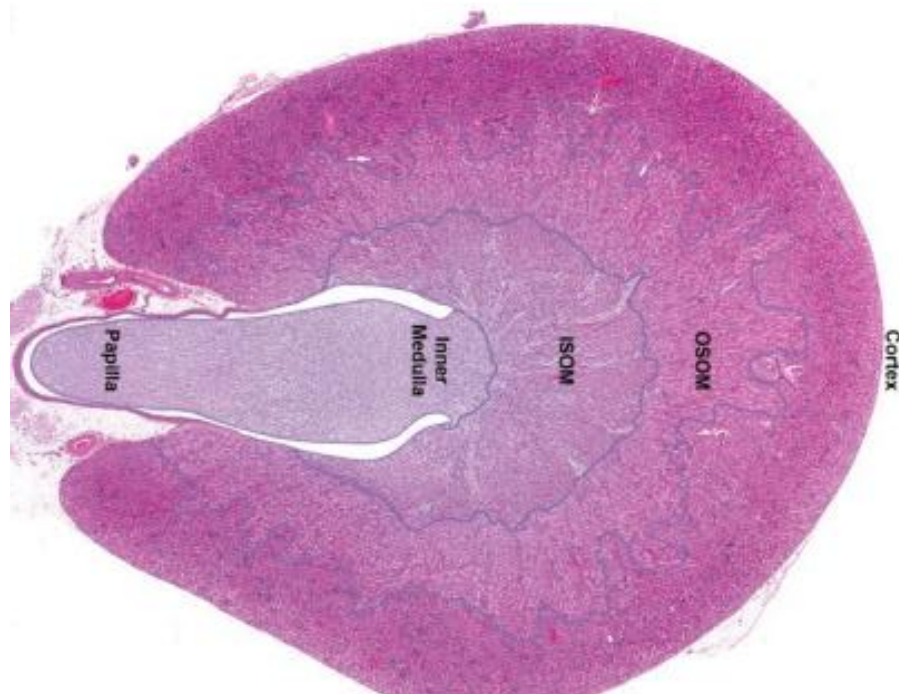


Figure 6: Demarcated regions of the kidney: cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM), inner medulla, papilla, and renal pelvis (Sands and Verlander, 2005)

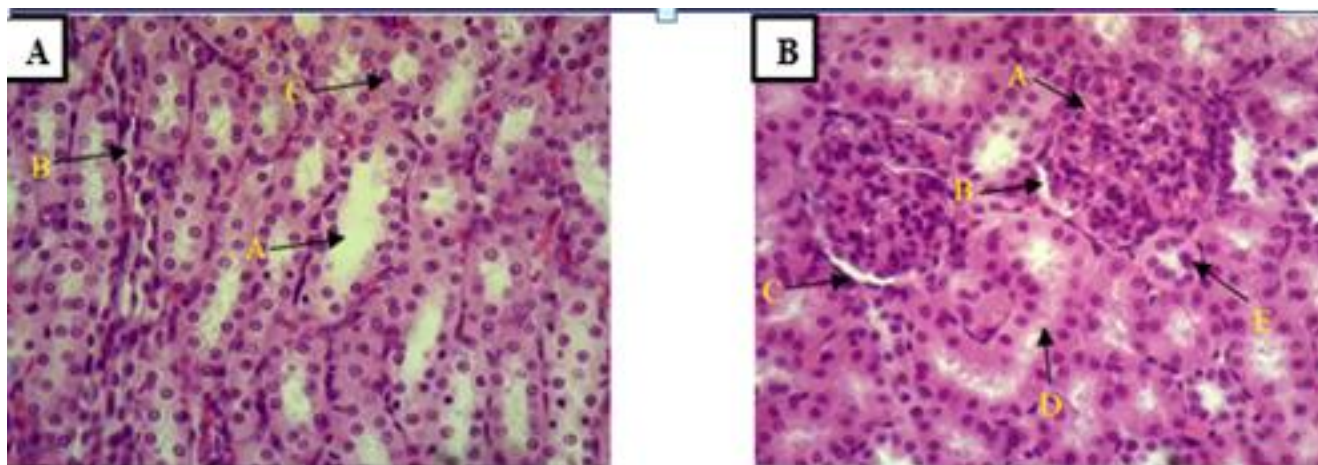


Figure 7: Kidney Histology of Rat taken from Al-Samway (2012)

A). A-collecting tubule, B-Thin limb, C-Thick limb: B) A-Glomerulus, B-Visceral layer, C-Parietal layer, D-Distal tubule, E-Proximal tubule; x400 (H &E).

The kidneys are one of the more important tissue examined for diagnostic purposes. Because of their role in the ultrafiltration, metabolism and excretion of compounds, they are often the site of test article induced lesions. In addition, as studied in the rats a wide range of spontaneous and age related diseases may be exacerbated by the chemical administration and is a confounding factor in the interpretation of renal toxicological and carcinogenic findings (Sands and Verlander, 2005). Several strains have been developed for studying genetic diseases, neuroanatomy, nutritional disorders, diabetes, hypertension, and others. The rat is also the most important animal used as a model for biological experiments in research laboratories worldwide (Pannabecker et al., 2004).

As one of the most heterogeneous tissues in the body, the kidney has a wide variety of cell types. For the most part, renal tubules and/or ducts comprise most of the renal parenchyma and are lined by specialized epithelial cells. Renal interstitial tissue is sparse in the cortex and gradually increases toward the papilla. The distribution of the renal vasculature is uniquely suited to supply more blood to the energy-active cortex (Sands and Verlander, 2005).

1.5. Significance of the Study

The leaves of *Moringa* have been used in the traditional medicine passed down for centuries in different cultures. As the *Moringa* tree has spread from the Indian sub-continent throughout the tropical and sub-tropical world, it has adapted itself to local conditions, resulting in many variations and widespread utilization. *Moringa* species have also recently attracted interest in the modern scientific community for their medicinal and nutritional purposes (Ghosh, 2014). Most of the studies are either nutritional analyses or efficacy on laboratory animals. However, there are only limited studies that investigated the safety and toxicity of *M. stenopetala* aqueous leaf extract. Ghebreselassie *et al.* (2011) reported that liver of mice treated at doses of 750 and 900 mg/kg body weight of the extract showed some mixed inflammatory cell infiltrations (mainly lymphocytes and eosinophils) around the portal triad and slight activation of Kupffer cells as compared with the control group. No significant histological changes were reported on liver of mice treated at a dose of 600 mg/kg body weight. Moreover, no significant histopathological changes were reported on the kidney tissues of mice treated at all doses of the extract after sub chronic (six weeks) treatment (Ghebreselassie *et al.*, 2011).

Musa *et al.* (2015) reported that in the acute toxicity study, rats treated with butanol fraction of *M. stenopetala* upto dose of 5000 mg/kg body weight showed no toxic signs on behavior, gross pathology, and body weight, as compared with the controls. Furthermore, Debela (2015) reported that the aqueous extract of *M. stenopetala* did not produce adverse effects on the experimental rats after acute and sub chronic treatments. However, all these studies related to acute and/or sub chronic administration of the extract and did not see if there is similar results following prolonged administration of the extract .

Investigation of the safety of the plant following a prolonged treatment with the plant is important as it is widely used for an extended period of time for its claimed traditional therapeutic values against many chronic diseases, such as hypertension and diabetes. Therefore, this study aimed to investigate if there is any effect on histopathology of liver and kidney and blood parameters following chronic administration of the extract on Wistar rats. The expected outcome of the study is hoped to contribute evidence on safety of the *M. stenopetala* aqueous leaves extract for its possible utilization for the various claims of its medicinal and nutritional values

2. Objectives

2.1. General objective

- ✓ To investigate the effects of *Moringa stenopetala* aqueous leaves extract on histology of Liver and Kidney, and determine hematological and biochemical parameters of Wistar rats upon chronic administration.

2.2. Specific objectives

- ✓ To assess the effects of extract on the general body weight.
- ✓ To assess the effects of extract on the organ weight particularly kidney and liver.
- ✓ To examine the general behavior of rats
- ✓ To evaluate the chronic toxic effect of *Moringa stenopetala* on histology of liver and kidney of Wistar rats.
- ✓ To explore any Hematological and biochemical changes on blood profiles at different doses of aqueous leaves extract.

3. Materials and Methods

3.1. Study Design

In this comparative experiment twenty four white Wistar rats were randomly divided into four groups of six animals each. Out of four groups, the first three groups I, II, III were the treated groups administered with *M. stenopetala* leaves extract suspended in distilled water orally with 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight respectively. The last group (group IV) was the control and administered with distilled water orally.

3.2. Study Area

The study was conducted at Traditional and Modern Medicine Research Directorate of EPHI and Addis Ababa University, College of Health Science, Department of Anatomy (Histology Laboratory).

3.3. Study period

The study was conducted from May 2016-October 2017.

3.4. Collections and extraction of plant materials

The fresh leaves of *Moringa stenopetala* were collected from Arba Minch, 502 km far to Southwest of Addis Ababa. Then the leaves were identified and authenticated by a taxonomist at EPHI. Fresh leaves were cleaned from extraneous materials, dried under shade light at room temperature, and ground by manual crusher to obtain fine particles (Debella, 2002).

The powdered leaves (200 gm of *Moringa stenopetala*) were macerated with distilled water in 2 liter flasks for 4hrs with intermittent agitation by orbital shaker. Then, the supernatant of agitated materials were separated from the un-dissolved portion of the plant. The supernatant portions were filtered with 0.1 mm² mesh gauze and whatman filter paper. The filtrate of the plant were freeze-dried at lower temperature (-46°C -51°C) and lower pressure (133x10⁻³ mbr) to form crude extract. A yield of 34 gm (17%) was obtained. It was kept in desiccators at room temperature until used (Debella, 2002).

3.5. Selection and Preparation of experimental animals

Normal Wistar rats were obtained from EPHI. Both sexes of healthy young adult Wistar rats of 8-12 week old were employed. Females were nulliparous and non-pregnant. They were randomly grouped into experimental and control groups. They were kept under standard conditions (temperature of 22°C ($\pm$ 3°C), relative humidity of 50-60%, with 12h light / 12h dark cycle,) (OECD, 2009). For feeding, conventional rodent laboratory diets were used with an unlimited supply of drinking water (*Ad libitum*). Then the animals were acclimatized to laboratory conditions for one week prior to the experiment to alleviate any non-specific stress (OECD, 2009).

3.6. Method of extract administration

The different doses of aqueous leaves extract of *M. stenopetala* for the treatment groups and distilled water for the control group were administered orally using intragastric catheter. Following the period of fasting, the animals were weighed weekly and the dose was calculated according to the body weight, and the test substances were administered each day (Dapar, *et al.*, 2007). All equipment were cleaned and placed in an oven after each administration to prevent any contamination.

3.6. Chronic toxicity study

The study was carried out using 24 rats randomly assigned in to four groups, one control and three experimental (each contains 6 rats, three males and three females). Rats in group I, II and III, respectively received an aqueous leaves extract of *M. stenopetala* at doses of 500, 1000, and 2000 mg/Kg body weight orally per day for consecutive 6 months.

3.7. Data Collection Method

Data were collected from the experimental animals before and after they were sacrificed.

3.7.1. Body weight measurement

Body weight of all experimental and control animals were recorded using digital electronic balance sensitive to 0.001 g (Precisa 125A, Switzerland) before commencing of the first oral administration and then weekly until the last day of oral administration of the extract.

3.7.2. Cage side observation

In each cage, animals were carefully observed individually before and after giving the different doses periodically for any changes in skin and fur, eyes, respiratory effects, autonomic effects (such as salivation), diarrhea, urination and CNS effects (such as tremors) (OECD, 2009).

3.7.3. Blood Samples collection

At the end of experiment, the experimental animals were fasted overnight, cervical dislocations were made and blood samples were collected into sterile tube by cardiac puncture. Part of the blood samples collected from each rat was then put in separate test tube with an anti-coagulant substance, EDTA and plain test tube without EDTA.

Hematological parameters such as White blood cells (WBCs) and its differential, Red blood cells (RBCs), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Platelets were analyzed from the blood samples collected with EDTA immediately by using Automated Hematological Analyzer Cell-DYN (SYSMEX RX 21, Japan). For biochemical analysis, the blood samples in the plain test tubes were allowed to stand for 2 hours for complete clotting and centrifuged at 5000 rpm for 10 minutes using a bench top centrifuge machine (HUMAX-K, HUMAN-GmbH, Germany). The serum from each sample was withdrawn through micropipette and transferred into other clean vial tube to analyze the function of liver and kidney. It was then kept at -20°C until the analysis was done. The concentrations of ALP, ALT, AST, total Bilirubin, Urea and Creatinine were determined by using (AUTO LAB 18, Clinical Chemistry Analyzer, Italy).

3.7.4. Organs Weight Measurements and Tissue processing

Target organs studied were dissected and weighed with the precision electronic digital balance (Precisa 125A, Switzerland).

Sample tissues from median lobe of liver and left kidney were collected randomly and fixed in a test tube containing 10% buffered formalin. The fixed tissues were dehydrated and cleared in ascending graded series of ethanol and xylene, respectively.

The tissues were infiltrated with molten paraffin wax and embedded in paraffin blocks. The blocks were sectioned at 5 µm thickness and fixed onto clean frosted slide glasses. The thin sections were stained with hematoxylin and eosin. Finally, the prepared slides were examined under Binocular compound light microscope for any possible histopathological findings and then were photographed under X400 total magnification using Photomicrograph (Leica ICC50, China).

3.8. Data processing and analysis

All data were entered and analyzed by SPSS 21 version of statistical software. All values of parameters were expressed in mean±SEM. Treatment over time were compared between control and treated groups by analysis of covariance. The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett test to identify possible difference of body, liver and kidney weight, and hematological and biochemical values. P values <0.05 are considered statistically significant.

3.9 Ethical Consideration

The study was conducted after obtaining ethical clearance from Department of Anatomy, College of Health Sciences and then EPHI. Animals that were used in this study were kept from any unnecessary painful and terrifying situations (OECD, 2009). To keep the pain and suffering minimal during extract administration and final sacrifice all procedures were done by well-trained person.

Animals were protected from pathogens; and placed in appropriate environment. The number of animals in each cage and room was reduced to the minimum consistent with achieving the scientific objectives of the study (OECD, 2009).

3.10 Communication of the Results

The result of the study will be trimmed in the form of thesis, and communicated to all concerned institutions including the department of Anatomy, SoM, AAU, Traditional and Modern Medicine Research Directorate (EPHI) and to advisors and published with reputable journal for the public.

4. Results

4.1. Effect of chronic oral administration of *M. stenopetala* aqueous leaves extract on general health, body weight and food intake

Cage side observations of the animals were carried out daily after administration of both the vehicle and the *M. stenopetala* leaves extract at doses of 500, 1000 and 2000 mg/kg body weight for general signs of abnormalities throughout the study period. During the first and second weeks, the rats treated with the extract at 2000 mg/kg body weight revealed low locomotion, weakness, dizziness, shivering and piloerection. During the third week, these symptoms disappeared and the animals completely recovered. Moreover, there was no morbidity and death throughout the study period. Rats that received high doses reduced their food intake during the first three weeks of the study.

As shown in the Tables 1 and 2, there were progressive body weight gains in nearly all groups of rats of both sexes with time over the whole period of experiment except female and male rats received 2000 mg/kg body weight decreased significantly in the first 2 and 3 weeks as compared with the controls respectively. No significant difference ($P>0.05$), however, was recorded in the pattern of body weight gain among the different groups of rats in both experimental groups and the controls.

Table 1: Mean body weight (gram) of male rats administered with 500, 1000 and 2000 mg/kg of the extract during the consecutive 24 weeks measurement as compared to the controls

Week	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
Initial mean body weight	139.67±4.63(1.000)	130.67±5.17(0.098)	118.67±1.76(0.062)	139.67±0.67
1	150.13±5.48(0.835)	133.9±7.86(0.307)	129.67±2.6(0.045)*	145.33±0.33
2	166.67±3.18(0.917)	144±3.06(0.024)	140±5.77(0.009)*	163.67±3.93
3	153.58±3.95(0.697)	161±5.69(0.379)	164.33±5.36(0.639)	171.67±2.85
4	182±3.46(0.692)	182±7.21(0.692)	182.33±4.37(0.660)	176±1.15
5	186±3.78(0.887)	192.67±3.67(0.219)	191.67±4.80(0.286)	183±1.53
6	206.67±3.76(0.638)	204.67±6.64(0.788)	193.67±8.67(0.895)	198.33±1.76
7	219.33±2.91(0.619)	217.67±2.90(0.465)	224±5.77(0.978)	226±0.57
8	241±3.06(0.292)	225±5.77(0.468)	236.33±3.67(0.767)	232±1.53
9	250.67±1.45(0.875)	235.33±9.38(0.558)	249±7.77(0.952)	245.33±1.85
10	265±2.31(0.790)	245.67±9.84(0.544)	265.67±9.68(0.750)	257.33±2.40
11	267.33±0.33(0.996)	251±8.96(0.232)	270.33±10.17(0.998)	269±3.05
12	285±2.08(0.752)	261.67±12.02(0.369)	282.33±6.06 (0.897)	276.67±4.25
13	290.67±0.88(0.887)	269.67±10.65(0.173)	291.67±3.33(0.822)	286±1.73
14	285.67±5.21(0.736)	271.67±7.26(0.071)	283.67±6.12(0.576)	292.33±2.18
15	293.33±4.37(0.591)	286.33±6.36(0.154)	291.67±4.41(0.448)	300.67±3.53
16	300.33±3.18(0.977)	291.67±4.98(0.187)	297.33±3.18(0.709)	302±3.05
17	292.67±7.42(0.087)	295.33±5.04(0.152)	295.67±2.33(0.163)	310.33±3.67
18	300±5.51(0.137)	303.33±4.37(0.304)	302.67±2.85(0.261)	313±3.51
19	303.67±6.98(0.057)	303.67±6.49(0.057)	310.33±3.18(0.214)	324.33±2.96
20	310±5.77(0.126)	312.33±18.33(0.192)	316±4.16(0.360)	328±2.64
21	313.33±5.78(0.149)	312±11.15(0.121)	319.33±5.21(0.364)	333.33±3.33
22	320±4.93(0.067)	321.67±7.79(0.093)	326±4.36(0.210)	340.67±3.84
23	324.67±3.53(0.235)	323.67±7.53(0.201)	332.67±5.21(0.676)	341.33±8.41
24	339.67±2.91(0.968)	334.33±5.29(0.942)	345.67±4.81(0.596)	342.33±7.17

Effect of *M. stenopetala* aqueous extract on body weight of various groups of male rats (expressed as mean± SDE, N=3). The figures under brackets indicate the P-values, N= Number of rats per group;*= significant (P<0.05).

Table 2: Mean body weight (gram) of female rats administered with 500, 1000 and 2000 mg/kg of the extract during the consecutive 24 weeks measurement as compared to the controls.

Week	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
Initial mean body weight	159.33±3.71 (.274)	141±12.34 (.996)	116.67±4.40 (.068)	142.66±2.03
1	163±1.00(.731)	146.67±11.92 (.709)	120±5.19 (.014)*	155±1.73
2	166.67±4.7(.953)	165.67±0.88 (.990)	130.33±5.89(.001)*	164.33.67±2.33
3	158.33±4.26(.281)	171.67±2.03(.857)	137±6.25(.002)*	168±2.00
4	166.67±11.57(.803)	182.33±4.40(.742)	151.67±6.01(.126)	174±2.522
5	170.33±6.33(0.562)	191.33±2.60(0.124)	167±5.13(0.289)	177.33±1.76
6	188±5.13(0.926)	198±4.04(0.111)	181.33±2.73(0.804)	185.33±2.91
7	182±1.15(0.199)	201.67±4.48(0.498)	184±6.93(0.311)	194±2.65
8	195.67±.67(0.731)	215.33±5.78(0.116)	197.67±5.46(0.907)	201±3.60
9	201.67±1.76(1.000)	216.33±4.67(0.058)	201.33±4.67(1.000)	201±3.78
10	210.33±3.18(0.997)	225.33±5.78(0.153)	212.33±6.33(0.997)	211.33±1.76
11	207.67±4.67(0.285)	221.33±5.78(0.998)	216.67±6.94(0.928)	220.33±3.18
12	214.67±3.76(0.256)	239±4.93(0.178)	224.67±5.49(0.993)	226±3.79
13	224±4.51(0.842)	237.33±7.42(0.674)	231.33±6.89(0.993)	229.67±2.60
14	214.67±3.84(0.092)	231.67±3.3(0.981)	231±9.29(0.962)	234±3.21
15	229.33±5.67(0.778)	235±4.73(1.000)	243±5.69(0.507)	234.67±2.40
16	220±5.00(0.406)	229.33±6.35(0.950)	237.67±10.11(0.938)	233.33±3.33
17	221.67±5.36(0.081)	233.67±0.88(0.681)	241.67±8.84(0.998)	240.67±1.76
18	224.33±5.67(0.072)	234.67±4.48(0.468)	245±8.19(1.000)	244.67±0.33
19	230.67±5.00(0.073)	240.33±2.33(0.570)	248±7.94(1.000)	248±0.58
20	236.67±4.91(0.174)	242.33±4.33(0.566)	249.67±5.81(1.000)	249.33±0.88
21	238.67±5.55(0.228)	253.67±4.18(0.991)	253.33±6.64(0.995)	252±3.51
22	239±7.81(0.236)	247.33±6.23(0.648)	256.33±8.99(0.999)	257.33±4.70
23	244.67±5.24(0.148)	254.67±6.74(0.578)	261.33±9.53(0.953)	265.33±4.70
24	251±3.78(0.095)	261±6.35(0.455)	265±9.24(0.728)	272.67±4.09

Effect of *M. stenopetala* aqueous extract on body weight of various groups of male rats (expressed as mean± SEM, N=3). The figures under brackets indicate the P-values, N= Number of rats per group;*= significant (P<0.05).

4.2. Effects of chronic administration of *M. stenopetala* aqueous leaves extract on organ weights

Postmortem macroscopic examination of the dissected liver and kidneys did not reveal any gross abnormalities except the liver of one male rat that received 2000 mg/kg of the extract, which showed a single white spot on the median lobe. Wet absolute weights of liver and kidney of both treated and control groups are shown in table 3 and 4. No significant difference ($p < 0.05$) was noted among the rats treated with the extract at doses of 500, 1000 and 2000 mg/kg body weight and control rats of either sex.

Table 3: Mean liver and kidney weights of male rats (gram) chronically administered with crude extract of *M. stenopetala* as compared to the controls.

Dose	Liver	Kidney (single)
500mg/kg	11.14±0.75(0.654)	1.09±0.08(0.700)
1000mg/kg	10.87±0.35(0.451)	1.0±0.01(0.302)
2000mg/kg	12±0.46(0.999)	1.06±0.03(0.549)
Control	11.48±0.62	1.19±0.13

Mean weight (gram; mean $\pm$ SEM; N=3). The figures under brackets indicate the P-values, N= Number of rats per group.

Table 4: Mean liver and kidney weight of female rats (gram) chronically administered with crude extract of *M. stenopetala* as compared to the controls.

Dose	Liver	Kidney (single)
500mg/kg	10.65±1.31(1.000)	0.91±0.01(0.435)
1000mg/kg	8.98±0.61(0.427)	0.87±0.61(0.235)
2000mg/kg	7.52±0.37(0.077)	0.81±0.46(0.069)
Control	10.63±0.82	1.00±0.66

Mean weight (gram; mean $\pm$ SEM; N=3). The figures under brackets indicate the P-values, N= Number of rats per group.

4.3. Effects of *M. stenopetala* aqueous leaves extract on hematological parameters

The effects of 24 weeks oral administration of *M. stenopetala* on hematological parameters of the rats are illustrated in Table 3 and 4. As indicated, chronic treatment with 500, 1000 and 2000 mg/kg body weight of the leaf of aqueous extracts did not significantly affect ($p>0.05$) almost any of the hematological parameters investigation. However, it induced significant ($p<0.05$) elevation in MCV of female rats at all doses when compared with the control. There is also decrement of MCH at doses of 1000 mg/kg and 2000 mg/kg significantly, ($p<0.05$) as compared to the control in male rats.

Table 5: Effects of chronic administration 500 mg/kg, 1000 mg/kg and 2000 mg/kg aqueous leaf extract of *M. stenopetala* on hematological parameters in male rats as compared to the controls (expressed as mean $\pm$ SEM).

Hematological parameters	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
WBC($\times 10^3/\mu\text{L}$)	8.7 $\pm$ 3.14(.890)	6.3 $\pm$ 0.67(.992)	6.3 $\pm$ 1.01(.991)	6.99 $\pm$ 0.50
NEUT($\times 10^3/\mu\text{L}$)	1.09 $\pm$ 0.18(.558)	1.14 $\pm$ 0.26(.445)	0.96 $\pm$ 0.03(.850)	0.78 $\pm$ 0.07
LYMPH($\times 10^3/\mu\text{L}$)	6.96 $\pm$ 2.62(.930)	4.41 $\pm$ 0.79(.919)	4.89 $\pm$ 0.89(.977)	5.72 $\pm$ 0.61
MONO($\times 10^3/\mu\text{L}$)	0.57 $\pm$ 0.24(.709)	0.60 $\pm$ 0.08(.605)	0.38 $\pm$ 0.11(.999)	0.35 $\pm$ 0.04
EO($\times 10^3/\mu\text{L}$)	0.17 $\pm$ 0.04(.792)	0.15 $\pm$ 0.05(.911)	0.03 $\pm$ 0.01(.614)	0.11 $\pm$ 0.61
BASO($\times 10^3/\mu\text{L}$)	0.05 $\pm$ 0.01(.898)	0.03 $\pm$ 0.01(.952)	0.04 $\pm$ 0.03(1.000)	0.04 $\pm$ 0.01
RBC($\times 10^6/\mu\text{L}$)	10.01 $\pm$ 0.57(0.603)	9.09 $\pm$ 0.88(1.000)	10.03 $\pm$ 0.22(.613)	9.07 $\pm$ 0.17
HGB(g/dl)	18.5 $\pm$ 0.85(.819)	16.23 $\pm$ 1.78(.898)	18.4 $\pm$ 0.42(.851)	17.23 $\pm$ 0.37
HCT (%)	57.57 $\pm$ 2.92(.428)	50.27 $\pm$ 4.21(.989)	56.23 $\pm$ 1.45(.613)	51.43 $\pm$ 0.95
MCV(fL)	57.33 $\pm$ 0.54(.945)	55.47 $\pm$ 1.39(.669)	56.03 $\pm$ 0.44(.917)	56.73 $\pm$ 0.03
MCH(pg)	18.47 $\pm$ 0.23(.259)	17.83 $\pm$ 0.27(.010)*	17.89 $\pm$ 0.07(.011)*	19 $\pm$ 0.10
MCHC(g/dL)	32.17 $\pm$ 0.39(.479)	32.13 $\pm$ 1.17(.460)	32.73 $\pm$ 0.15(.823)	33.5 $\pm$ 0.20
PLC($\times 10^3/\mu\text{L}$)	339.67 $\pm$ 229.7(.553)	351.67 $\pm$ 208.9(.580)	720.3 $\pm$ 93.2(.996)	669.3 $\pm$ 110.1

The figures under brackets indicate the P-values, N= Number of rats per group;*= significant ($P<0.05$).

Table 6: effects of chronic administration 500 mg/kg, 1000 mg/kg and 2000 mg/kg aqueous leaf extract of *M. stenopetala* on hematological parameters in female rats as compared to the controls (expressed as mean $\pm$ SEM).

Hematological parameters	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
WBC($\times 10^3/\mu\text{L}$)	5.10 $\pm$ 1.36(.967)	8.49 $\pm$ 0.86(.110)	4.18 $\pm$ 1.43(.996)	4.48 $\pm$ 1.11
NEUT($\times 10^3/\mu\text{L}$)	0.40 $\pm$ 0.08(.339)	0.84 $\pm$ 0.12(.965)	0.71 $\pm$ 0.38(.813)	0.98 $\pm$ 0.35
LYMPH($\times 10^3/\mu\text{L}$)	4.48 $\pm$ 1.29(.576)	6.05 $\pm$ 0.92(.131)	3.09 $\pm$ 1.05(.999)	5.72 $\pm$ 0.61
MONO($\times 10^3/\mu\text{L}$)	0.23 $\pm$ 0.03(.705)	0.60 $\pm$ 0.29(.381)	0.27 $\pm$ 0.05(.867)	0.37 $\pm$ 0.13
EO($\times 10^3/\mu\text{L}$)	0.12 $\pm$ 0.01(.781)	0.19 $\pm$ 0.02(.541)	0.09 $\pm$ 0.04(.567)	0.18 $\pm$ 0.10
BASO($\times 10^3/\mu\text{L}$)	0.06 $\pm$ 0.01(.732)	0.01 $\pm$ 0.06(.299)	0.02 $\pm$ 0.003(.998)	0.02 $\pm$ 0.01
RBC($\times 10^6/\mu\text{L}$)	8.03 $\pm$ 0.33(0.955)	8.73 $\pm$ 0.35(1.000)	7.21 $\pm$ 1.62(.685)	8.66 $\pm$ 1.38
HGB(g/dl)	15.7 $\pm$ 0.72(.928)	16.53 $\pm$ 0.48(.997)	15.67 $\pm$ 0.81(.918)	16.33 $\pm$ 1.41
HCT (%)	47.47 $\pm$ 2.24(.989)	52.23 $\pm$ 2.13(.754)	41.47 $\pm$ 8.62(.922)	45.5 $\pm$ 6.77
MCV(fL)	59.10 $\pm$ 0.79(.010)*	59.77 $\pm$ 1.13(.006)*	58.20 $\pm$ 1.60(.023)*	52.77 $\pm$ 0.74
MCH(pg)	19.53 $\pm$ 0.27(1.000)	18.97 $\pm$ 0.20(.999)	24.33 $\pm$ 5.79(.562)	19.47 $\pm$ 1.84
MCHC(g/dL)	33.07 $\pm$ 0.07(.889)	31.77 $\pm$ 0.52(.779)	41.3 $\pm$ 8.55(.821)	36.77 $\pm$ 2.89
PLC($\times 10^3/\mu\text{L}$)	120 $\pm$ 31.34(.703)	590 $\pm$ 113.66(.125)	256 $\pm$ 139.4(1.000)	254.67 $\pm$ 103.89

The figures under brackets indicate the P-values, N= Number of rats per group;*= significant (P<0.05).

4.4. Effects of *M. stenopetala* aqueous leaves extract on serum biochemical parameters

Effects of chronic oral treatment with aqueous leaves extract of *M. stenopetala* on serum biochemical parameters of rats are shown in Table 7 and 8. There was no significant ($p>0.05$) difference in ALP, AST, ALT and total bilirubin in female rats administered at all doses as compared to the controls. However, the ALP of male rats that received 2000 mg/kg body weight, AST and ALT of male rats that received 1000 and 2000 mg/kg body weight were found significantly ($p<0.05$) increased as compared to the controls. Chronic treatment with 500, 1000 and 2000 mg/kg body weight of the leaf of aqueous extract did not significantly affect ($p>0.05$) almost any of the urea and creatinine investigations in both sexes. However, female rats that administered the extract at 2000 mg/kg orally have shown significant change ($p<0.05$) decrease in Urea and increase in Creatinine levels as compared to the controls.

Table 7: Effect of 500, 1000 and 2000 mg/kg aqueous leaves extract of *M. stenopetala* on biochemical parameters of male rats as compared to the control group; (expressed in mean± SEM, N=3)

Biochemical parameters	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
ALP (IU/L)	109.33±8.65(.057)	109.67±16.76(.056)	119.67±21.67(.030)*	44.33±16.17
ALT (IU/L)	122.47±36.46(.187)	149.27±33.10(.035)*	136.07±42.74(.042)*	88.73±14.56
AST (IU/L)	274.67±97.95(.157)	521±57.27(.032)*	316.03±103.60(.048)*	196.9±48.54
Urea (g/dl)	46.5±1.21(.879)	49.67±2.48(.303)	38.67±0.45(.193)	44.67±3.30
Creatinine (mg/dl)	0.33±0.05(.668)	0.24±0.03(.787)	0.28±0.01(.998)	0.28±0.03
Bilirubin (mg/dl)	4.59±0.14(.994)	4.09±0.15(.184)	4.53±0.03(.810)	4.79±0.45

The figures under brackets indicate the P-values, N= Number of rats per group;*= significant (P<0.05).

Table 8: Effect of 500, 1000 and 2000 mg/kg aqueous leaves extract of *M. stenopetala* on biochemical parameters of female rats as compared to the control group; (expressed in mean± SEM, N=3)

Biochemical parameters	500 mg/kg	1000 mg/kg	2000 mg/kg	Control
ALP (IU/L)	84±22.05(.538)	79.33±6.64(.475)	144±49.52(.989)	133±24.7
ALT (IU/L)	175±54.17(.980)	96.33±14.78(.367)	93.43±12.41(.337)	161.73±23.16
AST (IU/L)	712.2±204.09(.438)	396.53±129.72(.990)	274.6±109.2(.733)	444.57±90.97
Urea (g/dl)	40.43±2.71(.075)	39.87±3.58(.062)	38.9±1.99(.044)*	51.53±3.49
Creatinine (mg/dl)	0.19±0.02(.997)	0.26±0.03(.397)	0.34±0.02(0.023)*	0.2±0.05
Bilirubin (mg/dl)	4.56±0.52(.887)	4.29±0.56(.999)	4.53±0.38(.922)	4.34±0.120

The figures under brackets indicate the P-values, N= Number of rats per group;*= significant (P<0.05).

4.5. Effect of *M. stenopetala* aqueous leaves extract on histology of liver

Histological examination of liver sections of rats treated with the aqueous leaves extract of *M. stenopetala* at a dose of 500 mg/kg body weight as shown in figures (8A and B) had a normal architectures with normal appearance of the hepatic plates, central vein and hepatic sinusoids lined by endothelial and Kupffer cells similar to the controls (Figure 8C and D). The hepatocytes appeared normal in size and shape, and no vacuoles were noted in their cytoplasm.

Rats treated with the extract at 1000 mg/kg (Figure 8E and F) and 2000 mg/kg (Figure 8G, H, I and J) body weight doses however, showed signs of bile retention indicating for induction of cholestasis (biliary stasis). These were observed as yellowish discoloring of hepatocytes (intrahepatic bile retention) and deposits in the bile canaliculi between the individual hepatocytes (extrahepatic bile retention). Moreover, around the central veins and portal triads, there were mononuclear leukocytic cells infiltration (Figure 8E and F) and cytoplasmic vacuolization, congestion of sinusoids and hydropic changes (Figure 8E, F and I), revealing mild hepatotoxicity. On the other hand, the liver lobules showed normal architecture; hepatocytes and Kupffer cells were also morphologically appeared normal. Furthermore, there was no treatment related changes in diameter and appearance of central veins, sinusoids and portal veins.

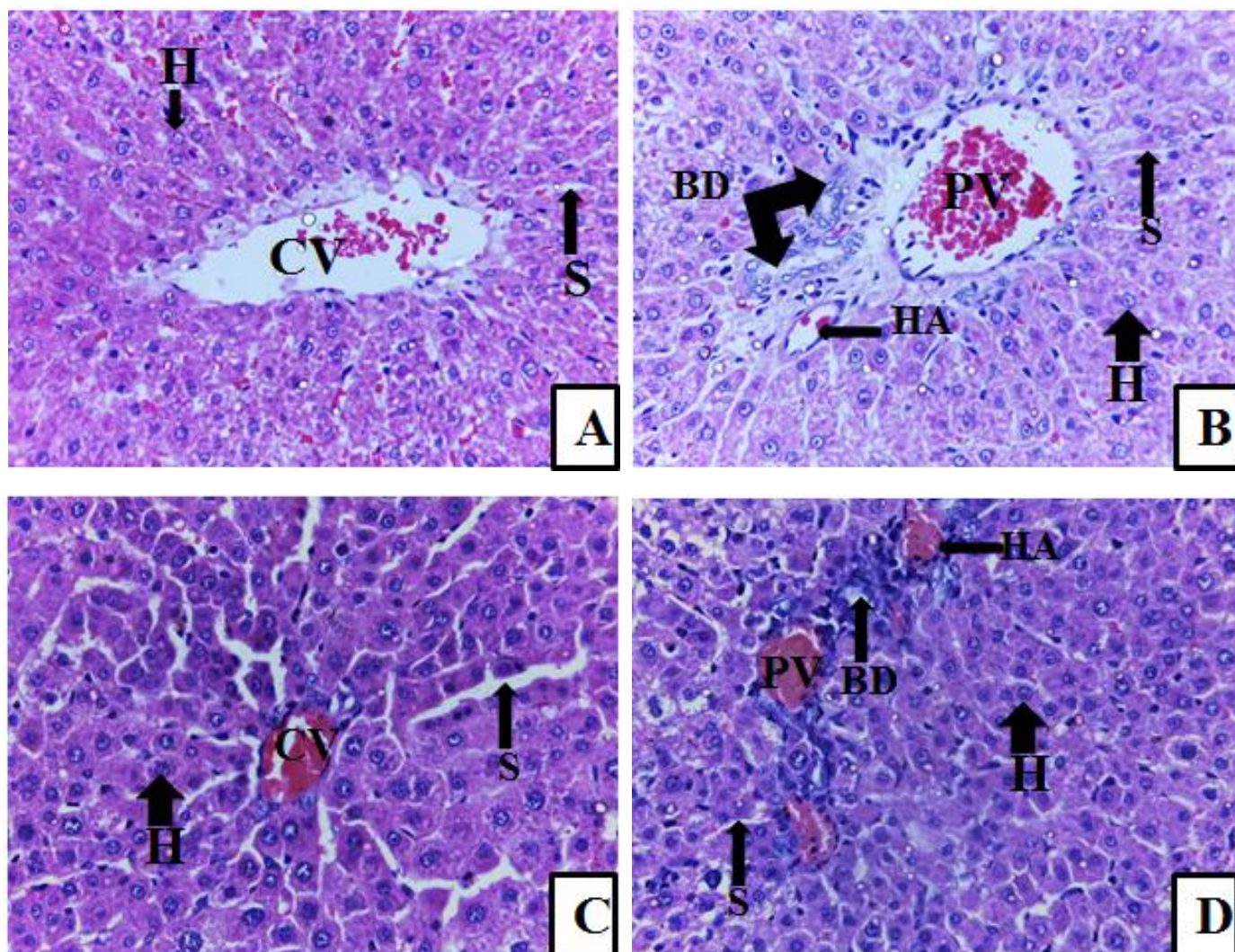


Figure 8: Photomicrographs of rat liver sections of control (A&B), and rat treated with 500 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (C&D) for 24 weeks. Sections are taken from male rats. CV= Central vein, S= Sinusoids, H= Hepatocyte, PV= Portal vein, HA= Hepatic artery, BD= Bile duct (H & E, X400).

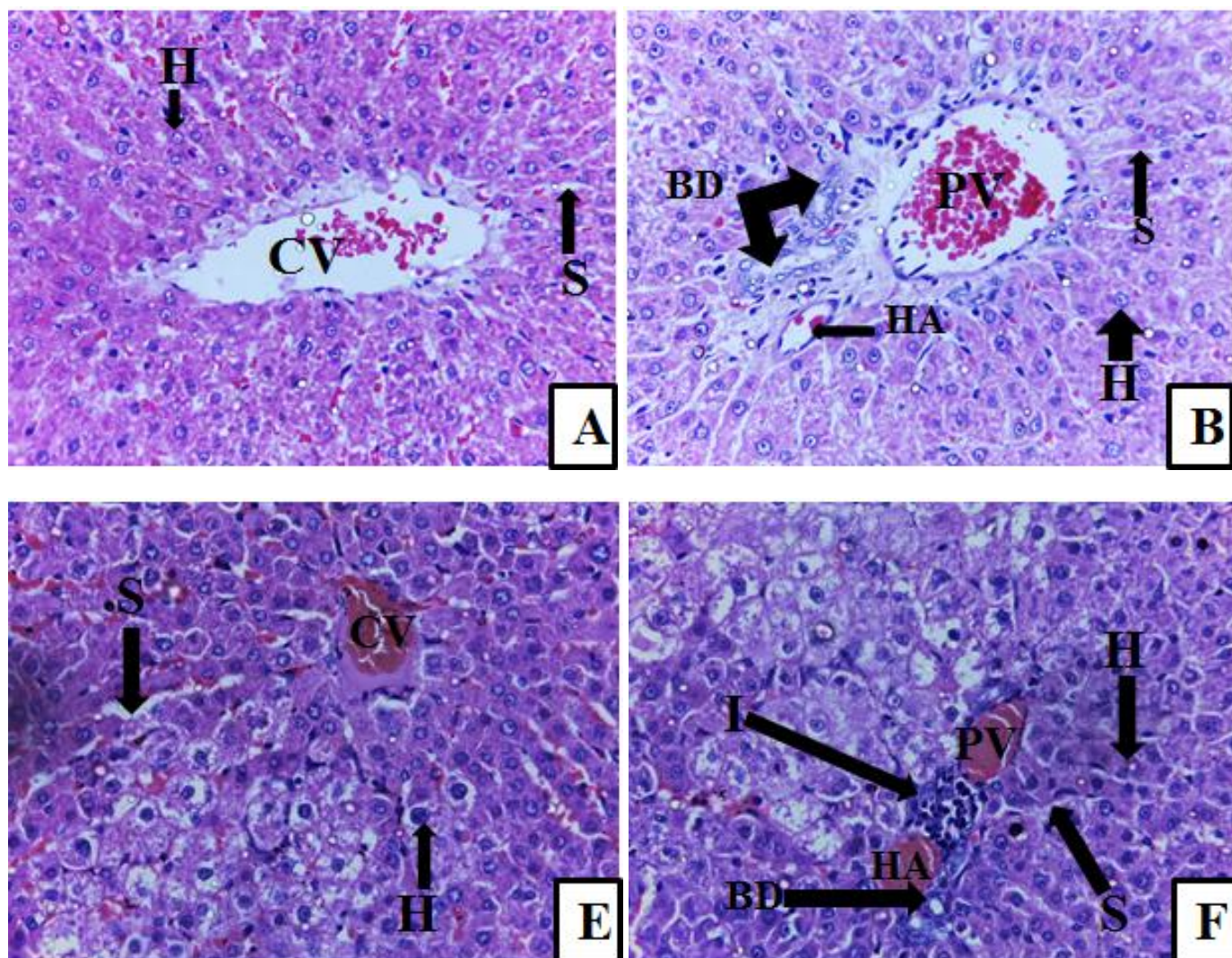


Figure 9: Photomicrographs of rat liver sections of control (A&B), and rat treated with 1000 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (E&F) for 24 weeks. Sections are taken from male rats. CV= Central vein, S= Sinusoids, H= Hepatocyte, PV= Portal vein, HA= Hepatic artery, BD= Bile duct, I=Infiltrations (H & E, X400).

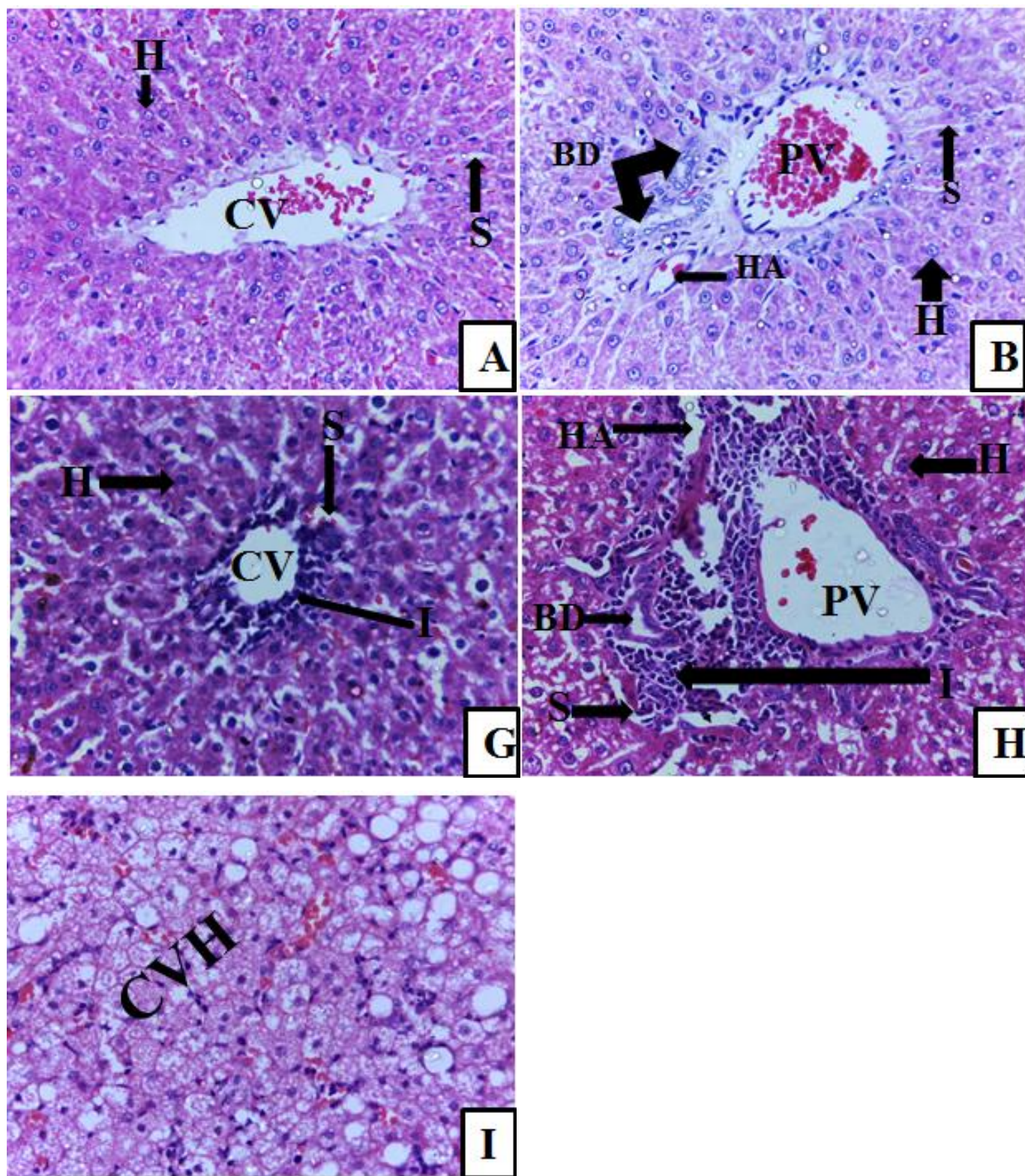


Figure 10: Photomicrographs of rat liver sections of control (A&B), and rat treated with 1000 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (G, H&I) for 24 weeks. Sections are taken from male rats. CV= Central vein, S= Sinusoids, H= Hepatocyte, I= Infiltrations PV= Portal vein, HA= Hepatic artery, BD= Bile duct (H & E, X400).

4.6. Effect of *M. stenopetala* aqueous leaves extract on histology of kidney

Histological evaluation of kidney sections of rats treated with the aqueous leaves extract of *M. stenopetala* for 24 weeks at a dose of 500 and 1000 mg/kg body weight (Figure 9C, D, E and F) showed no change as compared to the controls. Both the cortical and medullary regions appeared normal; there were intact glomerular and tubular structures, and normal sized urinary spaces. Moreover, the proximal convoluted tubules, distal convoluted tubules, loop of Henle and interstitial mesangial cells appeared normal as compared to the controls on both sexes. However, in the female rats treated with 2000 mg/kg body weight of the extract, there were minor tubulointerstitial leukocytic infiltrations, widened urinary space and congested glomeruli in some areas of cortex and medulla of the kidney sections (Figure 9).

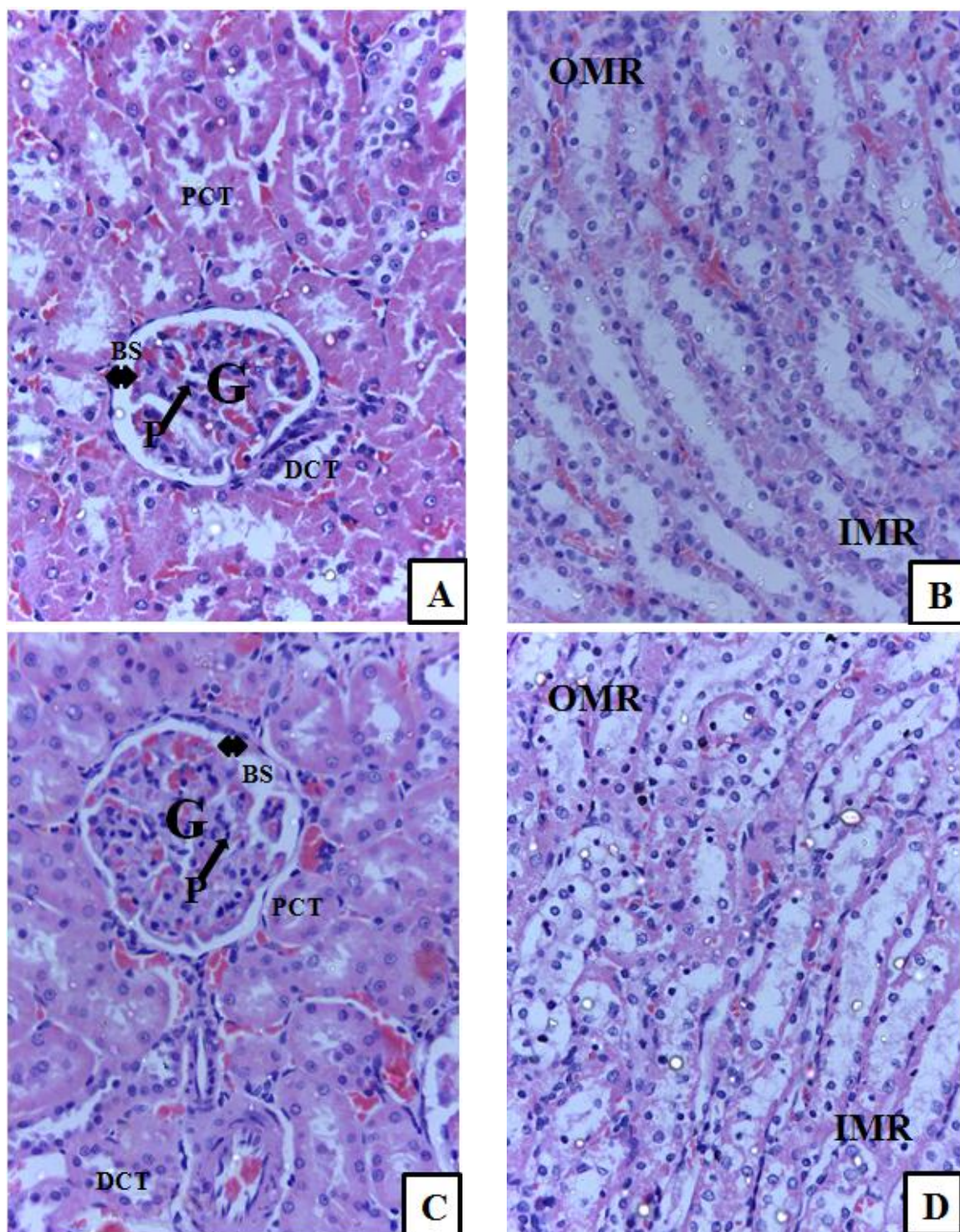


Figure 11: Photomicrographs of rat kidney sections of control (A&B), and rat treated with 500 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (C&D) for 24 weeks. Sections are taken from female rats. G= Glomerulus, P= Podocyte, DCT= Distal convoluted tubule, PCT= Proximal convoluted tubule, US= Bowman's space, OMR= outer medullary region, IMR= Inner medullary region (H & E, X400).

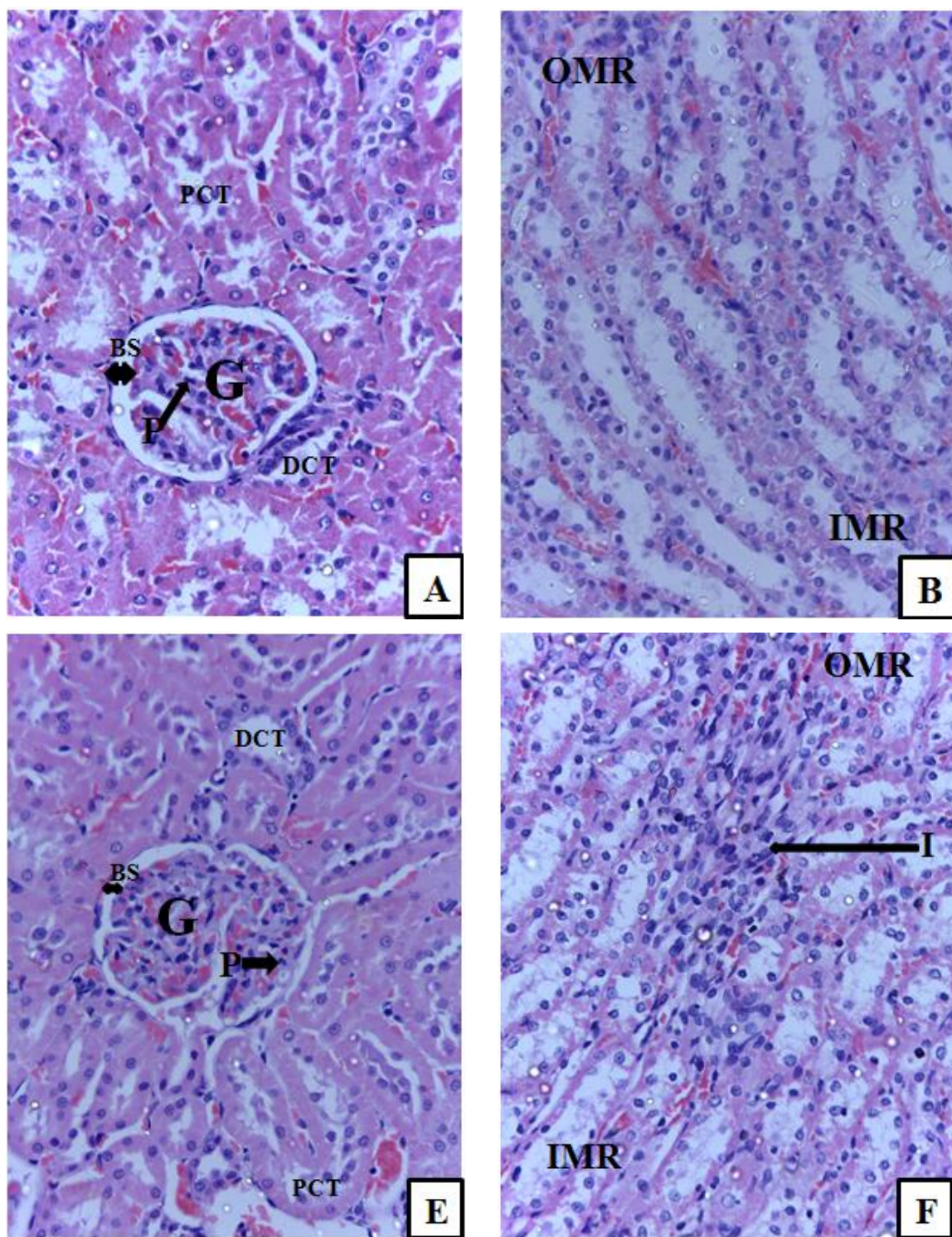


Figure 12: Photomicrographs of rat kidney sections of control (A&B), and rat treated with 500 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (E&F) for 24 weeks. Sections are taken from female rats. G= Glomerulus, P= Podocyte, DCT= Distal convoluted tubule, PCT= Proximal convoluted tubule, US= Urinary space, I= Infiltrations, OMR= outer medullary region, IMR= Inner medullary region (H & E, X400).

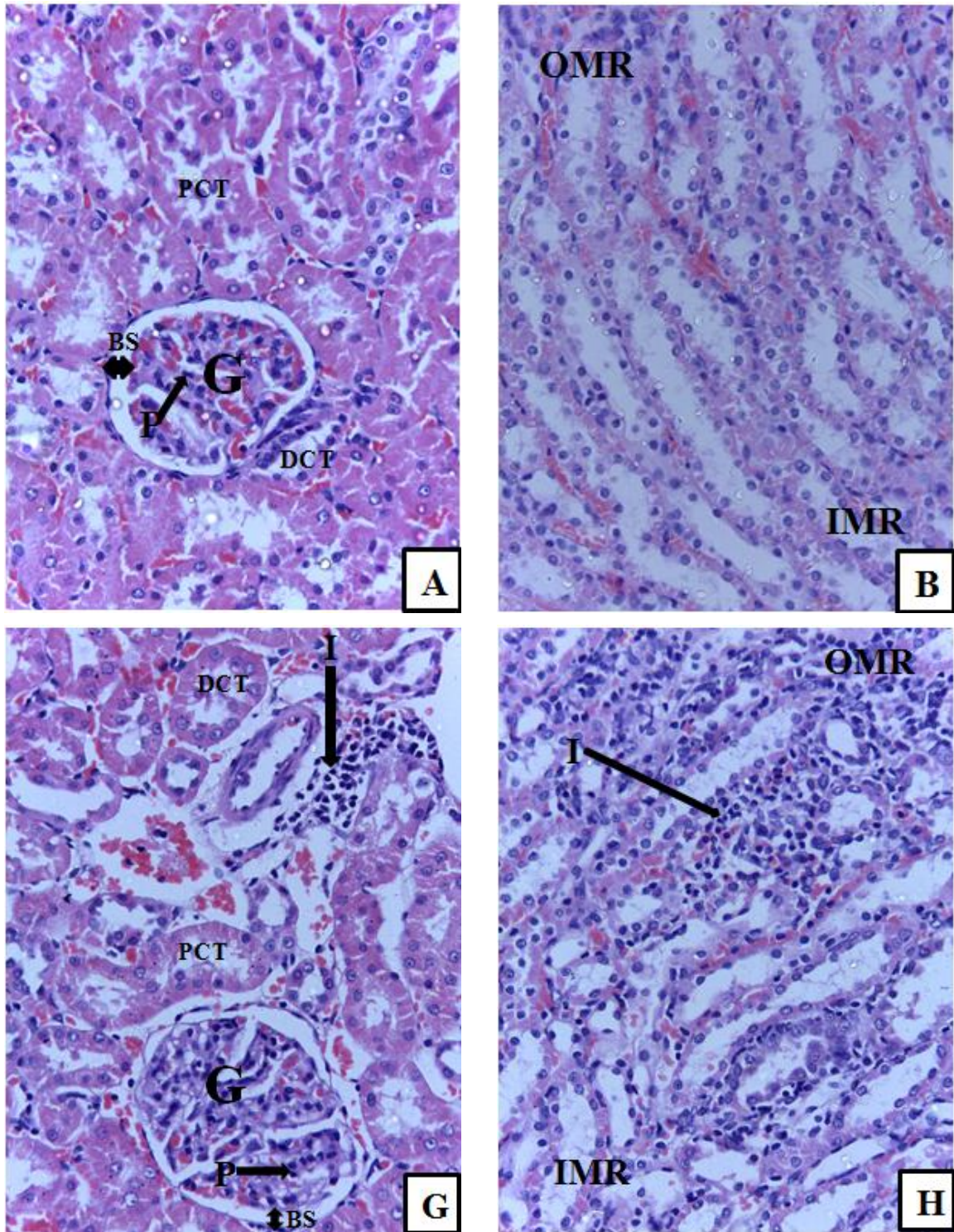


Figure 13: Photomicrographs of rat kidney sections of control (A&B), and rat treated with 2000 mg/kg of aqueous extract of *M. stenopetala* leaves orally administered (G&H) for 24 weeks. Sections are taken from female rats. G= Glomerulus, P= Podocyte, DCT= Distal convoluted tubule, PCT= Proximal convoluted tubule, US= Urinary space, OMR= outer medullary region, IMR= Inner medullary region and I= Infiltrations (H & E, X400).

5. Discussion

Some plants contain harmful chemicals that may cause serious side effects to the host system (Effendi, 2006). Therefore, it is necessary to investigate the toxicity of indigenous medicinal plants which are employed by traditional medical practitioners for therapeutic or diagnosis purposes even to maximize the potential contribution of traditional remedies to health care system in general.

In Ethiopia, especially in the southwest region *M. stenopetala* leaves are used as food and recommended for treatments of chronic diseases such as hypertension and diabetics in general (Toma *et al.*, 2012). Recent studies on aqueous and alcohol fraction leaf extract of *M. stenopetala* have found its antihyperlipidemics, antihypertensives and antidiabetics activities at an oral dose of 500 mg/kg/day in rat model (Mekoya, 2007; Nardos *et al.*, 2011; Sileshi *et al.*, 2014 and Toma *et al.*, 2012). However, such findings must be supported through further studies that investigate its safety. It is for this reason that this study was primarily designed to investigate the effect of long term treatment with different doses of the aqueous leaf extract of *M. stenopetala* on various parameters in Wistar rat. The effect of the extract on general health, food intake and body weight growth as well as on hematological and biochemical parameters of blood and gross and histological appearance of the liver and kidney were assessed.

Cage side observations of the animals were carried out daily after administration of both the vehicle and the *M. stenopetala* leaves extract at doses of 500, 1000 and 2000 mg/kg body weight for general signs of abnormalities throughout the study period. During the first and second weeks, the rats treated with the extract at 2000 mg/kg body weight revealed low locomotion, weakness, dizziness, shivering and piloerection. The recorded use of alkaloids for medicinal purposes stretches back some years and this chemical group has contributed the majority of the poisons, neurotoxins and cholinesterase inhibition (Goldman, 2001).

The results of 24 weeks chronic treatment of this study revealed that the extract was well tolerated by all rats, as there was no mortality and extract related toxicity signs and symptoms were observed throughout the study period in either sex. Throughout the study period, aqueous extract of *M. stenopetala* leaf treatment did not adversely affect food intake of both sexes of rats at all doses. The extract had no harmful effect on body growth patterns of treatment groups as

compared to the control group; although a transitional body weight decrement was observed in female and male rats treated with 2000 mg/kg body weight of the extract only during the first 2 and 3 weeks respectively. This might be due to the high dosage of *M. stenopetala* extract caused physiological variation in food intake and metabolism but tolerated afterwards. Hence, the *Moringa* plants are low fat and rich source of bioactive compounds may serve as a food supplement for weight reduction (Anwar *et al.*, 2007). This finding was in line with Debela (2015) and Ghebressilase *et al.* (2007) where there were no significant changes ($p>0.05$) in weight of both male and female rats that received 500, 600, 750, 900 and 1500 mg/kg of *M. stenopetala* aqueous leaf extract as compared to the controls. They also have reported that there were progressive non-significant weight increments in both experimental and control groups throughout the study period. Increment in body weight suggests for the positive health status of animals. Therefore, the overall weight gain in both experimental and control rats observed in the present study indicate the good health status of the treated animals.

According to Sellers *et al.* (2007), evaluation of organ weight in toxicology studies is an integral component in the assessment of pharmaceutical, chemical and medical devices. A remarkable change of organ weight between the experimental and control animal is an indicator of toxicity. In the present study there was no significant change ($p>0.05$) in both liver and kidney weight in the animals that received 500, 1000 and 2000 mg/kg body weight of the extract as compared with the controls. This finding is in line with the previous study of Ghebressilase *et al.* (2007).

Investigations on blood could serve as a diagnostic index of pathological, toxicological and physiological status in humans as well as in animals (Adeneye and Benebo, 2007). Blood profile usually provides important information on the response of the body to injury or lesion, deprivation and stress. Therefore, it is one of the most sensitive targets for toxic compounds (Everds, 2008). In this study, hematological parameters were evaluated to obtain any toxicity related information that may not be detected by gross examinations and analysis of organs and body weight.

In the present study, 24 weeks oral administration of aqueous leaves extract of *M. stenopetala* at doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight, there were no significant difference in the total red blood cells, HGB, HCT and MCVC of the experimental rats that received all the three doses of the extract in both sexes as compared to the controls. This was in

agreement with findings of previous studies by Toma *et al.* (2012), Ghebressilase *et al.* (2007) and Debela (2015) where hematological parameters did not show significant ($p>0.05$) changes in rats that received orally 500, 600, 750, 900 and 1500 mg/kg body weight of the aqueous leaves extract of *M. stenopetala* on both rat and mice models as compared with the controls. However, in the present study, in the male rats that received 1000 and 2000 mg/kg body weight there was a significant decrease of MCH as compared with the controls. The decreased value of MCH indicates the swelling of red blood cells and it may also occur by the less amount of hemoglobin in the circulation due to release of young erythrocytes (Olayemi *et al.*, 2016). In addition there were increases in the MCV values in the female rats treated with the extract at all the three doses as compared with the controls. This might be the defect in nuclear maturation, as seen in megaloblastic anemias due to foliate or B12 deficiency result large oval erythrocytes. Therefore, exposure to this plant at highest dose for a longer period of time may cause anemia (Asare *et al.*, 2012). This is in agreement with Adedapo *et al.* (2009) who described increased MCV and decreased MCH in rats that received 1600 and 2000 mg/kg body as compared with the controls. However, with the other hematological indices unchanged, an elevation of MCV and decrement of MCH were reported. The additional reasons for the observed changes in MCV and MCH should therefore call for further investigation.

The total white blood cells count and its differential as well as the platelets count in all treated rats did not show significant difference as compared with the controls which was again in agreement with those of the previous studies by Toma *et al.* (2012), Ghebressilase *et al.* (2007) and Debela (2015). In general, the absence of significant changes on these hematological indices may suggest that the extract does not possess toxic substances that can cause anemic and infection condition in rats.

Hepatotoxicity is one of the main reasons for withdrawal of drug from the market. 50% of all acute liver failures and 5% of all hospital admission are associated with drug induced hepatotoxicity (Dey *et al.*, 2013). Biochemical markers (ALT, AST, ALP, GGT and Bilirubin) are often used to assess the status of liver. Liver injury is defined as a rise in ALT, AST, ALP and total bilirubin levels more than the upper limit of the normal (Mumoli *et al.*, 2006). Liver damage is further characterized into hepatocellular (ALT and AST elevated) and cholestatic

(ALP raised). However, they are not mutually exclusive and mixed types of injuries are often encountered (Jaeschke *et al.*, 2002).

In this study, there was no significant ($p>0.05$) difference in ALP, AST, ALT and total bilirubin in female rats administered at all doses as compared to the controls. However, the ALP of male rats that received 2000 mg/kg body weight, AST and ALT of male rats that received 1000 and 2000 mg/kg body weight were found significantly ($p<0.05$) increased as compared to the controls. These findings are in line with previous studies by Geleta *et al.* (2015), Adedapo *et al.* (2009) and Ajibade *et al.* (2011) who reported there were increased levels of ALP, AST and ALT following treatment with aqueous leaves extract of *Moringa oleifera* at doses of 800, 1000, 1500, 1600 and 2000 mg/kg body weight as compared with the controls. Such findings might suggest for a mild hepatotoxicity of male rats even confirmed by histopathological slides. This is because male rats are sensitive to the hepatotoxic effect of alkaloid phytochemical present in *M. stenopetala* while female rats are resistant to its hepatotoxicity. This is due to the absence of isoform of cytochrome P450 involved in the bio-activation of alkaloid in female rats (Williams *et al.*, 1989).

Chronic treatment with 500, 1000 and 2000 mg/kg body weight of the leaves of aqueous extract did not significantly affect ($p>0.05$) almost any of the urea and creatinine investigations in both sexes. However, female rats that administered the extract at 2000 mg/kg orally have shown significant change ($p<0.05$) decrease in Urea and increase in Creatinine levels as compared to the controls. Such findings might suggest for a mild nephrotoxicity of female rats at dose of 2000 mg/kg body weight. However, this finding is discordant with a previous study by Geleta *et al.* (2015) where rats which received 500, 1000 and 1500mg/kg body weights of the extract had no significant change in urea and creatinine levels as compared with the controls. This is because female rats are often more sensitive to nephrotoxic compounds than males (Zbinden *et al.*, 1988 and OECD, 2009).

Many xenobiotics are capable of causing some degree of liver injury. Liver is prone to xenobiotic-induced injury because of its central role in xenobiotic/drug metabolism, its portal location within the circulation and its anatomical and physiological structures (Jones, 1996).

. Liver is the largest important organ and the site for essential biochemical reactions in the body (Ahsan, *et al.*, 2009). It has the function to detoxify toxic substances and synthesized useful biomolecules (Subramaniam *et al.*, 2015).

Histological examination of liver sections of rats treated with the aqueous leaves extract of *M. stenopetala* at dose of 500 mg/kg body weight showed a normal architecture with normal appearance of the hepatic plates, central vein and hepatic sinusoids lined by endothelial and Kupffer cells similar to the controls. Moreover, the hepatocytes appeared normal in size and shape, and no vacuoles were noted in their cytoplasm. In the rats treated with 1000 mg/kg and 2000 mg/kg body weight, there were an intrahepatic (as a yellowish discoloring of hepatocytes) and extrahepatic (as deposits in the bile canaliculi between the hepatocytes) bile retention indicating induction of cholestasis (Biliary stasis). Moreover, around the central veins and portal triads, there were some mononuclear leukocytic cells infiltration and cytoplasmic vacuolization's of hepatocytes, congestion of sinusoids and hydropic change at 1000 and 2000 mg kg body weight. Such finding is in agreement with that of a previous study by Ajibade *et al.* (2011) where rats administered with 800 and 1600 mg/kg body weight extract of *M. olifera* orally induced portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver.

The kidneys play a key role in the body by functioning as integrating and regulatory organs that maintain homeostasis of the extracellular fluid and the pH of blood in normal physiological range. They excrete metabolic wastes like blood urea, total protein and creatinine and regulate the threshold of electrolytes (bicarbonate, phosphate, potassium, sodium and chloride). Xenobiotics could interfere with the normal biochemical processes in the kidneys and lead to pathological changes and disruption of glomerular functions (Effendy *et al.*, 2006). Therefore, serum urea and creatinine levels, in addition to morphologic histology, were assessed in this study as markers of any nephrotoxicity of the extract. Female rats that received 2000 mg/kg body weight of the extract showed decrement of urea and increment of creatinine significantly as compared with the controls.

Kidneys are highly susceptible to toxicants for two reasons: a high volume blood flows through them and because they filter large amount of toxins which can concentrate in their tubules.

Nephrotoxicity lead to the kidneys' decreased ability to excrete body wastes, inability of body fluid and electrolyte balance and decreased synthesis of essential hormones (Emily, 2007).

In this study histological evaluation of sections of the kidneys from the rats treated with the aqueous leaf extract of *M. stenopetala* at oral doses of 500 and 1000 mg/kg body weight showed normal cortical and medulla regions with intact glomerular and tubular structures, and normal sized urinary spaces. Moreover, the proximal convoluted tubules, distal convoluted tubules, loop of Henle's and interstitial mesangial cells appeared normal as compared to the controls in both sexes. However, in the female rats treated with 2000 mg/kg, there were minor tubulointerstitial leukocytic infiltration, wider urinary space and congested glomeruli in some areas of both cortex and medulla of the kidney sections. This finding is in line with a study by Ajibade *et al.* (2011) who reported cortical congestion and interstitial hemorrhages in the kidneys of rats following treatment of *M. olifera* at doses of 600, 900 and 2000 mg/kg body weight. These findings may be related with the observed change in the values of urea and creatinine as well as minor histological changes at dose of 2000 mg/kg in the female rats in the present study and may be indicative of mild nephrotoxicity. Nevertheless, the extract is safe at lower doses in prolonged administration.

6. Conclusion

As found from the results of this study, the aqueous leaves extract of *M. stenopetala* at a dose of 500 mg/kg body weight does not show any adverse effect on body growth, organ weights, hematological, biochemical parameters and as well as on gross and histological appearances of liver and kidneys in Wistar rats. However, at doses of 1000 and 2000 mg/kg body weight/day the extract in male rats cause cytoplasmic vacuolization of hepatocytes, mononuclear leukocytic infiltration around the portal triad and central vein of liver as well as an elevated ALP, AST and ALT as compared to the control suggesting for a mild toxicity of liver. Furthermore, the extract also cause a mild nephrotoxicity in the female rats that received 2000 mg/kg body weight treatment of the extract as revealed from the decreased urea and elevated creatinine as well as congested glomeruli, wider Bowman's capsular space of kidneys and mononuclear leukocytic infiltrations in kidney histology as compared to the controls.

Therefore, from this study it can be concluded that prolonged administration of aqueous leaves extract of *M. stenopetala* at the dose of 500 mg/kg, which marks a therapeutic dose, are safe. However, as dose accumulates the extract was toxic.

7. Recommendations

- Further investigation on other vital organs (heart, brain, GIT) and on other higher animals is recommended at higher doses of the fractioned extract.
- Further study is also needed to identify and isolate the bioactive ingredients responsible for medicinal value from different parts of the plant (leaf, bark, stem, root, seed and flower).
- Reproductive and developmental toxicity investigations have to be carried out to investigate the possible side effects if there is any congenital anomaly associated factor in the use of this plant decoction during gravidity, particularly at the embryonic/critical period of development.
- As there is an apparent cytoplasmic vacuolization change in liver hepatocytes at high doses, further histological technique, such as immunocytochemistry may be needed for differential diagnosis.
- Further studies should be conducted with large sample size animal models

8. References

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9. Appendices

9.1. Appendix I: preparation of working chemicals and solutions

10% Neutral Buffered Formalin

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

Harris' hematoxylin (H)

Hematoxylin crystals.....	2.5gm
Absolute ethanol.....	25ml
Potassium alum.....	50gm
Mercuric oxide.....	1.25gm
Glacial acetic acid.....	20ml
Distilled water.....	500ml

1% Acid alcohol

70% Ethanol.....	250ml
Hydrochloric acid (Concentrated).....	2.5ml

1% Alcoholic Eosin (E)

Eosin Y, water soluble.....	1gm
95% Ethanol.....	100ml
Glacial acetic acid.....	0.5ml

Bluing solution

Sodium bicarbonate.....	2.5gm
Distilled water.....	1000ml

9.2. Appendix II: Tissue processing techniques and procedures

Fixation

Neutral buffered formalin, 10%.....24hrs

Washing

Running tap water.....24hrs

Dehydration

Ethanol, 70%.....2hrs

Ethanol, 90%.....2hrs

Absolute Ethanol I, 99.9%.....1 1/2hrs

Absolute Ethanol II.....1 1/2hrs

Absolute Ethanol III.....1 1/2hrs

Absolute Ethanol IV.....overnight

Clearing

Xylene I.....1 1/2hrs

Xylene
II.....1 1/2hrs

Infiltration (in hot oven, 60°C)

Paraffin wax I (56°C).....1 1/2hrs

Paraffin wax I (56°C).....1 1/2hrs

Paraffin wax I (56°C).....overnight

9.3. Appendix III Routine Hematoxylin and Eosin (H &E) staining procedures

Deparaffinization

Xylene I.....	5min
Xylene II.....	5min

Rehydration

Absolute alcohol I.....	4 min
Absolute alcohol II.....	4 min
95% Ethanol.....	3 min
70% Ethanol.....	3 min
Rinse in distilled water.....	5 min
Stain in Hematoxylin.....	15 min
Rinse in running tap water.....	5 min
Decolorized in acid alcohol.....	1-3 sec
Rinse in running tap water.....	5 min
Immerse in sodium bicarbonate solution.....	3-6 sec
Rinse in running tap water.....	5 min
Counterstain in Eosin.....	1 min

Dehydration

70% Ethanol.....	2 min
95% Ethanol.....	2 min
Absolute Alcohol II.....	2 min
Absolute Alcohol I.....	2 min

Clearing

Xylene II.....	4 min
Xylene I.....	4 min