ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF MEDICAL ANATOMY

Evaluation of the acute and sub-chronic toxicity of aqueous extracts of *Moringa stenopetala* seeds on kidneys, liver, and some blood parameters of Wistar rats

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 Human Anatomy.

By: EPHREM FISSEHA

Addis Ababa, Ethiopia

January, 2021

Evaluation of the acute and sub-chronic toxicity of aqueous extracts of *Moringa stenopetala* seeds on kidneys, liver, and some blood parameters of Wistar rats

By: Ephrem Fisseha Mamo (BSc.)

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 Human Anatomy.

Principal Advisor

Dr. Mekbeb Afework (PhD., Associate Professor of Anatomy)

Co-advisors:

1. Prof. Eyasu Makonnen (PhD., Professor of Pharmacology)
2. Dr. Yonas Bekuretsion (MD., Associate Professor of Pathology)
3. Mr. Abiy Abebe (MSc., Associate Researcher - II)

Addis Ababa, Ethiopia

January, 2021

Identification	
Name of Investigator	Ephrem Fisseha Mamo (BSc.)
Principal Advisor	Dr. Mekbeb Afework (PhD., Associate Professor of Anatomy, Department of Medical Anatomy, School of Medicine, CHSs, AAU)
Co-advisors	**1.** Prof. Eyasu Makonnen (PhD., Professor of Pharmacology, Department of Pharmacology, School of Pharmacy, CHSs, AAU) **2.** Dr. Yonas Bekuretsion (MD., Associate Prof of Pathology, Department of Pathology, School of Medicine, CHSs, AAU) **3.** Mr. Abiy Abebe (MSc., Associate Researcher - II, Department of TMMDRD, EPHI)
Study Area	AAU, CHSs, School of Medicine, Department of Medical Anatomy and EPHI, Department of TMMDRD
Full title of the Project	Evaluation of the acute and sub-chronic toxicity of aqueous extracts of *Moringa stenopetala* seeds on kidneys, liver, and some blood parameters of Wistar rats.
Duration of the project	March, 2019 – December, 2020
Address of the investigator	**E-mail:** ephremfisseha@gmail.com

Declaration

I declare that this thesis is my original work, and it has not been submitted to any other educational institution for the award of any academic degree or certificate. Every effort has been made to avoid plagiarism in the preparation of this thesis. All scholarly materials that are included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document.

Student's name	**Signature**	**Date**
Ephrem Fisseha	_____	_____

Advisors	**Signature**	**Date**
Dr. Mekbeb Afework (Main-advisor)	_____	_____
Prof. Eyasu Makonnen	_____	_____
Dr. Yonas Bekuretsion	_____	_____
Mr. Abiy Abebe	_____	_____

Examiner	**Signature**	**Date**
Dr. Amanuel Damie	_____	_____

ACKNOWLEDGEMENT

I would like to acknowledge my principal advisor Dr. Mekbeb Afework for giving me a good appreciation starting from proposal development to thesis work and his constructive and timely comments and suggestions.

Moreover, I wish to extend my most sincere gratitude to my co-advisors Prof. Eyasu Makonnen, Dr. Yonas Bekuretsion, and Mr. Abiy Abebe for guiding me through this thesis work and their valuable advices and comments.

Also, I would like to thank Ethiopian Public Health Institute (EPHI), Department of Traditional and Modern Medicine Drug Research Directorate (TMMDRD) for sponsoring my thesis work and allowing me to use all available laboratory instruments and chemicals.

Finally, I am highly indebted to all my family, colleagues, and friends for their encouragement, moral support, and unremarkable contribution to this thesis work and my academic success.

LIST OF ABBREVIATIONS AND ACRONYMS

AAU……	Addis Ababa University
ALB…….	Albumin
ALP…….	Alkaline phosphate
ALT…….	Alanine aminotransferase
ANOVA…..…….	Analysis of variance
AST…….	Aspartate aminotransferase
BA……...	Basophils
CHS…….	College of Health Science
EO……	Eosinophils
EPHI…….……...	Ethiopian public health institute
fL…….	femtoliter
HCT……...…….	Hematocrit
HGB……	Hemoglobin
IRB…….…….	Institutional Review Board
LD_{50}…….	Lethal dose fifty (lethal dose that kills half of the animals)
LY……	Lymphocytes
MCH……	Mean Corpuscular Hemoglobin
MCHC…….….....	Mean Corpuscular Hemoglobin Concentration
MCV……	Mean Cell Volume
MO..……	Monocytes
NEU……	Neutrophils
OECD…….…….	Organization of Economic Co-operation and Development
pg…….	Pictogram
PLT….....	Platelet
RBC...……...…....	Red blood cells
SPSS…….……	Statistical package for social sciences
TM…….….....	Traditional Medicine
TP……	Total protein
WHO……...	World Health Organization
WBC……	White Blood Cells
µL……	Microliter

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Traditional medicine is the oldest and culture-based method of the health care system. Among the traditional medicine practices, the use of herbal medicines is the most popular and used by most populations around the globe. *M. stenopetala* is one of the herbs used as a treatment for various illnesses in different Ethiopian societies. Although there are some data available regarding the various biological activities of the different parts of Moringa plants, that of safety study is scarce, especially relating to the extract of its seeds. The objective of this study was to evaluate the acute and sub-chronic toxic effect of orally administered aqueous extracts of *M. stenopetala* seeds on gross and histopathology of kidneys and liver, and some blood parameters of Wistar rats.

For the acute toxicity study, 15 female rats were randomly divided into five groups with three rats in each group. The experimental groups (Groups I - IV) received a single dose of 300mg/Kg, 2000mg/kg, 3500mg/Kg, and 5000mg/kg of the extract, respectively, while the control group (Group V) received distilled water orally. For the sub-chronic toxicity study, 24 rats for each sex were randomly divided into four groups, each group comprising of six rats. The rats in the experimental groups (Groups I - III) received 250mg/Kg, 500mg/Kg, and 1000mg/kg oral dose of the extract for 90 days, and the control group (Group IV) received distilled water for a similar duration.

In an acute toxicity study, LD_{50} of the aqueous extract of *M. stenopetala* seeds was found to be above 5000mg/Kg dose. There were no observed apparent significant differences between the experimental groups and the control group in body weight gain, relative kidneys and liver weight, and gross pathological changes at the end of the 14 days experimental period. In the sub-chronic toxicity study, there was no mortality in all experimental rats of both sexes. Besides, there were no substantial changes in the general condition, hematological and biochemical values, and relative organ weight of the kidney and liver. Moreover, the light microscopic histopathological examination of sections of the kidney and liver showed no change in the treatment groups as compared with the control group in both sexes. Therefore, this study demonstrated that 90 days oral administration of aqueous *M. stenopetala* seeds extracts are relatively safe in rats up to 1000mg/Kg dose.

Keywords: *M. stenopetala* seeds, Acute and sub-chronic toxicity, Traditional medicine

1. INTRODUCTION

1.1. Traditional Medicine

Traditional medicine (TM) is the oldest form of the health care system and culture-based method of healing that humans have used to cope and deal with various ailments and diseases that have threatened their existence and survival. Different societies have developed different forms of indigenous healing methods that are captured under the broad concept of TM, e.g., Indian, Chinese, and African traditional medicines. These indigenous methods are known as folk medicine, ethnomedicine, native healing, and alternative medicine or complementary medicine. Therefore, TM is a broad and diverse term. This explains the reason why there is no single universally accepted definition of the term TM. One of the most accepted definitions of TM has been provided by the World Health Organization (WHO) as "the sum total of the knowledge, skill, 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." According to WHO the terms "complementary medicine" or "alternative medicine" refer to a broad set of health care practices that are not part of that country's own tradition or conventional medicine and are not fully integrated into the dominant health-care system (WHO, 2013).

TM is an important part of the health care system, although previously it has been underestimated. TM practitioners include herbalists, herb sellers, village midwives or traditional birth attendants, bonesetters, traditional psychiatrists, and other specialists (Sofowora, 1996). Among the TM practices, the use of herbal medicines which is defined as plant-derived preparations claimed to have therapeutic benefits, is the most popular and used by most populations around the globe (Furnharm, 1996, Ernst, 2003, WHO, 2003). Herbal medicine includes herbs, herbal preparations, herbal materials, and finished herbal products that contain parts of plants or other plant materials as active ingredients.

TM has been used since the existence of mankind worldwide in all nations. In low and middle-income countries, the number of modern medicine practitioners may not be enough to meet the

health care needs of the country; hence TM and its practitioners are considered an important resource and choice for the population health system. TM is perceived to be more affordable, accessible, and acceptable relative to modern medicine to the communities in which it operates (Sato, 2012).

About 65% of the world's populations have incorporated TM into their primary modality of the health care system (Fabricant and Farnsworth, 2001). Many millions of people use herbal medicines, traditional treatments, and traditional practitioners as their main source of health care, and sometimes as the only source of care especially in developing countries. Many people in developing countries, particularly those in rural areas, have more access to TM than modern medicines and use them more frequently as their primary health care system. The main reasons for preferring traditional medicine over modern medication are its accessibility, efficacy, availability, and affordability in getting the services (Konno, 2004). In one study done by the WHO and Health Action International (HAI) in 36 low and middle-income countries, drugs were reported far beyond the reach of large sections of the population (Cameron *et al*., 2009).

In developed countries also, there is a widespread use of TM. As stated by WHO anxiety about the adverse effects of chemical drugs, improved access to health information, changing values and reduced tolerance of paternalism are some of the factors responsible for the growing demand for CAM in developed countries (WHO, 2002). The other reason is that there is increasing evidence that TM is effective in the management of chronic illnesses (Thorne *et al*., 2002). Therefore, in countries like the USA, TM is taught as part of school curriculum activities in medical schools (Wetzel *et al*., 1998).

Irrespective of reasons for seeking out traditional & complementary medicine, interest towards its use has grown and will continue to grow around the world (WHO, 2013). WHO has also described TM as it is one of the surest means to achieve total health care coverage of the world’s population (CAMH, 2004). As a result, there is an increasing trend worldwide to integrate TM with the primary health care system.

In Africa, TM is a part of the people's culture but it is not well organized, for example, as such in China and India. TM is the mainstay of primary health cares for the majority of Africans, especially for those in the rural areas of Africa. According to the WHO data in Africa, up to 80%

of the population uses TM to help meet their health care needs (WHO, 2002). The ratio of traditional health practitioners to population is 1:500, whereas the ratio of medical doctors to population is 1:40,000 (WHO, 2013). Therefore, for millions of people in rural areas of Africa, native healers remain their primary health care providers.

The widespread use of TM can be attributed to the safety, acceptability, affordability, compatibility, and suitability of the medications for the treatment of various diseases, particularly chronic ones (Okigbo and Mmeka, 2006). Research done in Nigeria has shown that several traditional medicines are important and effective in the management of a wide spectrum of diseases, some of which may not be effectively managed using Western medicines (Lawal and Banjo, 2007). Inadequate accessibility to modern medicines and drugs to treat and manage diseases also may have contributed to the widespread use of TM in these regions, especially in poor households (Cameron *et al*., 2009). Therefore, the widespread use of TM in Africa can be attributed to its accessibility and affordability. Furthermore, TM provides an avenue through which cultural heritage is preserved and respected. Indeed, in Africa TM practice is in line with the socio-cultural and environmental conditions of the people who use it (Owumi, 2002).

Like the rest of African countries, in Ethiopia, 80% of the population is dependent on TM and traditional practitioners as a source of health care (WHO, 2013). In 2005 one research reported an estimated 90% of the people in Ethiopia use traditional medicine to meet their primary health care needs (Patwardhan, 2005). The Ethiopian people's reliance on TM is also reflected by the fact that continued use of TM by Ethiopian migrants in developed countries. TM in Ethiopia is demanded due to its culturally linked traditions in using it, the trust the communities have in its values, its accessibility, and its relatively low cost (Patwardhan, 2005). In Ethiopia, more than 95% of traditional medical preparations are of plant origin (Tadele, 2018).

The primary health care system of Ethiopia has different strategies to fulfill its goal of health for all. Among these strategies, one is the incorporation of traditional medicine into modern medicine. Moreover, the health sector strategy also states that the incorporation of TM into the official health care system is advantageous for improving the health coverage of the country (MOH, 1995).

TM 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). The safety and efficacy of several commercially available herbs have recently come into question due to reports of adverse effects and potential interactions with prescribed drugs (Popat *et al*., 2001). Some scientific researches have shown that many plants used as food or in TM are potentially toxic, mutagenic, and carcinogenic (Schimmer *et al*., 1988, Higashimoto *et al*., 1993, Kassie *et al*., 1996, Fernandes and Vargas, 1999). WHO also notes that inappropriate use of traditional medicines or practices can have negative or dangerous effects and that further research is needed to ensure the efficacy and safety of the several practices and medicinal plants used by TM systems (WHO, 2008).

Hence the growing interest in herbal medicine demands information about the effects of the various plant preparations used in the management of diseases on the human body (Atawodi, 2005, Toma *et al*., 2012). The occurrence of adverse effects and sometimes life-threatening conditions allegedly emanating from herbal medicines have been reported among various ethnic groups (Elvin-Lewis, 2001). Moreover, there are numerous examples of potential side effects associated with the most commonly used herbal medicines and other types of complementary and alternative medicines (Rokos, 1969, Popat, 2001, Teshome *et al*., 2008, Ajibade and Famurewa, 2012). Therefore, it is an issue requiring attention and action to ascertain the toxicity profiles of medicinal herbs.

An inappropriate dosage of TM could result in chronic damage to the blood composition and tissue of various organs. Some herbs used in Herbal medicine are found to contain toxic constituents with various potential adverse effects. Furthermore, the concept of side effects is probably more elaborated in TM (Gilani, 2005). For instance, blindness and changes in central nervous system function have been repeatedly reported in people who consumed *Hagenia abyssinica* (Rokos, 1969). Dobb and Edis (1984), reported neuropathy and coma in patients who took herbal laxatives to control body weight.

Traditional healers have considerable knowledge of medicinal plants and how to avoid acute poisonings (Savage and Hutchings, 1987). Hence poisoning from TM is not due to innate risks of using traditional healthcare rather it appears to occur frequently as a result of inappropriate use

because of self-administration (Popat *et al*., 2001). Also, it occurs as a result of misidentification, incorrect preparation, or inappropriate administration and dosage of the medications (Stewart and Steenkamp, 2000).

For these reasons, information about the safety and efficacy of traditional medicines is required. This study evaluates the acute and sub-chronic toxic effect of seeds of *M. stenopetala*.

More than 800 plant species have been employed as traditional medicinal plants in Ethiopia (Tesema *et al*., 2002). Most of the medicinal plants are prepared in fresh form, and methods of their applications include squeezing, grinding, boiling, chewing, crushing, and tying. Most frequently oral route of administration is used, dermal and nasal routes are also the other routes of application of the remedies (Yirga and Zeraburk, 2011).

One of the herbs used as a treatment for various illnesses in different Ethiopian society is *M. stenopetala*. The oral route of administration of this plant preparation is the most common. In addition to its medicinal values, the seeds of this plant have nutritional and water purification uses. This research investigates if frequent use of *M. stenopetala* seeds has any histopathologic effect on the kidneys, liver, and some blood parameters of the rat.

1.2. *Moringa stenopetala*

1.2.1. Ethnobotany

Moringa stenopetala (Baker f.) Cufodontis is a green, drought-resistant, branched, and multipurpose tree; which has significant nutritional, industrial, and medicinal importance (Jahn, 1991). As shown in (**figure 1**), the tree is thick at the base and grows up to 6 to 10 m tall, with smooth white to pale gray or silvery color bark. It is domesticated in the east African lowlands and indigenous to southern Ethiopia, as the result, it is often referred to as the African Moringa tree. It is widely distributed in the southwestern part of Ethiopia at an altitude range of about 1100 to 1600 meters above sea level and found widely distributed in Konso, Wolayta, Derashe, Gamogofa, Borana, Sidama, and Bale areas (Seifu, 2015).

Figure 1: *M. stenopetala* plant in the backyard of a farmer in southern Ethiopia (Eyassu S., 2015).

M. stenopetala is native to Ethiopia, and it is known by various names in different localities of Ethiopia: “Haleko” in Gofa, “Shelagda” in Konso, and “Shiferaw” in Amharic (Seifu, 2015). There are two most common English vernacular names for the tree: based on the shape of its

pods, it is called “drumstick tree” and based on the taste of its roots, it is called “horseradish tree” (Sutherland *et al*., 1994).

M. stenopetala has a wide range of adaptation from the arid to humid climates and can grow in various land-use patterns. It grows in the lowlands of the west of the Great Rift Valley Lakes from arid to semi-humid areas, altitudinal ranging from 390 to about 2200 meters. It is a strategic multi-purpose tree, being a unique food tree in drought-prone areas and has recently been distributed to other regions of Ethiopia beyond its place of origin (Melesse *et al*., 2011).

M. stenopetala is often called “cabbage tree” and is an important indigenous vegetable in southwestern Ethiopia where it is cultivated as a food crop. The Gofa, Konso, Burji, and Gamo tribes consume its leaves as a vegetable, especially during the dry season (Seifu, 2015). The seed of this plant is also edible in some parts of the country (Schneemann, 2011, Kumssa *et al*., 2017).

As shown in (**figure 2a**), the undehulled seeds of *M. stenopetala* are triangular and have three wings. It is covered with a yellowish thick spongy seed coat. It has a 17.6 mm length and 8.2 mm width. The dehulled seed has an oval shape, its thickness decreases from the highest point at the center towards either end of the seed (**figure 2b**). The average weight of the seed is o.6 gm (Seifu, 2012).

Figure 2: *M. stenopetala (a).* Undehulled seeds (b). Kernel (Seifu, 2012).

1.2.2. Ethno-medicinal values

The various parts of the Moringa tree, such as leaves, flowers, seeds, pods, roots, and barks are used as traditional medicine for the treatments of more than 157 different diseases (Hiawatha, 2010). Its leaves are being used to expel the retained placenta in women who have just given birth, and the seeds are used to clear muddy water. Various parts of the *M. stenopetala* tree are claimed to contain disease-preventing chemicals (Endeshaw, 2003, Mekonnen, 2003).

A study conducted in Arbaminch, Jinka, Konso, and Yabello showed evidence for medicinal and water treatment values of the seed (Megersa *et al*., 2015). The seeds are used in clearing muddy water in Negelle, Wolayta Sodo, and especially in a village called Kola Shara near Arbaminch (Jahn, 1991). A handful of seed is taken and put in a bucket containing about 20 liters of muddy water. After about an hour, all the dirt accumulates at the base of the bucket and settles down. Besides, the seeds serve as adherents to coagulate all the impurities in turbid water (Mekonnen and Gessesse, 1998).

The water-soluble Moringa seed proteins possess coagulating properties similar to those of alum and synthetic cationic polymers. The use of Moringa species for water clarification is a part of African indigenous knowledge. Women in Sudan use seed powder from *M. stenopetala* to clarify the turbid water of the Nile (Oluduro and Aderiye, 2007). Jahn in 1991 first studied and confirmed the coagulating properties of Moringa seeds, after observing women in Sudan use the seeds of Moringa to clarify the turbid Nile water. Church World Service observed that powder from crushed Moringa seed kernels worked as a natural flocculent by binding to the solids in water and causing them to sink to the bottom (Fuglie, 1999). Bacteria in the water are mostly attached to solid particles; thus, 90-99% of the bacteria can be purified by the treatment of water with Moringa powder (Yisehak *et al*., 2011). A recent study by Hellsing *et al.* (2014), also indicated that proteins extracted from the seeds of the *M. stenopetala* tree are effective flocculents for particles dispersed in water and are attractive as a natural and sustainable product for use in water purification.

Seeds of *M. stenopetala* have natural flocculating and antimicrobial properties (Seifu, 2015). Crude seed extracts have shown anti-bacterial activity by strongly inhibiting the growth of *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* species, and *Candida albicans* (Bosch, 2004).

Methanol and n-hexane extracts of *M. stenopetala* seeds showed potential antimicrobial activity against some human pathogenic bacteria (*Salmonella typhi, Vibrio cholera,* and *Escherichia coli*) that are known to cause water-borne diseases (Walter *et al*., 2011). Such findings may suggest that the seed extracts of *M. stenopetala* could be promising natural antimicrobial agents, with potential applications in controlling bacteria that cause water-borne diseases. Besides, the essential oils of the seeds displayed significant anti-trypanosomal activity and anti-cancer activity against human promyelocytic leukemia cells (Nibret and Wink, 2010).

M. stenopetala seed powder could also be used to remove heavy metals from water and industrial wastes. A study by Mataka *et al*. (2010), indicated that *M. stenopetala* seed powder reduced the concentration of cadmium from water. The result of this study indicated that *Moringa* seeds could be used as a less expensive bio-sorbent for the removal of cadmium (Cd) from polluted water. Earlier reports indicated that *M. stenopetala* seed powder could remove lead from contaminated water (Mataka *et al*., 2006). Another study indicated that *M. stenopetala* seed powder could be used to remove chromium (Cr) from tannery effluents (Gatew and Mersha, 2013). A similar study also showed that the seed powder of *M. stenopetala* was found to be effective in the removal of chromium from tannery wastewaters (Degefu and Dawit, 2013). The use of bio-adsorbents like *M. stenopetala* seed powder that is easily available and effective for the removal of heavy metals could be an innovative and economical approach for the treatment of industrial wastewater.

All parts of the Moringa tree except the wood are edible, and also providing highly nutritious food for both humans and animals. The edible parts are exceptionally nutritious (Ram, 1994, Jiru *et al*., 2006). Roasted seeds of *M. stenopetala* are edible in Showa Robit (Schneemann, 2011). The seeds are also edible in Konso and northern Kenya (Kumssa *et al*., 2017).

The seed of *M. stenopetala* is an important source of oil that could be used for cooking (Lalas *et al*., 2006). In some areas of southern Ethiopia, the seed oil is used as a lubricant in perfumery and soap production (Bosch, 2004).

1.2.3. Bioactive ingredients

Studies done on the chemical composition of *M. stenopetala* indicated the seed of this plant is rich in glucosinolates, isothiocyanates, and 5,5-Dimethyloxazolidine-2-thione (Mekonnen and Dräger, 2003, Nibret and Wink, 2010).

The seed of *M. stenopetala* contains Fat (saturated and unsaturated fatty acids), protein (essential and non-essential amino acids), sugar, ash, soluble and insoluble fibers, and minerals such as Phosphorus, Calcium, Magnesium, Potassium, Sodium, and Manganese (Melesse *et al*., 2009, Seifu, 2015).

Chemical composition of the essential oil of *M. stenopetala* is Isobutyl isothiocyanate, Benzene, Cyclopropane, Nonanoic acid, Benzyl isothiocyanate, ɗ-Cadinene, Myristic acid, Methyl palmitate, Palmitic acid, Methyl 9-octadecenoate, and Oleic acid (Nibret and Wink, 2010).

1.3. Liver Structure and Function

The liver is the largest internal organ constituting 2.5 % of adult human body weight. It has a reddish-brown color. It is located in the right upper quadrant of the abdomen below the diaphragm, where it occupies almost the entire right hypochondriac region, part of the epigastric region, and extends into the left hypochondriac region. In this position, the liver is well protected by the rib cage in the dome of the diaphragm; and this position is maintained through peritoneal reflections (attachments) (Moore *et al*., 2013, Tortora and Derrickson, 2018). Most part of the liver is covered by a dense irregular collagenous connective tissue capsule known as "Glisson's capsule"; which is in turn covered by the visceral layer of peritoneum. The Glisson's capsule forms septae and divides the liver into lobes and lobules (Mescher, 2013). The liver has 2 major (the smaller left and the larger right) lobes and two small (caudate and quadrate) lobes. The liver has dual blood supply: the hepatic artery, which contributes about 20-30% of the blood supply, and the portal vein, which contributes the remaining 70-80% of the blood supply (Ross and Pawlina, 2011, Mescher, 2013).

The rat liver is large and multilobulated. It occupies the cranial third of its abdominal cavity and comprises approximately 5% of the total weight. In the rats weighing between 250 – 300 g, the mean liver weight is 13.6 g. The rats' liver has a transverse diameter measuring 7.5 to 8.0 cm, a superior-inferior diameter measuring 3.8 to 4.2 cm, and an anterior-posterior diameter ranging from 2.2 to 2.5 cm. When the rat is in the decubitus position, its liver fundamentally has three surfaces: superior, inferior, and posterior. A sharp and well-defined margin divides the inferior surface from the superior surface. Unlike in the human liver, the other margins are also sharp. Although the rat liver is lobulated, it has rather uniform surfaces as lobes lie flat against each other. The posteriorly located caudate lobe is the only exception to this, which is separated from the rest of the liver by the stomach (Martins and Neuhaus, 2007). Like in humans, a thin connective tissue capsule that is externally lined by peritoneal mesothelial cells covers the visceral and parietal surfaces of the rat's liver (Thoolen *et al*., 2010).

The superior (parietal) surface of the rat liver comprises a part of the left lateral and medial lobes; and as a whole is convex, and fits under the vault of the diaphragm. It is completely covered by the peritoneum, except along the line of attachment of the falciform ligament. The line of attachment of the falciform ligament divides the liver into two parts, termed the right and

left lobes. Unlike the human liver, in which the right lobe is much larger than the left one, the rats' left and right lobes of the liver have approximately the same volume.

The inferior (visceral) surface is uneven, concave, and is in relation to the stomach, duodenum, right colic flexure, the superior part of the pancreas, the right kidney, and the right suprarenal gland. The rat liver inferior surface does not have the fossae in the shape of the letter H as in humans. This surface is almost completely invested by the peritoneum. Through the porta (transverse fissure) go the portal vein, the hepatic artery and nerves, the hepatic duct, and lymphatics. Liver impressions (colic, renal, duodenal, and suprarenal) are not as evident as in the human liver (Martins and Neuhaus, 2007).

The posterior surface is not covered by the peritoneum over some part of its extent and is in direct contact with the diaphragm. It extends obliquely between the caudate lobe and the bare area of the liver. The inferior vena cava is completely intrahepatic (Martins and Neuhaus, 2007). Similar to the human liver, the rat liver is also connected to the undersurface of the diaphragm and the internal surface of the anterior abdominal wall by five ligaments: the falciform, the coronary, and the two laterals are peritoneal folds; the fifth, the round ligament is a fibrous cord, which is the obliterated umbilical vein. The liver is also attached to the lesser curvature of the stomach by the hepatogastric ligament and the duodenum by the hepatoduodenal ligament (Gershbein and Elias, 1954, Martins and Neuhaus, 2007).

Like the human liver, the rat liver lobes, are named after the portal branches that supply them. As among mammals, the portal system is the most constant anatomical reference. As shown in (**figure 3**) the rat liver has 4 lobes: the middle (median), the right, the left lateral, and caudate lobes (Aller *et al*., 1999, Martins and Neuhaus, 2007).

The middle or median lobe is the largest, accounting for approximately 38% of the liver weight. It has a trapezoidal shape and is fixed to the diaphragm and abdominal wall by the falciform ligament. It is in continuity with the left lateral lobe and as shown in (**figure 3**) it is subdivided by a vertical fissure (main fissure or umbilical fissure) into a large right medial lobe (2/3 of the volume of the medial lobe) and a smaller left medial lobe (1/3 of the volume). The right medial lobe has both left and right hepatic vascular components.

The right lobe is located on the right side of the vena cava and posteriorly in the right hypochondrium and is almost completely covered by the medial lobe. It comprises about 22% of the liver weight and is divided by a horizontal fissure into two pyramidal-shaped lobules: the superior (also called the right posterior lobe) and inferior (inferior right lobe, also called the right anterior lobe) lobules.

The left lateral lobe has a rhomboid shape, is flattened and situated in the epigastric and left hypochondriac regions over the anterior aspect of the stomach. Its medial portion is covered by the left part of the medial lobe. Its upper surface is slightly convex and is molded on the diaphragm and it has no fissure.

The caudate lobe is situated behind the left lateral lobe and on the left side of the vena porta and inferior cava vein. It constitutes 8–10% of the liver weight and is divided into two portions: the paracaval portion (caudate process), which encircles the inferior vena cava and bridges the caudate lobes and the right lateral lobe; and the Spiegel lobe, which has an anterior (superior) and a posterior (inferior) portion in the form of discs. The anterior part of the caudate lobe is located anterior to the esophagus and stomach, and its pedicle lies superior, while the posterior is located behind these structures, and its pedicle lies inferior. Both are covered by a very thin layer of peritoneum, the hepatoduodenal and hepatogastric ligaments (Kogure *et al*., 1999, Martins and Neuhaus, 2007).

A study done by Martins and Neuhaus, 2007, has shown that the origin and course of the major vessels of the liver of rats are similar to those of humans, and no variability in vessel origin has been identified. Unlike a human, the rat does not have a gallbladder.

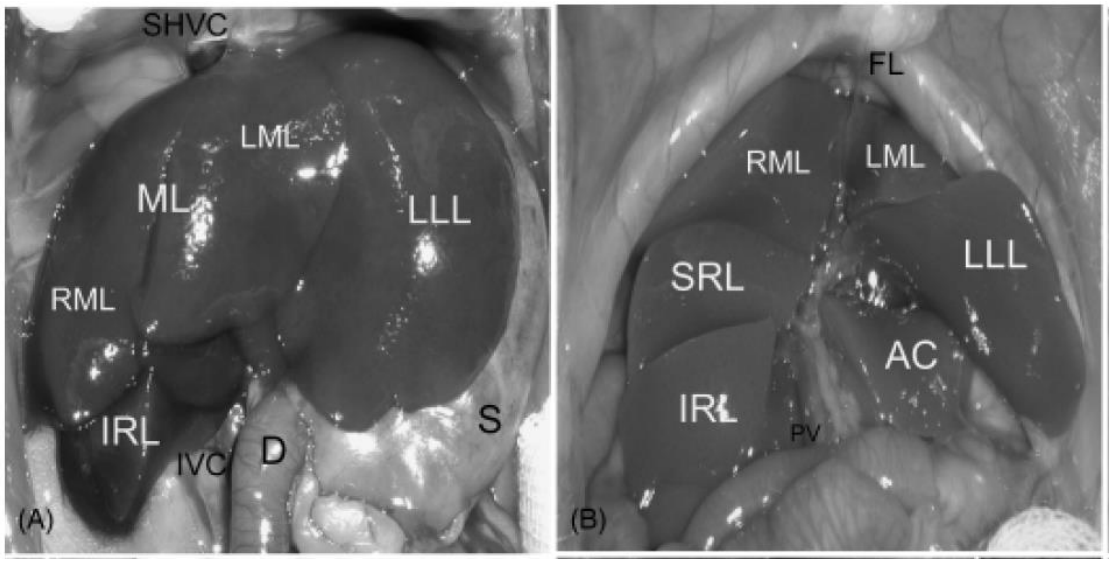
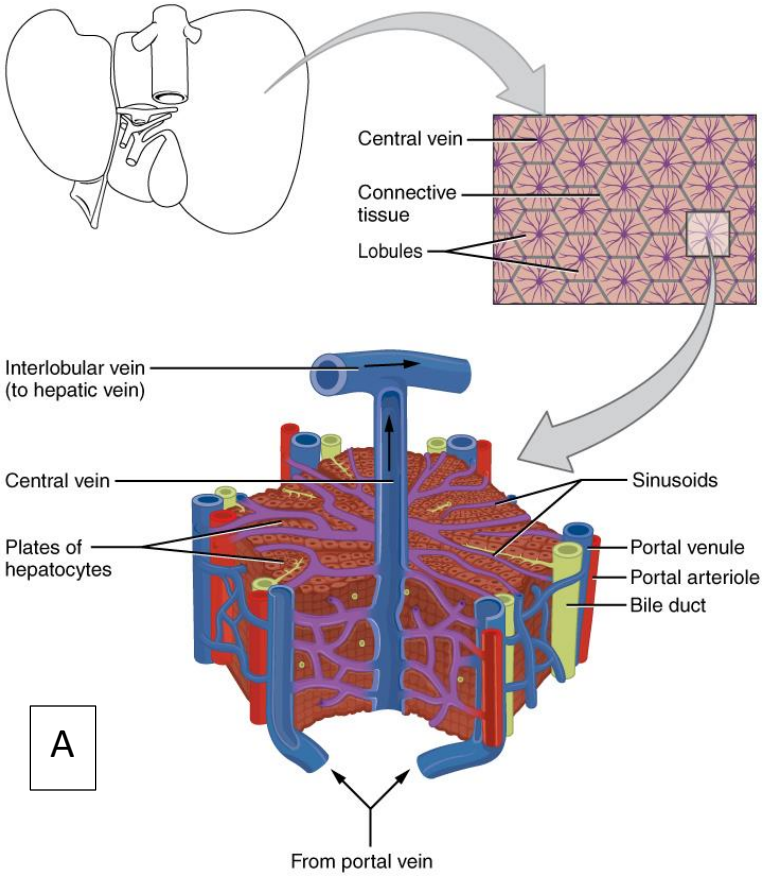


Figure 3*: Rat liver in situ. (A) Anterior view, (B) Anterior view after separating the lobes (Martins and Neuhaus, 2007).* AC, anterior caudate lobe; SRL, superior right lateral lobe; IRL, inferior right lateral lobe; ML, median lobe; RML, right portion of the medial lobe; LML, left portion of the medial lobe; LLL, left lateral lobe; FL, falciform ligament; S, stomach; D, duodenum; PV, portal vein and IVC, inferior vena cava.

Regardless of gross differences, the microscopic features of the rat's and human livers are more or less similar. The liver is structurally organized into polygon-shaped classic hepatic lobules as seen in light-microscope sections. As shown in (**figure 4a**) each lobule is formed of the central vein located at the center and cords of hepatic cells radiating peripherally from the central vein. The cell cords are separated by narrow blood sinusoids. Each lobule is bound by scanty connective tissue. As shown in (**figure 4a & b**) at the apices of lobules there are portal islands of connective tissue, each containing a branch of the hepatic artery, hepatic portal vein, and bile ductile forming portal triads (Demetris and Schiff, 2008). Blood always flows from the periphery to the center of each hepatic lobule. Consequently, oxygen and metabolites, as well as all other toxic or nontoxic substances absorbed in the intestines, reach the lobule's peripheral cells first and then the more central cells (Mescher, 2013). The parenchymal cells of rat liver, hepatocytes, are polyhedral cells with acidophilic cytoplasm and a rounded pale stained nucleus. The sinusoidal surface of hepatocytes has numerous microvilli that increase the available surface area for the exchange and absorption of substances from portal blood (Malarkey *et al*., 2005, Demetris and Schiff, 2008).

As shown in (**figure 4a**) hepatic sinusoids are capillaries that are larger and more irregular in shape than ordinary capillaries. These sinusoids conduct nutrient/hormone-rich portal venous blood and high-oxygenated arterial blood to pass the hepatocytes slowly. Cells lining the hepatic sinusoids include liver macrophages (Kupffer cells), endothelial cells, and fat-storing stellate cells (Ito cells) (Laskin, 1996, Demetris and Schiff, 2008, Thoolen *et al*., 2010). The main functions of Kupffer cells are to break down aged erythrocytes, remove bacteria or debris that may enter the portal blood from the gut, and clear foreign materials, in particular, endotoxin from the portal circulation. Endothelial cells are long slender cells with extended processes, and they constitute the major cellular element of the hepatic sinusoidal lining with a fenestrated endothelium (Laskin, 1996, Mescher, 2013).



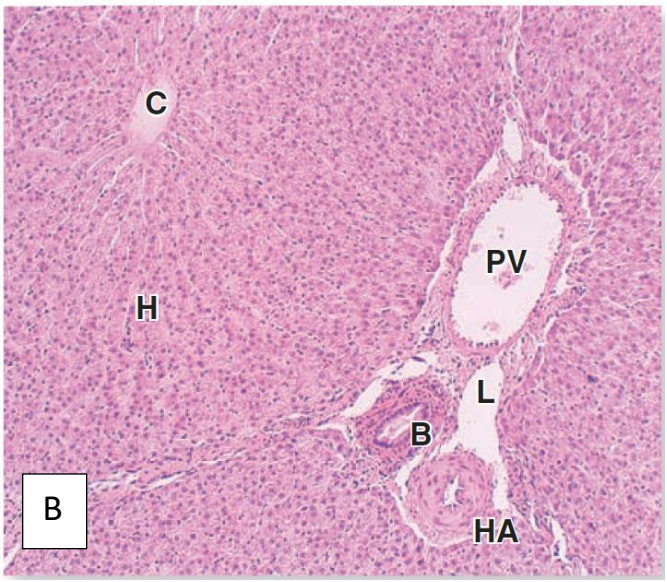


Figure 4*: (A) Diagrams of the human liver Hepatic lobules, Hepatocytes, and Sinusoids (J. Gordon Betts, 2013). (B) Micrograph of portal triad X220. H&E (Mescher, 2013).* C, central vein; H, plates of hepatocytes; L, lymphatic; PV, portal venule; HA, hepatic arteriole; and B, bile ductule.

The liver is a vital organ that has four major functions; secretory, storage, metabolic, and excretory. Its secretary function involves the production and release of 600 to 1200 mL of bile per day for emulsification of the large fat particles of food into smaller particles. It manufactures most of the plasma proteins and blood clotting factors (Abol-Munafi *et al*., 2006). Liver cells store metabolites such as large amounts of glucose in the form of glycogen, amino acids, and fat-soluble vitamins from the diet. Whenever needed it also breaks down glycogen into glucose to supply the body with energy (Guyton and Hall, 2006). It detoxifies and eliminates drugs such as pesticides, herbicides, and toxic substances, and removes damaged red blood cells from the blood in coordination with the spleen (Abol-Munafi *et al*., 2006, Bigoniya *et al*., 2009).

Because of the livers' central role in xenobiotic metabolism and portal location within the circulation, accumulation of toxins within the liver is common. Accumulation of toxins in the body faster than the capacity of the liver to process and remove them results in hepatic damage (Bigoniya *et al*., 2009). Early evidence of liver damage is usually manifested by the fatty change that is indicated by the form of cytoplasmic vacuoles in hepatocytes. These vacuoles will displace the nucleus to one side, and hepatocytes will enlarge and their nuclei appear darkly stained (Ebaid *et al*., 2007). Cytoplasmic vacuolation occurs mainly as a consequence of a considerable disturbance in lipid inclusions and fat metabolism occurring during pathological changes (Chen *et al*., 1984). When there is more severe metabolic disruption, the hepatocytes will undergo hydropic degeneration and become swollen. These affected cells will gradually undergo necrosis, which is an indication of severe hepatic damage (Abol-Munafi *et al*., 2006).

1.4. Kidney Structure and Function

Kidneys are paired, reddish-brown, bean-shaped retroperitoneal organs lying on the posterior wall of the abdominal cavity on each side of the vertebral column between the level of the twelfth thoracic and the third lumbar vertebrae, with an adrenal gland sitting on the top or superior pole of each kidney like a small-cap (Landau R., 1980). The right kidney lies slightly lower than the left kidney. A typical single adult human kidney measures 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, and the weight ranges from 125 g to 170 g in the male and from 115 g to 155 g in the female. The kidneys are concave medially and convex laterally, and the medial or concave surface of each kidney has a slit, called the hilus, through which the renal pelvis, the renal artery and vein, the lymphatics, and a nerve plexus pass into the sinus of the kidney. The kidneys are closely invested by a strong fibrous capsule, which gives the fresh kidney a glistening appearance and it is easily removable under normal conditions. Each kidney has anterior and posterior surfaces, medial and lateral borders, and superior and inferior poles (Moore *et al*., 2013).

The kidneys of the rat are paired, bean-shaped, smooth, reddish-brown organs, which are covered by a thin connective tissue capsule adherent to subcapsular connective tissue. The rat kidneys lay on either side of the vertebral column in the abdominal cavity retroperitoneally. The right kidney of the rat is larger and heavier, also located more anteriorly and cranially than the left kidney (Cook, 2008). Each kidney has dorsal and ventral surfaces, medial and lateral borders, and an upper and lower pole. The lateral border is convex, while the medial border is concave with indentation, called the hilus, where major renal vessels enter and leave, and the ureter originates (Onyeanusi *et al*., 2009, Al-Samawy, 2012). Male rats have relatively larger kidneys than do females; kidney weight varies between inbred strains (Popesko *et al*., 1990).

A coronal section of the kidney reveals two major distinct regions: the outer pale region the cortex, which has a light color and granular appearance, and the inner medulla, which has a dark reddish-brown color. The medullas are a series of pyramidal structures. The cortex or cortical parenchyma extends into spaces between adjacent pyramids, and these extensions are known as the columns of Bertin. A medullary pyramid with two adjacent columns of Bertin plus the sub-capsular cortex constitutes a renal lobe. The base of each pyramid is positioned at the

corticomedullary boundary, and the apex extends toward the renal pelvis to form the renal papilla (Eddy and Fogo, 2006).

In humans, the medulla is divided into 8 to 18 renal pyramids. Each pyramid has its own renal papilla, which extends into a minor calyx. Several minor calyces converge into 2-3 major calyxes. The major calyces converge into the renal pelvis. Unlike the human kidney, as in (**figure 5**) the rat kidney has a single renal pyramid and is therefore termed as "unipapillate.". The unipapillate kidney of the rat is directly surrounded by the renal pelvis and extends to the ureter. Otherwise, the rat kidney resembles the human kidney in its gross and microscopic appearances (Webster *et al*., 1947, Eddy and Fogo, 2006, Sowash, 2009, Al-Samawy, 2012).

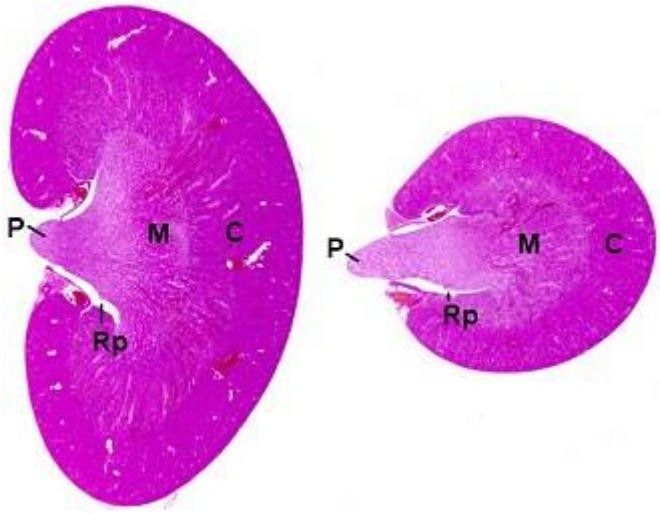


Figure 5: Photograph showing the microscopic structure of rat kidney (Piper *et al.,* 2012). **P,** papilla; **M**, medulla; **C,** cortex; **Rp,** renal pelvis.

The nephrons are the basic structural and functional units of the kidney. A single human kidney contains about 1 - 1.4 × 10^6 nephrons (Mescher, 2013). In contrast, each adult rat kidney contains approximately 30,000 nephrons (Rytand, 1938, Webster *et al*., 1947). Nephron has two parts: the renal corpuscle, where blood plasma is filtered, and the renal tubules, into which the filtered fluid passes (Tortora and Derrickson, 2018).

As shown in **figure 6b**, each renal corpuscle consists of a tuft of capillaries (the glomerulus), which is surrounded by a double-walled epithelial capsule called a glomerular (Bowman's)

capsule. The internal (visceral) layer of the capsule envelops the capillaries of the glomerulus. The external (parietal) layer forms the outer limit of the renal corpuscle. Between the two layers of Bowman's capsule is the urinary space, which receives the fluid filtered through the capillary wall and the visceral layer. Each renal corpuscle has a vascular pole, where the afferent arteriole enters and the efferent arteriole leaves, and a urinary pole, where the proximal convoluted tubule begins.

The renal tubule can be subdivided into several distinct parts in the cortex and medulla. These include the proximal convoluted tubule, the thin and thick limbs of Henley's loop, the distal convoluted tubule, the collecting tubules, and collecting ducts (Al-Samawy, 2012, Mescher, 2013). The proximal convoluted tubule is lined by cells that have abundant long microvilli on their luminal surfaces forming a prominent brush border for reabsorption (**figure 6b**). The loop of Henley is a U-shaped structure, which interposed between the proximal and distal convoluted tubules. It has descending and ascending limbs that are composed of simple cuboidal epithelial cells near the cortex and simple squamous epithelial cells deeper in the medulla (**figure 6a**). The distal convoluted tubule is lined by simple cuboidal cells, which are flatter, smaller, and have no brush border in contrast to the cells of the proximal convoluted tubule (**figure 6b**). The initial straight part of the distal tubule makes contact with the vascular pole of the renal corpuscle of its parent nephron and forms part of a specialized structure, the juxtaglomerular apparatus. At the point of contact with the arterioles, the cells become columnar and more closely packed, and called the macula densa (Mescher, 2013).

The collecting tubules are not part of the nephron, and these tubules are lined by simple cuboidal epithelium with rounded nuclei and basophilic cytoplasm. Cell boundaries are normally clear when compared with the cells of the proximal and distal convoluted tubules in progress toward the renal papilla (William and Linda, 2000). The terminal portion of these tubules is lined by simple or pseudostratified columnar epithelium and it is called the papillary duct. The renal pelvis appears as a dilated cavity at the proximal end of the ureter lodged in the renal sinus and facing the renal papilla (Charmi *et al*., 2009).

The space between uriniferous tubules and blood and lymph vessels is called the renal interstitium. It occupies a very small volume in the cortex but increases in the medulla. In the renal interstitium, there is a very flimsy and scant amount of loose connective tissue, housing

three types of cells. These cells are fibroblasts, macrophages, and interstitial cells (Gartner and Hiatt, 2007, Mescher, 2013).

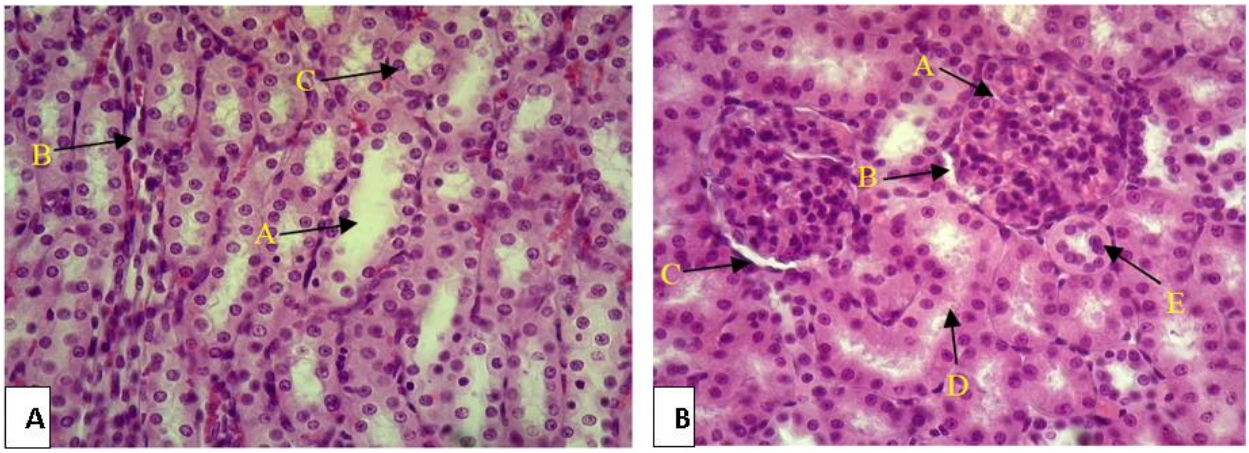


***Figure 6**: Photomicrograph of kidney tissue, (400× H & E), (Al-Samawy, 2012).* (A); A-Collecting Tubule; B-Thin limb; C-thick descending limb. (B); A-Glomerulus. B-Visceral layer. C-Parietal layer. D-Distal tubule. E-Proximal tubule.

The kidneys clear the blood plasma of metabolic wastes, regulate fluid osmolality and volume, maintain electrolyte balance, eliminate foreign chemicals, and help maintain the acid-base balance of the body. In addition to these, the kidneys have properties of endocrine organs (Gartner and Hiatt, 2007). The kidneys are the primary organs for the clearance and excretion of foreign chemicals, including drugs and drug metabolites from the body. Damage to the kidney may arise due to the administration of plant extracts (Nwanjo, 2007). In Africa, the use of traditional herbal remedies has been implicated for about 35% of all cases of acute kidney failure (Colson and De Broe, 2005). Nephrotoxicity can result in systemic toxicity, causing decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance, and decreased synthesis of essential hormones (Abdelhafiz *et al*., 2010). As a result, a study of the effect of drug extracts on the kidney is essential. Therefore, the assessment of the histology of the kidney tissues and the determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys; such metabolites include urea and creatinine (Nwanjo, 2007).

1.5. Blood Composition and its Function

Blood is a specialized connective tissue that consists of cells surrounded by a liquid extracellular matrix. The extracellular matrix is called blood plasma and accounts for 55% of total blood volume, and it suspends various cells and cell fragments collectively known as formed elements, which accounts for 45% of total blood volume. The formed elements circulating in the plasma are erythrocytes (red blood cells), leukocytes (white blood cells), and platelets. Blood is propelled mainly by rhythmic contractions of the heart. About 5 L of blood in an average adult human moves unidirectionally within the closed circulatory system (Mescher, 2013, Tortora and Derrickson, 2018).

The total blood volume of an average normal adult rat is about 5.6 - 7.1 ml/100g of body weight, and it is estimated to account for 7% of its total body weight. Younger rats usually have a larger blood volume relative to their body weight than older rats (Garcia, 1957, Pass and Freeth, 1993).

Erythrocytes or red blood cells (RBC) are terminally differentiated, flexible, biconcave, anucleated cells devoid of typical organelles, and filled with oxygen-carrying hemoglobin. Hemoglobin is a protein, which is involved in the transport of O_2 and CO_2, and function exclusively within the vascular system (Burkitt *et al*., 2006, Mescher, 2013). There are about 25 trillion erythrocytes in the human body (Carola *et al*., 1992). The normal range of erythrocytes in a typical blood sample is from 4.1 - 6.0 million/µl in human adult males and 3.9-5.5 million/µl in females (Mescher, 2013). Blood of a normal adult rat contains about 7-10 million RBC/µl of blood sample (Pass and Freeth, 1993). Morphology of human and rat RBC appears similar, and rat RBC measures 4–7µm in diameter, while that of human RBC measures 6–8 µm in diameter (Diggs *et al*., 1954).

The reference values of RBC indices such as hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in adult rat measures about 11-19.2g/dl, 35 - 64%, 14.3 – 19.5 pg. and 26.2 – 40 g/dl, respectively (Pass and Freeth, 1993). Hematocrit is defined as an estimate of the volume of packed erythrocytes (sedimented material) per unit volume of blood or the percentage of total blood volume occupied by RBCs (Mescher, 2013, Tortora and Derrickson, 2018).

Leukocytes or white blood cells (WBC) are the diffuse units of the body's defense system. They have nuclei and a full complement of other organelles. Leukocytes function outside the blood within the tissue, and they use blood as a means of traveling from one region of the body to another. When they reach their destination, they leave the bloodstream by migrating between the endothelial cells of the postcapillary venules and capillaries by a process known as diapedesis; and head directly towards the site of injury or invasion to perform their function (Ross and Pawlina, 2011, Mescher, 2013, Tortora and Derrickson, 2018). The number of leukocytes is much smaller than that of RBCs, and it varies according to age, sex, and physiologic conditions. Healthy adult humans have 4,500 - 11,000 leukocytes per microliter of blood (Mescher, 2013). In a healthy adult rat, there are about 3,000 - 17,000 white blood cells per microliter of blood (Sharp and Villano, 2012).

Based on the presence or absence of specific granules in the cytoplasm, leukocytes are sub-classified into two general groups: the granulocytes (include the neutrophils, eosinophils, and basophils) and agranulocytes (include monocytes and lymphocytes). Neutrophils are the most common granulocytes as well as the most numerous WBC and they constitute 40-75% of circulating leukocytes of adult healthy humans (Ross and Pawlina, 2011). In the rat, neutrophils account for 14-20% of total leucocytes (Pass and Freeth, 1993). Their primary function is to attack and destroy invading bacteria and other small injurious particles, and they eliminate tissue debris by the way of phagocytosis. They are usually the first leukocytes to arrive at sites of infection (Mescher, 2013).

Eosinophils contain large fragile eosinophilic granules in their cytoplasm. In humans, they constitute 1 to 3% of total leukocytes (Mescher, 2013), while in rats they account for 1 - 4% of the total leucocytes (Bernstein, 1963). They release enzymes, such as chemokines, cytokines, and lipid mediators that combat the effects of histamine and other substances involved in the inflammatory process during allergic reactions. Besides, they contain eosinophilic peroxidase and toxins in their cytoplasm, that act to kill parasitic worms or helminths. Eosinophils also phagocytize antigen-antibody complexes (Mescher, 2013).

Basophils contain large basophilic cytoplasmic granules. In humans, they are the least abundant components of WBCs. Basophils are involved in allergic reactions and they produce heparin, histamine, and serotonin. These chemicals intensify the inflammatory reactions and are involved

in hypersensitivity (allergic) reactions (Tortora and Derrickson, 2018). Like humans, the percentage composition of basophils is rare in total circulating leukocytes of rats (Pass and Freeth, 1993).

Monocytes are the largest leukocytes, and they are the precursors to the cells of the mononuclear phagocytotic system. They originate from the bone marrow stem cells and migrate to different tissues using the blood as a means of transportation. The monocytes constitute 2-10% of total leukocytes and differentiate into the various phagocytes. For example, when they are found in the liver they are called perisinusoidal macrophages or Kupffer cells. All monocyte-derived cells are antigen-presenting cells, and they have important roles in the immune defense of tissues (Ross and Pawlina, 2011).

Lymphocytes are the main functional cells of the lymphatic or immune system, and they are the most common agranulocytes that account for about 30% of the total blood leukocytes (Ross and Pawlina, 2011). In rats, the lymphocytes are the major component of blood and constitute 69-86% of the total circulating leukocytes (Pass and Freeth, 1993). They are the major soldiers in lymphatic system battles, and they respond against foreign substances that are introduced to the body. Most lymphocytes continually move among lymphoid tissues, lymph, and blood, spending only a few hours at a time in blood. Thus, only a small proportion of the total lymphocytes are present in the blood at any given time.

There are three functionally distinct types of lymphocytes in the body: T-lymphocytes, B-lymphocytes, and Natural killer (NK) cells. B-lymphocytes are involved in the production of circulating antibodies and are particularly effective in destroying bacteria and inactivating their toxins. T-lymphocytes attack viruses, fungi, transplanted cells, cancer cells, and some bacteria. They are also responsible for transfusion reactions, allergies, and the rejection of transplanted organs. NK cells are programmed during their development to kill certain virus-infected cells and some types of tumor cells (Ross and Pawlina, 2011, Tortora and Derrickson, 2018).

Platelets (thrombocytes) are not whole cells. They are very small, non-nucleated, disk-like, membrane-bound cell fragments that are formed in the bone marrow from megakaryocytes (Landau R., 1980). Platelets play an important role in the control of bleeding (homeostasis) by promoting blood clotting and helps in repairing gaps on the walls of blood vessels, thus

preventing the loss of blood. Normal platelets count ranges from 150,000 to 400,000 per microliter of blood in healthy adult humans (Mescher, 2013), while in a healthy adult rat platelets count ranges from 500,000 to 1,000,000 per microliter of blood (Pass and Freeth, 1993).

Plasma is a pale straw-colored liquid in which blood cells are suspended. It consists of 90% water and a variety of substances of low or high molecular weight that makes up 10% of the plasma. These include proteins, dissolved gases, electrolytes, nutrients, regulatory substances, and waste materials. Plasma consists of plasma proteins, such as albumin, globulin, and fibrinogen. Plasma is involved in the transport of nutrients from their site of absorption or synthesis and distributing them to various parts of the body. It also transports metabolic residues which are removed from the blood to excretory organs (Ross and Pawlina, 2011).

Plasma is obtained after the treatment of blood with an anticoagulant. In contrast, the serum is obtained from clotted blood from which serum biochemical parameters such as Glucose, Urea. creatinine, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and others are studied to test renal, hepatic, and cardiac functions (Mescher, 2013). Pass and Freeth (1993), reported that the composition of the rat's serum is similar to that of human serum.

AST and ALT concentrations in plasma indicate the health status of the liver and heart. ALP is commonly found in the biliary tree and bile duct, a block in this system will cause an elevated concentration of ALP in the serum. Urea and creatinine are good 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 (Wasan *et al*., 2001).

Administration of herbal medicine may result in reduced or increased RBC, HCT, and HGB concentration indicating that the herbs may produce some anemic or polycythemic effect. In one study treatment of rats with 800 mg/kg extract of *Gnidia stenophylla* Gilg root induced a significant rise in the RBC, HGB, and HCT counts when compared to the control levels (Nigatu *et al*., 2017). Another study done by Alebachew and his colleagues (2014), showed methanolic leaf extract of *V. bipontini* decreased RBC, HCT, HGB, Platelet, MCV, MCH, and MCHC at the dose of 800mg/Kg. Similarly, it was indicated that the number of lymphocytes may be reduced in the blood due to the presence of some chemicals in certain medicinal plants (Aniagu *et al*.,

2005). In a study done by Nigatu *et al*. (2017), 800mg/kg dose of *Gnidia stenophylla* Gilg root extract significantly reduced lymphocytes count. Biologically active ingredients of administered herbal medicine contain biologically active components that stimulate the immune system by increasing the number of defensive WBC (Taylor *et al*., 1997). One study showed 250mg/Kg dose of seed extract of *Albizia gummifera* resulted in an increased neutrophil count (Debebe *et al*., 2017).

It was also indicated that some herbal medicines cause elevation of blood urea nitrogen, AST, ALT, ALP, urea, creatinine, total protein, and albumin in the serum of animal blood. Elevation of those values is an indication of its toxic effect in the animal model (Oyewole and Massaquoi, 2008). A study on the aqueous extract of *Artemisia afra* showed a dose-dependent significant decrease in the level of AST in the female rats (Eshetu *et al*., 2016). Another study done by Alebachew *et al*. (2014), showed 800mg/kg dose of the methanolic leaf extract of *Vernonia bipontini* caused a significant increment in serum AST, ALT, and ALP levels. Oral administration of the aqueous *M. stenopetala* leaves extract at 2000mg/Kg dose has resulted in a significant decrement in the concentration of urea and a significant increment in the concentration of creatinine level in female rats (Bayu *et al*., 2020).

1.6. Toxicological Studies

Toxicity is defined as "the ability of a substance to cause poisonous effects resulting in severe biological harm or death after exposure to or contamination with that substance" (UN, 1997). There are only very few clinical data on the safety and efficacy of herbal medicines, and there is also no consensus even among traditional healers, on which plant preparations and dosages are most effective. Parallel with the recent increasing interest in herbal medicine for the prevention and treatment of various human illnesses and animal diseases, there is increasing concern about the safety of medicinal plants. There are general and herb-specific concerns regarding medicinal plants and their ability to produce toxicity and adverse effects (Saad *et al*., 2006).

Several authors have suggested the utilization of *M. stenopetala* seeds for drinking water purification as a safe option, as there was no known toxicity to humans that has ever been recorded. Seeds powder from 50 seeds per 100 mL water has been reported to have no toxic effect on rodents (Berger *et al*., 1984, Mayer and Stelz, 1993). In a 6-week study by Berger *et al*. (1984), the seeds of *M. stenopetala and M. oleifera* neither showed lethality in rats nor altered macroscopic and histologic features of 28 organs. This research was confined only to the non-toxic dose of the seed with a concentration of 50 & 500 mg/kg, but the toxic dose has to be evaluated. Research done by Ajibade and his colleagues (2013), on the effects of methanol extract of the seeds of *M. oleifera* in rats revealed that acute toxicity was observed at a dose of 4,000 mg/kg and the median lethal dose of the extract in the rat was 3,873 mg/kg.

Many findings show that the extracts of *M. stenopetala* seed could be promising natural antimicrobial agents with potential applications in controlling water-borne diseases. The extracts can provide a cheap and sustainable method of disease reduction in developing countries. However, very little is known about the toxicity of the seed of *M. stenopetala*, thus the safety and toxicity of the extracts need to be evaluated before using them to treat human diseases (Seifu, 2015), which is an issue this study is aimed to investigate.

1.7. Significance of the Study

It is well known that herbal medicines contain ingredients that maintain health and cure ailments. However, they may also contain toxic substances that are harmful or even dangerous to health. WHO (2008), notes that the inappropriate use of traditional medicines or practices can have negative or dangerous effects. Hence, research is needed to ascertain the efficacy and safety of several practices and medicinal plants used by traditional medicine systems.

Several studies on the seed of *M. stenopetala* have been done regarding its importance in the treatment of helminthic and bacterial infections, water coagulation, chemical removal from water, and anticancer and antioxidant effects. However, research related to the toxic effects of *M. stenopetala* seed in different organs is insufficient and incomplete. Therefore, the rationale of conducting this study is to investigate if there is any toxic effect of *M. stenopetala* seed which is used as traditional medicine and for water purification.

Generally, this research will fill the gap in knowledge on histopathologic effects of the seed of *M. stenopetala* on the kidneys, liver, and some blood parameters which can be used as a baseline for further toxicity study in higher animals. The outcome of the study will serve as a premise for further investigation on this plant's seed for better utilization of its claimed nutritional, therapeutic, and water purification values. It would also help in the formulation of regulatory legislation regarding the use of the plant.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

- To evaluate the acute and sub-chronic toxicity of the aqueous extracts of *M. stenopetala* seeds on kidneys, liver, and blood parameters of rats.

2.2. Specific Objectives

- To evaluate the acute and sub-chronic toxic effects of aqueous *M. stenopetala* seeds extract on the general behavior and body weight of rats.
- To evaluate the acute and sub-chronic toxic effect of aqueous extracts of *M. stenopetala* seeds on the gross anatomy of kidneys and liver of rats.
- To determine the LD_{50} value of the aqueous extracts of *M. stenopetala* seeds on rats.
- To evaluate the histopathological effect of the sub-chronic toxicity of aqueous extracts of *M. stenopetala* seeds on kidney and liver tissues of rats.
- To examine sub-chronic toxic effects of *M. stenopetala* seeds on blood parameters of rats.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted at the Traditional and Modern Medicine Drug Research Directorate, EPHI, and Anatomy Department of AAU.

3.2. Study Period

The study was conducted from May, 2019 to December, 2020.

3.3. Study Design

The laboratory-based experimental study design was used in this study.

3.4. Collection of Plant Material

The fresh seeds of *M. stenopetala* were collected from Arbaminch, 500km far from the capital city (Addis Ababa), Ethiopia. Then the seeds were recognized and authenticated by a taxonomist in the EPHI, and a small sample was deposited at the herbarium with a voucher number (GH/24) for future reference.

3.5. Preparation of Extract

The seeds were dried at room temperature and crushed to powder and weighed by electronic digital balance. The powdered seeds were macerated in water with 1 to 10 ratios for 24 hours with intermittent agitation by an orbital shaker. The supernatant of the mixture was decanted and filtered from the un-dissolved portion with mesh gauze. The filtrates were freeze-dried in a lyophilizer to yield a crude extract. From 3 Kg dry seed, which was dissolved in a total of 30 liters distilled water (1:10), 702.3 g (23.41%) of crude extract was obtained. The crude extract was kept in a desiccator at -20^{o}C until used (Debella, 2002).

3.6. Experimental Animals

For acute toxicity study (one-day administration of the extract and 14 days follow-up) only female rats were used, while both male and female rats were used for sub-chronic toxicity (90 days administration of the extract and follow-up) study. The rats used were aged between 8 - 12 weeks at the start of initial dosing and females were nulliparous and non-pregnant. The rats were obtained from the animal house of Addis Ababa University. The rats were housed in cages made from stainless steel and in an environmentally controlled room at 22 - 23°c and relative humidity of 50% ± 10. The room was illuminated with artificial light of 12 hours light and 12 hours dark cycle.

The rats were assigned randomly to control and treatment groups and marked with a unique identification color and kept in their cages for 7 days before the start of dosing to allow for adaptation to the new environment in the laboratory. During the period of adaptation, rats received food (Standard pellets) and water *ad-libitum*.

3.7. Grouping of Rats and Dosing of the Extract

In the pilot and acute toxicity study, 3 (three) female rats per group were used. Dosing of the crude extract was started with the initial dose of 300mg/Kg, followed with 2000mg/kg, 3500mg/Kg, and 5000mg/Kg. The doses were increased after 24 hours of observation of the previous dose effect (OECD, 2001).

For the sub-chronic toxicity study, a total of 48 rats of both sexes (n = 48; 24 males and 24 females) were used. The rats were randomly divided into four groups of each sex, with six rats in each group comprising of three experimental groups (Group I, II & III), and one control group (Group IV). Rats in the control group were given the vehicle (distilled water). Determination of the dose of the seeds' extract was done after the observation of the effect of acute toxicity and the result of LD_{50} of the extract (Wamburu *et al*., 2013, Prasanth *et al*., 2015, OECD, 2018). This was done as 10% of the LD_{50} as the medium dose, half of it as the minimum dose, and double of the medium dose as the higher dose. The first experimental group (Group I) rats were administered daily with 250mg/Kg body weight of the seeds' extract which is half of 10% of LD_{50}. The second experimental groups (Group II) received daily 500mg/Kg body weight of the

seeds' extract which is 10% of LD_{50}, while the third experimental groups (Group III) were administered daily with 1000mg/Kg body weight of the seeds' extract which is a double of the 10% of LD_{50} of the extract by gavage. During the days of the experiment, the crude extract was restructured with the vehicle (distilled water).

3.8. Observations of Toxic Effects of the Extract

For the acute toxicity study, mortality and clinical signs of toxicity were monitored continuously for 4 hours after dosing on day one. For the rest of the 14 days of the study period, animals were monitored once daily for signs like increased motor activity (increased grooming, scratching, and ambulation), tremors, lacrimation, piloerection, excretion, and salivation. Body weight was measured prior to initial dosing, then it was repeated on days 7 and 14. On day 15, all animals were sacrificed. Detailed examination of gross pathological changes of organs was done, with special attention to the kidneys and liver.

In the sub-chronic toxicity study, general clinical observations were made once a day, and the clinical condition of the animals was recorded. At the beginning and end of each day, all animals were inspected for signs of morbidity and mortality. Body weight was measured once before the initial dosing, then it was repeated at the end of every week. Once before the first exposure and once a week thereafter at similar times outside the home cage in a standard arena, detailed clinical observations which include changes in skin, eyes, mucous membranes, fur, gait, posture, and occurrence of secretions, excretions, and tonic or clonic movements. Besides, changes in autonomic activities (e.g., piloerection, lacrimation, and unusual respiratory pattern), response to handling, and bizarre behaviors (e.g., self-mutilation, repetitive circling, walking backward, and excessive grooming) were made and carefully recorded.

3.9. Determination of Hematological and Biochemical Blood Parameters

After 90 days of the experimental period, while fasted for about 12 hours, the rats were deeply anesthetized and blood samples were taken by cardiac puncture. The blood samples were analyzed using the UniCel DxH 800 (Beckman Coulter, USA) automatic hematology analyzer, and Red blood cells (RBC), White blood cells (WBC), Hemoglobin (HGB), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration

(MCHC), Platelet (PLT), Neutrophils (NEU), Eosinophils (EO), Basophils (BA), Lymphocytes (LY), and Monocytes (MO) count were evaluated. The concentrations of Urea, Creatinine, Total protein (TP), and Albumin (ALB), and enzymes indicative of hepatocellular effects (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphate (ALP)) were determined using Hitachi Cobas 6000 (Roche Diagnostics, Germany) blood chemistry analyzing machine.

3.10. Gross Necropsy

After 14 days of acute toxicity study and 90 days of sub-chronic toxicity study, all rats were sacrificed and subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents, by giving special attention to Liver and Kidneys (OECD, 2018).

For both acute and sub-chronic study, after dissection and examination of gross pathology of liver and kidney actual weight of these organs were measured and relative weight was calculated as follow.

$$\text{Relative weight of organ} = \frac{\text{Actual organ weight} * 100\text{g of body weight}}{\text{Total body weight}}$$

3.11. Study on Histopathology of the Liver and Kidney

After 90 days of sub-chronic toxicity study, rats were sacrificed; parts of the liver and kidney tissues were collected for histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of 24 hours, then dehydrated with increasing concentrations of alcohol, embedded in paraffin, cut into 5 micrometer thick sections by microtome, and finally stained with hematoxylin-eosin dye for photo microscopic observation (Appendix II and III). The microscopic features of the organs of male and female rats of experimental groups were compared with the control group.

3.12. Light Microscopy and Photomicrography

The stained tissue sections of the kidney and liver were examined carefully with the guidance and supervision of a pathologist using a binocular compound light microscope with an in-built digital photo camera (LEICA DM750 made in Germany). Tissue sections of the experimental groups were examined and compared with the controls for any evidence of histopathological changes blindly. After examination, photomicrographs of selected samples of liver and kidney sections from both the experimental and control rats were taken.

3.13. Data Analysis

All data were organized and digitally analyzed using computer statistical software, SPSS version 25.0. The results of analyzed data values were expressed using mean + SEM (standard error of the mean). The possible difference in body weight, relative liver and kidney weights, and hematological and biochemical values between experimental and control groups were identified using a one-way analysis of variance (ANOVA), followed by Dunnett's test to determine the level of significance. Variables that do not meet the assumptions of ANOVA were analyzed using a non-parametric Kruskal-Wallis test. Statistical significance was considered at $p < 0.05$.

3.14. Ethical Issues

The study was approved by the Research Committee of the Department of Anatomy, AAU and Institutional Review Board of EPHI. All animals were handled according to the OECD guideline.

3.15. Dissemination of the Result

The findings of this study will be compiled as a thesis and submitted to the Department of Anatomy, AAU, CHSs and EPHI. It will also be published on scientific reputable journals.

4. RESULTS

4.1. Acute Toxicity Study

4.1.1. Findings of acute toxicity of *M. stenopetala* seeds aqueous extract on the behavior of rats

During the 14 days acute toxicity study period, signs of toxicity were not observed at the doses of 300mg/kg and 2000mg/kg. Mild signs of toxicity like piloerection and decreased movement within the cage were observed on two out of three rats of 3500mg/kg dose group, these signs appeared after 15 – 30 minutes of administration of the extract and disappeared within the first 3 hours of observation. At the dose of 5000mg/kg initially all rats started circling the cage and repetitive grooming immediately after administration of the extract, later on signs of toxicity like diarrhea, decreased response to touch, staying at the back of the cage, piloerection and self-mutilation were seen on one out of three rats. The rat started to develop these signs after 30 minutes of administration, and the signs completely disappeared after 8 hours. The remaining two rats also showed these signs mildly. However, no death occurred during the 14 days observation period.

4.1.2. Findings of acute toxicity of *M. stenopetala* seeds aqueous extracts on body weight of rats

Throughout the 14 days follow up there was a gradual increase in body weight of both treated and control groups. However, as shown in **table1** there was no statistically significant difference in body weight gain between the treated and the control rats.

Table 1: Comparison of the mean body weight of female rats administered with the aqueous *M. stenopetala* seeds extract and controls during the 14 days observation

Group	Dose	Initial wt.	Wt. at end of 1st wk.	Wt. at end of 2nd wk.
I	300mg/Kg	225.3 ± 7.88(0.529)	232.3 ± 8.33(0.567)	238.7 ± 7.69(0.787)
II	2000mg/Kg	224.3 ± 10.75(0.573)	231.8 ± 11.65(0.590)	238.0 ± 14.0(0.812)
III	3500mg/Kg	213.0 ± 10.69(0.978)	223.3 ± 10.17(0.936)	229.7 ± 10.71(0.994)
IV	5000mg/Kg	227.7 ± 10.68(0.433)	236.0 ± 7.84(0.410)	242.3 ± 7.75(0.637)
V	Control	207.0 ± 9.54	215.3 ± 10.04	225.0 ± 12.66

Numbers in bracket indicate P-value, Wt.: weight, wk.: week

4.1.3. Findings of acute toxicity of *M. stenopetala* seeds aqueous extract on gross pathology

After 14 days of follow up both control and experimental rats were sacrificed and the gross appearance of internal organs including kidneys and liver were assessed. Afterward, the weight of the liver and kidney were measured, and the mean relative weight of these organs was analyzed. There was no statistically significant relative mean liver and kidney weight differences between the experimental groups and the control group (**table 2**). There was no observed change in the gross appearance of internal organs. There were also no abnormal changes in color, size, shape, or texture and nor was necrosis of organs of experimental group rats in comparison to control group rats.

Table 2: Comparison of the mean relative weight of Kidney and Liver of female rats administered with the aqueous *M. stenopetala* seeds extract and controls during the 14 days observation

Group	Dose	Relative Kidney Weight	Relative Liver Weight
I	300mg/Kg	0.332 ± 0.009(0.921)	3.415 ± 0.039(0.968)
II	2000mg/Kg	0.333 ± 0.008(0.880)	3.483 ± 0.073(1.000)
III	3500mg/Kg	0.338 ± 0.006(0.600)	3.468 ± 0.109(1.000)
IV	5000mg/Kg	0.339 ± 0.009(0.523)	3.276 ± 0.196(0.483)
V	Control	0.325 ± 0.003	3.487 ± 0.039

Numbers in bracket indicate P-value

4.2 Sub-Chronic Toxicity Study

4.2.1. General observations and effect of *M. stenopetala* seeds aqueous extract on the behavior of rats

Throughout the 90 days study period there were no observed signs of toxicity among 250mg/Kg and 500mg/Kg treated groups of female rats, as well as among the male rats treated with the extract at all three doses. However, there were mild signs of toxicity like piloerection and decreased activity within the cage on one rat out of six rats among female 1000mg/Kg dose group. The rat developed these signs after 5 – 10 minutes of administration of the extract in the first three days of treatment, which disappeared after 30 - 45 minutes of administration. Also, three rats of this group were developed mild intermittent diarrhea within the first three days of treatment. This sign disappeared after one week of treatment. Otherwise, there was no observed toxicity-related death throughout the experimental period.

4.2.2. Effect of *M. stenopetala* seeds aqueous extract on body weight

During the 90 days follow-up period, there was a general gradual increment in the mean body weight of both experimental and control groups in both female and male rats (**figure 7**). However, there was a decrease in the body weight of female rats that received the extract at the doses of 500mg/Kg and 1000mgKg, respectively during the 11^{th} week, and the 9^{th} and 11^{th} weeks. Similarly, in the male rats which were treated with the extract at the dose of 1000mg/Kg the mean body weight decreased from their respective previous week during the 10^{th} week though the changes were not statistically significant.

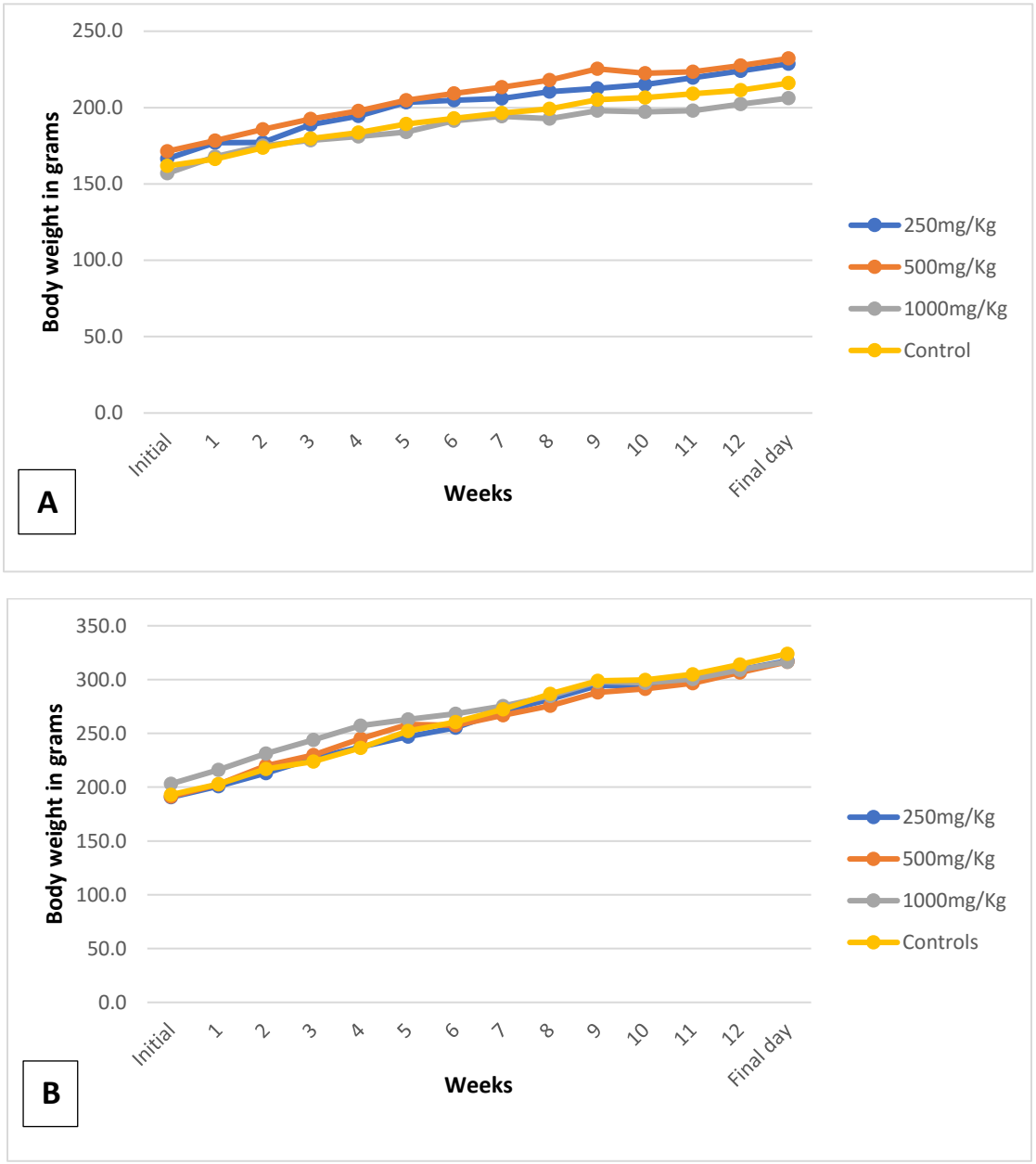


Figure 7: Comparison of the gradual mean body weight increment of (A) female and (B) male rats administered with 250mg/Kg, 500mg/Kg, and 1000mg/Kg dose of the aqueous *M. stenopetala* seeds extract and control rats for 13 weeks

At the end of 90 days study period of the sub-chronic toxicity study, the percentage weight gain of the experimental groups of both female and male rats showed no significant difference from their respective control group. However, relatively male rats showed higher body weight gain compared to female rats in both treated and control rats (**table 3**).

Table 3: Comparison of the mean percentage of weight gain of female and male rats administered with 250mg/Kg, 500mg/Kg, and 1000mg/Kg of the aqueous *M. stenopetala* seeds extract and controls during the 90 days observation

Group	Dose	Wt. gain of female rats (%)	Wt. gain of male rats (%)
I	250mg/Kg	40.62 ± 5.445(0.867)	66.32 ± 2.427 (0.985)
II	500mg/Kg	31.38 ± 4.547(0.691)	65.55 ± 4.755 (0.956)
III	1000mg/Kg	31.43 ± 3.17(0.697)	55.65 ± 6.048 (0.118)
IV	Control	36.87 ± 3.46	67.94 ± 1.719

Numbers in bracket indicate P-value, Wt.: Weight

4.2.3. Findings of *M. stenopetala* seeds aqueous extract on gross pathology and weights of the kidney and liver

At the end of the 90 days study period, no abnormal gross pathological findings were observed upon gross examination of the liver and kidneys. There were no changes in color, texture, shape, or size, and no abnormal dark and white spots in any of the treatment and control groups of both female and male rats. Besides, as shown in **table 4,** there was no statistically significant mean relative kidney and liver weight difference between experimental and control groups in both sexes.

Table 4: Comparison of the mean kidneys and liver relative weight of female and male rats administered with 250mg/Kg, 500mg/Kg, and 1000mg/Kg of the aqueous *M. stenopetala* seeds extract and controls during the 90 days observation

Group	Dose	Relative Kidney Weight		Relative Liver Weight	
		F	M	F	M
I	250mg/Kg	0.318±0.011(0.998)	0.286±0.005(0.571)	2.662±0.04(0.621)	2.619±0.053(0.987)
II	500mg/Kg	0.288±0.008(0.301)	0.284±0.01(0.469)	2.579±0.04(0.257)	2.487±0.041(0.252)
III	1000mg/Kg	0.306±0.016(0.915)	0.274±0.007(0.095)	2.865±0.169(0.900)	2.567±0.105(0.754)
IV	Control	0.316 ± 0.013	0.298 ± 0.006	2.793 ± 0.039	2.643 ± 0.046

F: female rats, M: male rats, Numbers in bracket indicate P-value

4.2.4. Effect of aqueous seeds extract of *M. stenopetala* on hematological parameters

The sub-chronic effect of the aqueous seeds extracts of *M. stenopetala* on hematological parameters of blood for both female and male rats is illustrated in **table 5**. Most of the tested hematological parameters of both male and female experimental groups were not significantly different from the control group, except in the female rats for values of RBC and HCT. RBC count of female rats treated at 500mg/Kg and 1000mg/Kg increased significantly by 7.92% and 7.4% respectively. Furthermore, the RBC count of female rats treated at 250mg/Kg increased by 1.5%, but it is not statistically significant. HCT of female rats treated at 1000mg/Kg increased by 6.4% and it was found statistically significant. Although the HCT of female rats treated at 500mg/Kg increased by 5.3% and those treated at 250mg decreased by 1%, these changes were not statistically significant.

Table 5: Comparison of the mean values of tested hematological parameters of female and male rats administered with 250mg/Kg, 500mg/Kg, and 1000mg/Kg of the aqueous *M. stenopetala* seeds extract and controls during the 90 days observation

Hematological	**250mg/Kg**		**500 mg/Kg**		**1000 mg/Kg**		**Controls**	
parameters	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
WBC ($x10^3/\mu L$)	1.62 ± 0.122(0.686)	4.7±1.433(0.624)	3.28±0.878 (0.993)	5.33±1.905(0.414)	5.02±1.498(0.384)	4.85±1.119(0.571)	2.97 ± 1.13	2.77±0.589
RBC ($x10^6/\mu L$)	7.56 ± 0.17(0.880)	8.65±0.19(0.748)	8.04±0.082(0.007*)	8.79±0.089(0.315)	8.0±0.125(0.013*)	8.69±0.157(0.626)	7.45 ± 0.091	8.49±0.068
HGB (g/dl)	14.8 ± 0.349(0.913)	15.78±0.375(0.999)	15.45±0.308(0.145)	16.27±0.138(0.507)	15.51±0.373(0.111)	15.9±0.304(0.992)	14.57±0.193	15.82±0.183
HCT (%)	44.17±1.021(0.951)	47.12±1.16(0.985)	47.0 ±0.343(0.091)	48.78±0.353(0.506)	47.48±0.889(0.036*)	48.12±0.85(0.877)	44.62±0.575	47.43±0.606
MCV (fL)	58.45±0.394(0.064)	54.47±0.721(0.152)	58.75±0.163(0.194)	55.55±0.494(0.944)	60.68±0.304(0.154)	55.38±0.313(0.837)	59.68±0.497	55.87±0.39
MCH (pg)	19.58±0.079(0.993)	18.25±0.246(0.250)	19.65±0.134(0.866)	18.53±0.143(0.947)	19.62±0.095(0.953)	18.32±0.091(0.390)	19.55±0.134	18.63±0.123
MCHC (g/dL)	33.48±0.138(0.191)	33.48±0.135(0.706)	33.62±0.095(0.074)	33.35±0.123(0.999)	32.4 ± 0.211(0.084)	33.08±0.128(0.339)	33.0±0.257	33.33±0.084
PLT (x103/μL)	827.3±31.56(0.975)	776.3±44.1(0.999)	869.0±21.58(0.584)	814.3±24.51(0.720)	834.5±54.21(0.939)	718.3±30.35(0.564)	808.7±43.95	772.0±35.69
MPV (fL)	5.53±0.08(1.000)	5.82±0.419(0.800)	5.5±0.063(0.975)	5.58±0.065(0.989)	5.62±0.075(0.741)	6.38±0.513(0.157)	5.5±0.061	5.47±0.021
NEU (%)	9.67±1.975(0.273)	12.67±2.321(0.981)	14.65±2.405(0.986)	13.3±3.1(0.997)	14.38±1.102(0.996)	14.65±4.476(0.998)	13.93±1.714	14.03±2.401
LY (%)	88.93±1.88(0.288)	86.28±2.347(0.968)	83.62±2.507(0.941)	85.18±3.177(0.999)	83.8±0.698(0.963)	83.57±4.307(0.989)	84.8±1.792	84.67±2.396
MO (%)	0.35±0.096(0.155)	0.23±0.042(0.983)	0.12±0.048(0.976)	0.25±0.096(0.998)	0.22±0.079(0.852)	0.42±0.105(0.436)	0.15±0.056	0.27±0.067
EO (%)	1.05±0.118(0.976)	0.82±0.233(0.765)	1.48±0.162(0.202)	1.27±0.217(0.725)	1.22±0.18(0.927)	0.92±0.18(0.949)	1.12±0.101	1.03±0.115
BA (%)	0.0±0.0(1.000)	0.0±0.0(1.000)	0.0±0.0(1.00)	0.0±0.0(1.000)	0.38±0.383(0.375)	0.45±0.285(0.092)	0.0 ± 0.0	0.0±0.0

*F: female rats, M: male rats, Numbers in bracket indicate P-value, *: statistically significant at p<0.05*

4.2.5. Effect of aqueous seeds extract of *M. stenopetala* on biochemical parameters

The sub-chronic effect of aqueous seeds extract of *M. stenopetala* on biochemical parameters of blood is illustrated in **table 6**. In the sub-chronic toxicity study, most of the tested biochemical parameters of both female and male rats of treated groups were not significantly different from their respective sex control groups, except in female rats for values of ALP and Creatinine, and ALB value of male rats.

The mean ALP values of female rats treated at 250mg/Kg, 500mg/Kg, and 1000mg/Kg dose groups were decreased by 25.3%, 34.2%, and 22.4%, respectively, and all of these changes were found to be statistically significant ($p<0.05$). The creatinine level of female rats treated at 500mg/Kg also showed a statistically significant increment by 20.9% from the control group.

The mean ALB value of male rats was decreased by 10% at the 1000mg/Kg dose group, and this change was statistically significant. Even though it was not statistically significant, the ALB value of 250mg/Kg and 500mg/Kg dose groups also decreased by 5.6% and 3%, respectively.

Table 6: Comparison of the mean values of tested biochemical parameters of female and male rats administered with 250mg/Kg, 500mg/Kg, and 1000mg/Kg of the aqueous *M. stenopetala* seeds extract and controls during the 90 days observation

	250mg/Kg		500 mg/Kg		1000 mg/Kg		Controls	
Biochemical parameters	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
ALT(IU/L**)**	45.03±3.205(0.479)	52.97±4.296(1.000)	49.07±4.191(0.922)	47.93±2.219(0.582)	51.57±4.689(1.000)	55.62±4.377(0.925)	51.87±3.377	53.18±2.269
AST(IU/L**)**	208.8±26.222(1.000)	239.3±30.78(0.925)	171.2±16.248(0.347)	197.2±13.41(0.119)	244.3±18.412(0.453)	218.2±11.4(0.440)	210.3±11.24	252.5±11.41
ALP(IU/L**)**	65.83±5.023(0.006*)	98.83±7.255(0.983)	58.0±5.19(< 0.001*)	78.0±6.256(0.361)	68.45±1.498(0.015*)	77.67±12.803(0.347)	88.17±5.186	95.33±5.829
UREA(g/dl)	48.28±1.368(0.851)	52.58±1.47(0.060)	55.4±2.478(0.391)	52.32±1.594(0.072)	51.03±3.583(0.998)	40.85±3.194(0.262)	50.57±1.872	45.57±1.213
CRE(mg/dl)	0.42±0.024(0.971)	0.4±0.019(0.816)	0.52±0.011(0.004*)	0.4±0.012(0.816)	0.44±0.013(0.897)	0.43±0.015(0.168)	0.43±0.016	0.39±0.012
TP(g/dl)	6.56±0.163(0.683)	6.53±0.086(0.985)	6.87±0.135(0.995)	6.46±0.067(0.999)	6.98±0.181(0.881)	6.33±0.172(0.699)	6.82±0.269	6.48±0.108
ALB(g/dl)	4.71±0.115(0.700)	4.35±0.135(0.221)	4.86±0.078(1.000)	4.47±0.062(0.693)	4.71±0.142(0.724)	4.15±0.131(0.015*)	4.87±0.162	4.61±0.061

*F: female rats, M: male rats, Numbers in bracket indicate P-value. CRE: Creatinine, *****: statistically significant at p<0.05*

4.2.6. Effect of aqueous seeds extract of *M. stenopetala* on histology of the kidney

Histopathological examination of kidney sections of both male and female rats treated with the aqueous seeds extract of *M. stenopetala* at all three doses showed no structural changes (**figure 8**). The Light microscopic structures of the kidneys were normal and similar to that of the control group rats. All the nephrons were normal and clearly visible with no degeneration, bleeding, tubular necrosis, hydropic change, loss of microvilli or infiltration with leukocytes. The appearance of glomeruli and distal and proximal tubules appeared normal. Furthermore, signs of toxicity like interstitial and intraglomerular congestion or tubular atrophies were not observed.

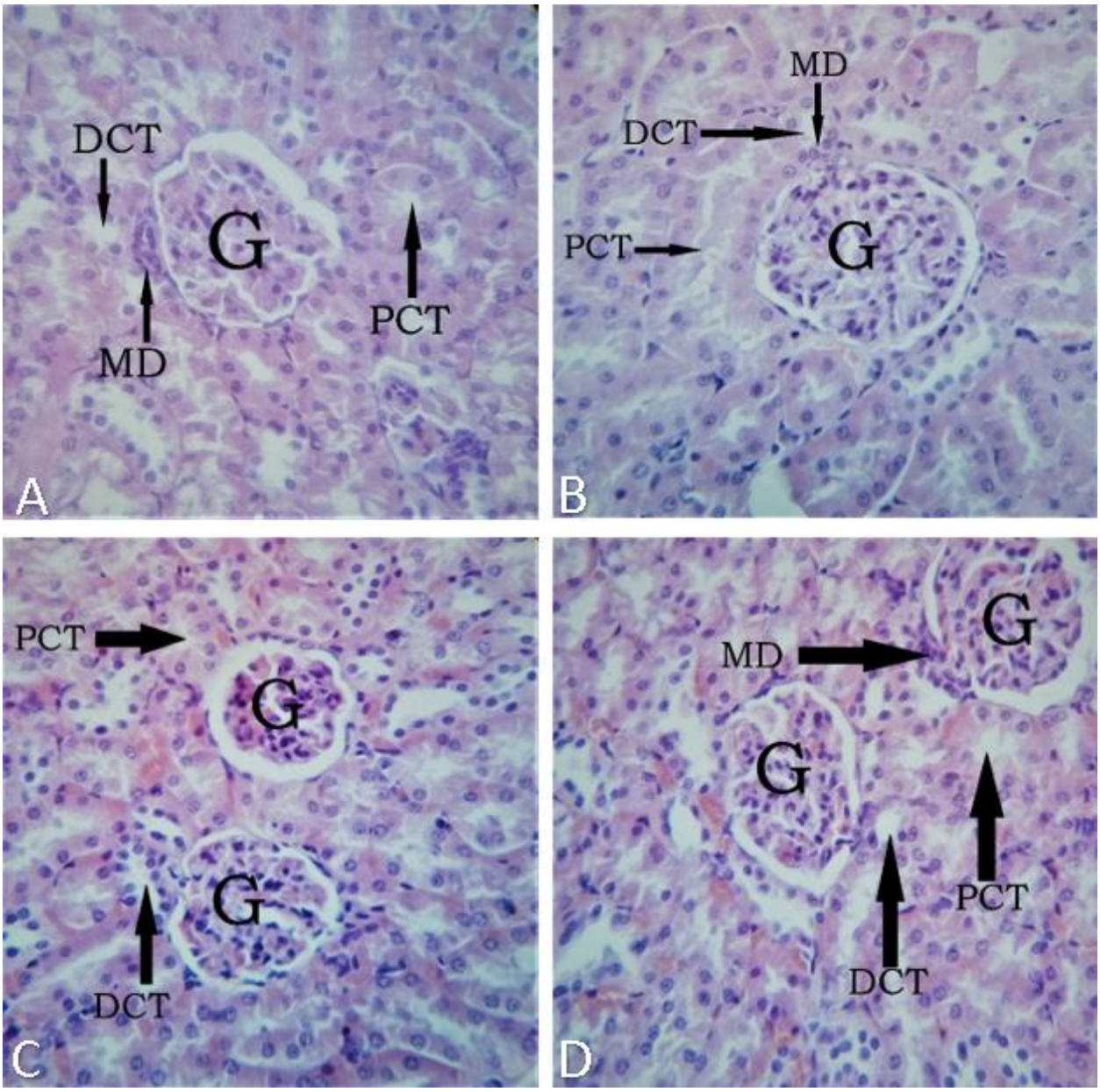
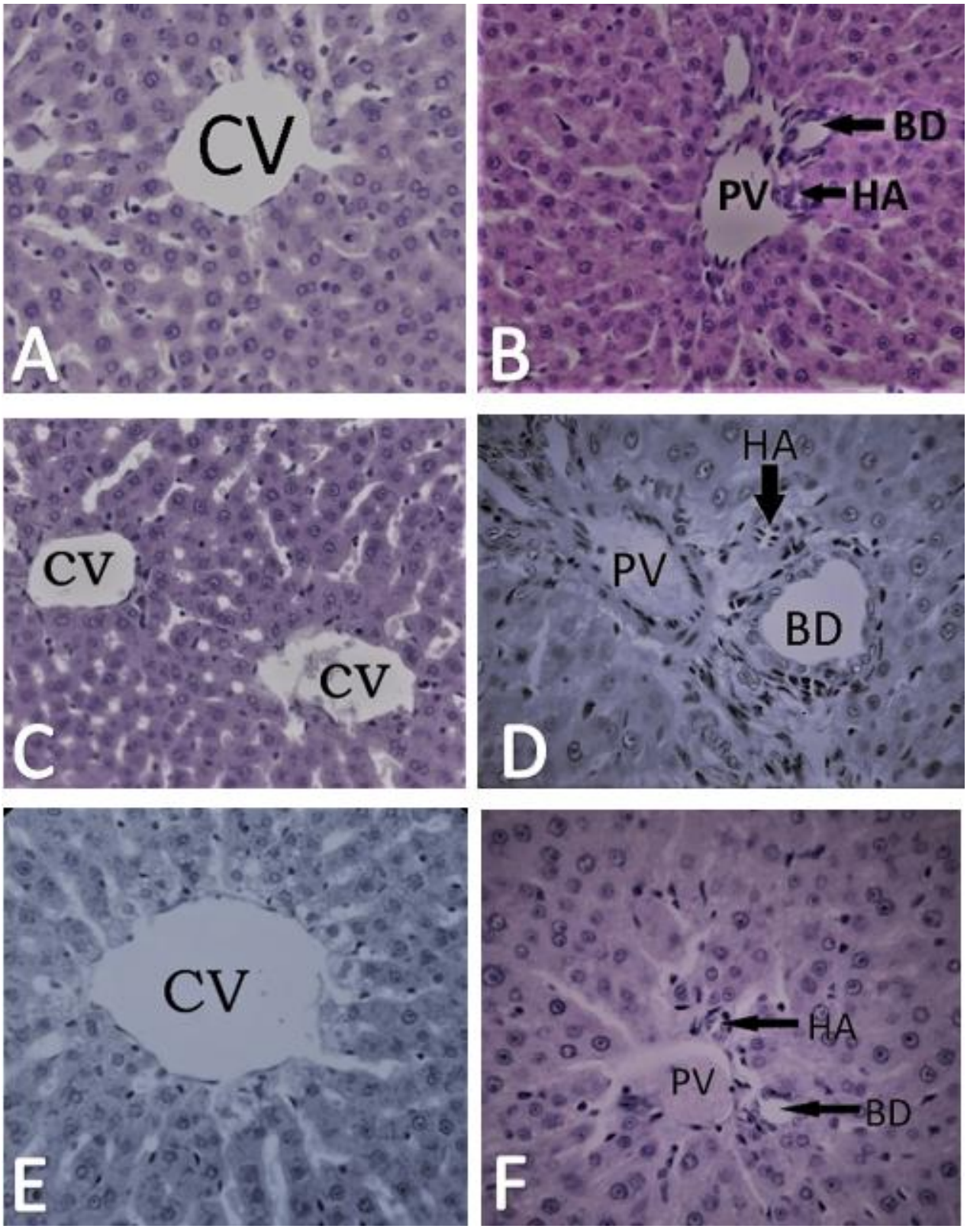


Figure 8: Photomicrographs of kidney sections from female control rats (A) as compared to those administered with aqueous *M. stenopetala* seeds extract at doses of 250mg/Kg (B), 500mg/Kg (C), and 1000mg/Kg(D). (H&E stain, 400x). G: Glomerulus, MD: Macula densa, DCT: Distal convoluted tubule PCT: Proximal convoluted tubule

4.2.7. Effect of aqueous seeds extract of *M. stenopetala* on histology of the liver

Histopathological examination of liver sections of both male and female rats treated with the aqueous seeds extract of *M. stenopetala* at all three doses showed normal architecture of the liver **(figure 9)**. Examination of the sections of liver tissue under the light microscope revealed the normal cellular architecture of hepatocytes arranged in cords surrounded by the sinusoids. Furthermore, necrosis, congestion, fatty changes, or hemorrhagic regions around the central vein or sinusoids of the liver, and lyses in the blood cells were not observed. In general, there was no observed significant histological architectural difference in the shape and size of the hepatocytes, sinusoids, and central vein between the liver section of treated and control rats.

However, light microscopic examination of the liver sections of female rats administered with 1000mg/kg dose showed portal lymphocytic infiltration **(figure 9)**.


CV
A
BD
PV
HA
B
CV
CV
C
HA
PV
BD
D
CV
E
HA
PV
BD
F

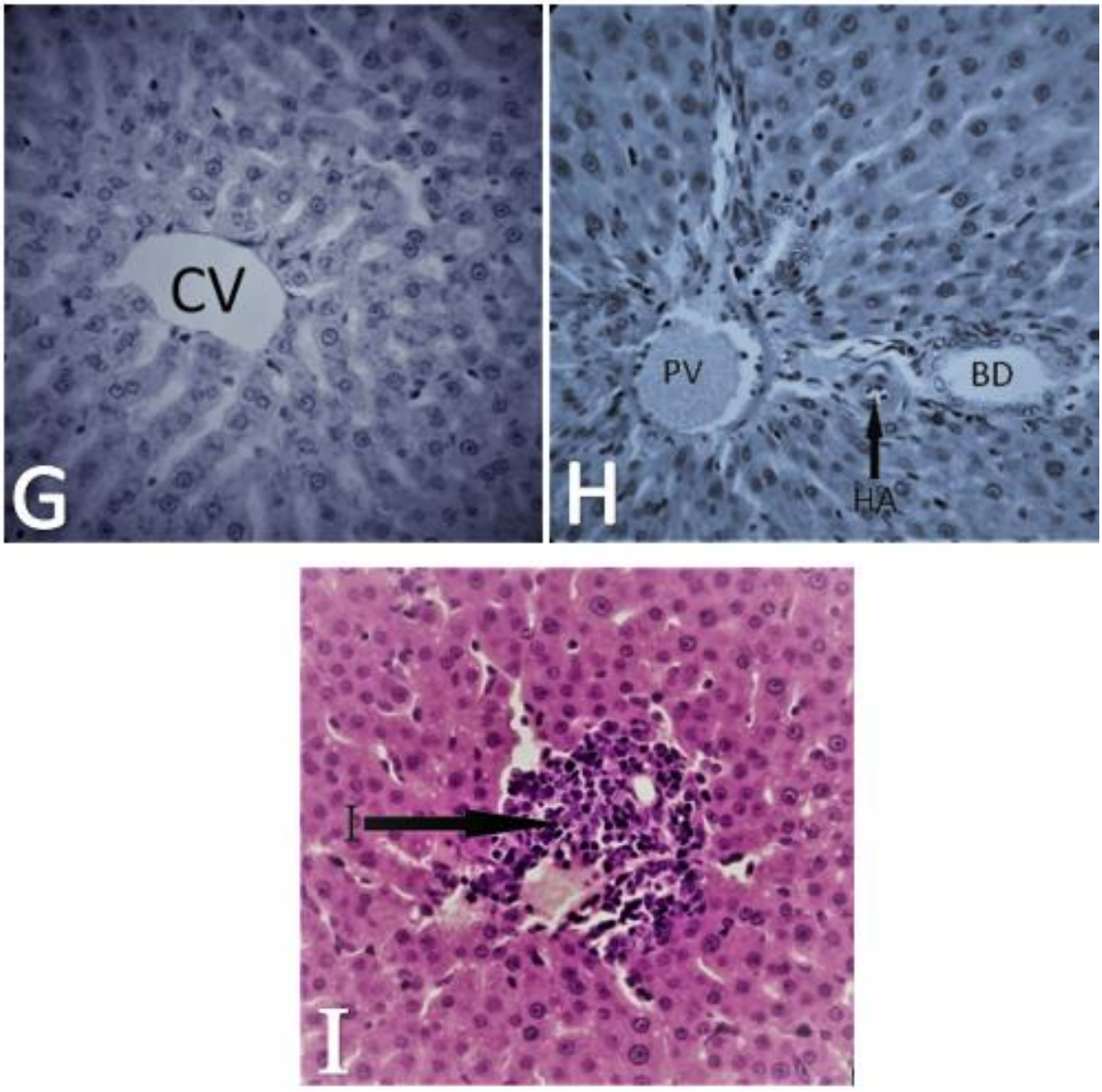


Figure 9: Photomicrographs of Liver sections from female control rats (A & B) as compared to those administered with aqueous *M. stenopetala* seeds extract at doses of 250mg/Kg (C & D), 500mg/Kg (E & F), and 1000mg/Kg (G, H, & I). (H&E stain, 400x). CV: Central vein, BD: Bile duct, HA: Hepatic artery, PV: Portal vein, I: Lymphocytic infiltration

5. DISCUSSION

Acute toxicity tests are the first tests to be conducted to evaluate the toxicity of any substance. It is an initial assessment of any toxic manifestations and also is the initial screening experiment performed with all compounds. These tests provided data on the relative toxicity of the substance to be tested which may arise from a single exposure to a substance (Akhila *et al*., 2007).

In the present study, no signs of acute toxicity and mortality were recorded at 300mg/Kg and 2,000 mg/kg doses. This shows that up to 2,000 mg/kg dose the seeds of *M. stenopetala* are safe in rats. Nevertheless, the mild toxicity signs like piloerection and decreased movement within the cage observed at 3,500 mg/kg, and signs of toxicity like diarrhea, decreased response to touch, staying at the back of the cage, piloerection, and self-mutilation observed at 5000mg/kg suggest that aqueous seeds extract of *M. stenopetala* could be toxic at the higher doses. No death was recorded at all tested doses up to 5000mg/Kg. Therefore, according to this study, the maximum safe dose of aqueous seeds extract of *M. stenopetala* is 2,000mg/Kg body weight. Furthermore, the LD_{50} of the aqueous seeds extract of *M. stenopetala* is greater than 5000mg/Kg body weight. As the LD_{50} is more than 5000mg/Kg according to OECD guidelines this substance is grouped under category 5 or unclassified according to Globally Harmonized Classification System (OECD, 2001).

In this investigation, the single administration of aqueous seeds extracts of *M. stenopetala* did not produce any significant change in the mean body weight of rats treated at all doses compared to the control group. On gross examination of the liver and kidneys, there were no observed treatment-related gross findings. Besides, there were no relative kidney and liver weight differences between all experimental groups and the control group.

Deltamethrin (2001), has stated that any compound or drug with the oral acute toxicity test of LD_{50} estimate greater than 1000mg/kg body weight is taken as low toxic and relatively safe. Therefore, the current study result indicates that the aqueous seeds extract of *M. stenopetala* is relatively safe on acute exposure. The result of this study goes in line with a previously done study by Berger *et al.* (1984), where *M. stenopetala* seeds powder at doses of 50 mg/kg and 500 mg/kg did not cause any acute toxicity to rats.

The sub-chronic toxicity study provides information on the possible health hazards likely to arise on the body systems as a result of repeated exposure over 90 days or 12 weeks period of time (OECD, 2018). In the sub-chronic phase of this research, the effect of oral administration of aqueous seeds extracts of *M. stenopetala* on body weight, kidneys, liver, and some blood parameters was studied.

During the 90 days study period the rats treated with aqueous seeds extract of *M. stenopetala* at doses of 250mg/Kg, 500mg/kg, and 1000mg/kg showed no signs of morbidity and mortality. The few signs of toxicity such as piloerection, decreased activity, and mild intermittent diarrhea observed in the female rats treated with 1000mg/Kg dose within the first three days of treatment also did not last long and disappeared. This implies that the aqueous seeds extract of *M. stenopetala* is well tolerable up to 1000mg/Kg for 90 days in the rats.

Differences in the change of body weights of animals can be used as an indicator of the effects of drugs and chemicals (Teo *et al*., 2002). At the end of this experiment, there was no significant mean weight gain difference between the control and treatment groups of both sex rats. This indicates that the extract did not have any adverse effects on body weight growth. Such a result is in line with previous research conducted on sub-acute oral toxicity tests where *M. stenopetala* seeds powder has not shown any effect on the weight of the rats (Berger *et al*., 1984).

In toxicity studies, organs weight analysis is usually employed to determine if there is a change in the weight of those organs as a result of the effect of the substance of interest. The kidneys and liver of rats are usually used to assess the effect of drugs or plant materials (Satyapal *et al*., 2008). In this study, both male and female rats after 90 days of oral administration of the aqueous seeds extract of *M. stenopetala* at all three doses showed no sign of gross abnormalities nor significant relative weight difference of the kidneys and liver as compared to the control group. The result of this study is in line with the absence of apparent toxicity-related changes in kidneys and liver after 6 weeks of oral toxicity tests on rats administered with *M. stenopetala* seeds powder (Berger *et al*., 1984).

Some medicinal plants affect the body of animals and these can be reflected by changes in various blood components (Ofem *et al*., 2012). Hematological parameters play a critical role in the diagnosis of the physiological and pathological status of animals as a result of the effect of

toxicants (Yakubu *et al*., 2008, Joshp *et al*., 2002). In this study, the treatment of rats with the extract at all tested doses did not alter most of the tested hematological parameters. However, the RBC count of female rats treated at 500mg/Kg and 1000mg/Kg dose increased significantly compared to control rats. There was also a statistically significant increment in HCT of 1000mg/Kg dose group of female rats. Such an increase in RBC count and HCT at the higher doses might be due to the rich nutritional value of the plant as was stated by (Seifu, 2015, Melesse *et al*., 2009). The adequate amount and quality of dietary proteins, vitamins, and minerals are necessary to maintain normal hematopoiesis in experimental animals (Dinning, 1962). The high vitamins, proteins, and minerals content of the seed may have increased the erythropoiesis process and resulted in an increase in the RBC and HCT of those groups. However, why the observed changes were observed only in the female rats, while the values in the male rats remained unchanged is interesting and is a matter that deserves investigation. Other studies have also previously reported that female rats are more susceptible in comparable toxicological studies (Otitoju *et al.*, 2014). The absence of significant changes in most of the tested hematological parameters as found in this study may suggest that the aqueous seeds extract of *M. stenopetala* has no significant toxic effect on the hematological parameters or hemopoietic system (Miller and Harley, 1996, Guilhermino *et al*., 1998, Mdhluli, 2003).

Another blood marker to the toxic effect of a substance is the level of serum biochemical profile. The effect of the substance in a toxicological evaluation can be detected or quantified by measuring the level of various serum biochemical parameters (Yakubu *et al*., 2008, Etuk *et al*., 2009).

The plasma concentration of urea and creatinine have been used as markers of the glomerular filtration rate or renal function (Tietz *et al*., 1983). In the present study, analysis of plasma urea and creatinine level showed that female rats of 500mg/Kg dose group have an increased mean creatinine level compared to the mean of the control group. The increment in creatinine level observed was not considered to be treatment-related because the change was not in a dose-dependent manner. Sometimes the level of creatinine increases without kidney injury, because of increased muscle mass, high protein diet, increased exercise, and fasting (Samra and Abcar, 2012). The increase of creatinine in this group also might have happened because of other confounding factors; for example, even if it is not significant, this group of rats has a relatively

higher body weight than the other groups of the same sex rats. Other than this change there was no observed significant change in the plasma level of urea and creatinine in all experimental groups of both sexes compared to their respective sex control groups. This suggests that the sub-chronic administration of aqueous *M. stenopetala* seeds extract is relatively safe to the kidneys.

Some medicinal plants have toxic effects on the liver, and damage to the liver often results in a change of some blood biochemical parameters. AST, ALT, and ALP are among some of the commonly used biochemical markers for the evaluation of hepatotoxicity (Coolborn *et al*., 2012, Oduola *et al*., 2010, Hall, 2013). In the present study, the mean value of ALP for the 1000mg/Kg dose treated group of female rats showed decrement compared to the control group. This finding is comparable to that of a previous sub-acute study, where oral administration of a related plant, *M. oleifera* methanolic seeds extract resulted in a significant reduction in the mean ALP value (Ajayi *et al*., 2016). A low level of ALP value is a rare condition and it occurs mostly because of malnutrition, severe anemia, and some autoimmune disorders. If there was hepatotoxicity the level of ALP would increase instead of decrease (Sharma *et al*., 2014). In addition, the levels of ALT and AST the most sensitive hepatotoxicity markers did not show change. Otherwise, sub-chronic treatment of the extract at all doses have not produced a significant difference in the level of blood biochemical parameters of the tested hepatotoxicity markers (AST, ALT, and ALP) compared to the control in both female and male rats.

The other tested blood biochemical parameters were albumin and total protein. Low albumin levels can indicate a problem with kidneys or the liver. Increased albumin levels may indicate dehydration (Tietz *et al*., 1983). In the present study, the level of albumin in 1000mg/Kg dose treated male rats decreased significantly compared to the controls. This result may not show the toxic effect of the extract on the kidney and liver as the value of the mean albumin level in the blood is within the reference range for rats. It is also not supported by other tested hepatotoxicity and nephrotoxicity markers in this group. Maybe it is caused by the effect of the extract on the secretory function of the liver; thus, it is an issue requiring further investigation on the secretory functional tests of the liver. Other than this difference the mean value of total protein and albumin has not shown a significant difference between the experimental groups and the control group rats of both sexes. This indicates that the sub-chronic oral doses of *M. stenopetala* seeds aqueous extract administration did not affect the blood total protein and albumin level of the rats

and was well tolerated over the 90 days study period, but the decrease of albumin in 1000mg/Kg dose group male rats needs further secretory liver function investigations.

The kidneys and liver have fundamental roles in the metabolism and excretion of drugs or plant products. During the biotransformation process of drugs and plant products, there is a generation of reactive metabolites, and the presence of secondary metabolites in the plant materials might result in toxicity or cell and tissue damage on these two organs (Belay, 2008, Marcela *et al*., 2001). Histopathological investigation of these organs therefore may reveal changes caused by novel drugs that may not be easily revealed by hematological and biochemical markers.

Histopathological changes observed in the microscopic examination of the kidney section include necrosis, urinary space obliteration, an increase in cellularity of the glomerulus, tubular degradation, hydropic changes, loss of microvilli, and inflammatory cellular infiltration (Alebachew *et al*., 2014). In the present study, there were no such changes in both female and male rats treated with *M. stenopetala* seeds extract at all three doses, the same as in sections from those of the controls. This shows that 90 days of oral administration of the extract has no marked effect on the kidney tissue of rats at the studied doses. This is in line with the findings from the biochemical parameters investigated in the current study.

Some of the main histopathological changes observed under a microscope during hepatotoxicity include necrosis, fatty changes, congestion, lyses in the blood cells, WBC infiltration, and vascular lesions around the central vein or sinusoids of the liver (Singh *et al*., 2011). The current study revealed none of such changes in both female and male rats treated with the extract at all three doses. However, female rats of 1000mg/Kg dose group revealed portal lymphocytic infiltration. As the viral infection is not excluded either by Viral antibodies or viral proteins and genetic material tests, such lymphocytic infiltration may be inflammation, a response to cell death which is caused by exposure to infectious agents (López Panqueva, 2016). Thus, it needs further investigation to exclude the viral infection. The observed inflammatory change was not accompanied by statistically significant changes in tested biochemical hepatotoxicity markers, which are considered to be sensitive indicators of hepatocellular damage (Jeschke *et al.*, 2018). Therefore, the 90 days oral administration of aqueous *M. stenopetala* seeds extract appears not to cause damage to the liver tissue of rats up to 1000mg/Kg daily dose.

6. CONCLUSION

From this investigation, it can be concluded that a single exposure of rats to aqueous *M. stenopetala* seeds extract does not cause mortality up to 5000mg/Kg dose. Hence, the oral LD_{50} of the aqueous extract of *M. stenopetala* seed is greater than 5,000mg/Kg. In the sub-chronic toxicity study, there was no mortality in all experimental rats of both sexes. Besides, there were no substantial changes in the general behavior, hematological and biochemical values, relative organ weight, and histopathological examination of the kidneys and liver in the extract-treated as compared to the control rats in both sexes. Therefore, this study demonstrated that 90 days oral administration of aqueous *M. stenopetala* seeds extracts is relatively safe in rats up to 1000mg/Kg dose.

7. RECOMMENDATIONS

- Further study including secretory liver function test investigation for the decrease in the albumin level of 1000mg/Kg dose group male rats.
- A further sub-chronic study including liver viral infection markers investigation is needed to exclude the viral infection of the liver, for the liver section of the 1000mg/Kg dose group female rats showed portal lymphocytic infiltration.
- Further detailed acute, sub-acute, and sub-chronic toxicity studies on other organs of similar species and with other extraction solvents are recommended.
- Further sub-chronic and chronic toxicity studies on other animal models are needed to be investigated.
- Further studies to determine the reproductive and genetic toxicity of *M. stenopetala* seeds should be carried out.
- Further studies to determine the carcinogenic and tumorigenic effect of *M. stenopetala* seeds may also need to be investigated.

REFERENCE

ABDELHAFIZ, A. H., BROWN, S. H., BELLO, A. & EL NAHAS, M. 2010. Chronic kidney disease in older people: physiology, pathology or both? *Nephron Clinical Practice,* 116**,** c19-c24.

ABOL-MUNAFI, A., LIEM, P., VAN, M., AMBAK, M., EFFENDY, A. & SOH, M. A. 2006. HIST OLOGICAL ONTOGENY OF THE DIGESTIVE SYSTEM. *Journal of sustainability Science Management,* 1**,** 79-86.

AJAYI, T. O., MOODY, J. O. & AKINTAYO, C. O. 2016. Toxicological evaluation of Moringa oleifera Lam seeds and leaves in Wistar rats. *Pharmacognosy Communications,* 6**,** 100-111.

AJIBADE, V. A. & FAMUREWA, O. 2012. Histopathological and Toxicological effects of crude saponin extract from Phyllanthus niruri, L (Syn. P. franternus. Webster) on Organs in animal studies. *Global Journal of Medical Research,* 12.

AKHILA, J. S., SHYAMJITH, D. & ALWAR, M. 2007. Acute toxicity studies and determination of median lethal dose. *Current science***,** 917-920.

AL-SAMAWY, E. R. 2012. Morphological and Histological study of the kidneys on the Albino rats. *Al-Anbar Journal of Veterinary Sciences,* 5**,** 115-119.

ALEBACHEW, M., KINFU, Y., MAKONNEN, E., BEKURETSION, Y., URGA, K. & AFEWORK, M. 2014. Toxicological evaluation of methanol leaves extract of Vernonia bipontini Vatke in blood, liver and kidney tissues of mice. *journal of African Health Sciences,* 14**,** 1012-1024.

ALLER, R., MOYA, J., MOREIRA, V., BOIXEDA, D., CANO, A., PICHER, J., GARCIA-RULL, S. & DE LUIS, D. 1999. Diagnosis of Hepatopulmonary Syndrome with Contrast Transesophageal Echocardiography (Advantages over Contrast Transthoracic Echocardiography). *Digestive diseases sciences,* 44**,** 1243-1248.

ANIAGU, S., BINDA, L., NWINYI, F., ORISADIPE, A., AMOS, S., WAMBEBE, C. & GAMANIEL, K. 2005. Anti-diarrhoeal and ulcer-protective effects of the aqueous root extract of Guierasenegalensis in rodents. *Journal of ethnopharmacology,* 97**,** 549-554.

ATAWODI, S. 2005. Antioxidant potential of African medicinal plants. *African Journal of Biotechnology,* 4**,** 128-133.

BAYU, F., AFEWORK, M., GELETA, B., ERGETE, W. & MAKONNEN, E. 2020. Effect of Chronic Administration of Aqueous Leaves Extract of Moringa Stenopetala on Blood Parameters and Histology of Liver and Kidney in Rats. *Ethiopian Journal of Health Sciences,* 30**,** 259.

BELAY, T. 2008. *In vivo antimalarial activity of the root extracts and fractions of Clerodendrum myricoides in Plasmodium berghei infected mice.* MSc thesis. Addis Ababa: Addis Ababa University.

BERGER, M., HABS, M., JAHN, S. A. A. & SCHMAHL, D. 1984. Toxicological assessment of seeds from Moringa oleifera and Moringa stenopetala, two highly efficient primary coagulants for domestic water treatment of tropical raw waters. *East African Medical Journal,* 61**,** 712-7.

BERNSTEIN, S. 1963. Problems and potentialities of hematopoietic tissue transplants. *Transactions of the New England Surgical Society,* 44**,** 76-83.

BIGONIYA, P., SINGH, C. & SHUKLA, A. 2009. A comprehensive review of different liver toxicants used in experimental pharmacology. *International Journal of Pharmaceutical Sciences and Drug Research,* 1**,** 124-35.

BOSCH, C. 2004. Moringa stenopetala (Baker f.) Cufod. *Plant Resources of Tropical Africa,* 2**,** 395-397.

BURKITT, H. G., YOUNG, B., HEATH, J. W., WHEATER, P. R. & DEAKIN, P. J. 2006. Wheater's functional histology: a text and colour atlas. *In:* EDITION, T. (ed.). Churchill Livingstone.

CAMERON, A., EWEN, M., ROSS-DEGNAN, D., BALL, D. & LAING, R. 2009. Medicine prices, availability, and affordability in 36 developing and middle-income countries: a secondary analysis. *The Lancet,* 373**,** 240-249.

CAMH 2004. Overview on medicinal plants and traditional medicine in Africa.

CAROLA, R., HARLEY, P. & NOBACK, R. 1992. *Human Anatomy and Physiology. 2nd edition,* U.S.A, Mc Graw-Hill, Inc.

CHARMI, A., BAHMANI, M., SAJJADI, M. & KAZEMI, R. 2009. Morpho-histological study of kidney in farmed juvenile beluga, Huso huso (Linnaeus, 1758). *Pakistan Journal of Biological Sciences,* 12**,** 11.

CHEN, H. H., ZHANG, W., WANG, S. & CARUANA, J. 1984. Twenty-two year experience with the diagnosis and treatment of intrahepatic calculi. *Surgery, gynecology & obstetrics,* 159**,** 519-524.

COLSON, C. R. & DE BROE, M. E. 2005. Kidney injury from alternative medicines. *Advances in chronic kidney disease,* 12**,** 261-275.

COOK, M. J. 2008. The anatomy of the laboratory mouse. *Academic press.* London.

COOLBORN, A. F., BOLATITO, B. & CLEMENT, A. F. 2012. Study of acute and sub chronic toxicity of Spathodea campanulata P Beav leaf. *International Proceedings of Chemical, Biological and Environmental Engineering,* 41**,** 76-80.

DEBEBE, M., AFEWORK, M., MAKONNEN, E., DEBELLA, A., GELETA, B. & GEMEDA, N. 2017. Evaluations of biochemical, hematological and histopathological parameters of subchronic administration of ethanol extract of Albizia gummifera seed in Albino Wistar Rat. *Clinical Toxicology,* 7**,** 337-345.

DEBELLA, A. 2002. Manual for phytochemical screening of medicinal plants, Department of Drug Research, Ethiopian Nutrition and Research Institute, Addis Ababa, Ethiopia. 45-71.

DEGEFU, D. M. & DAWIT, M. 2013. Chromium removal from Modjo Tannery wastewater using Moringa stenopetala seed powder as an adsorbent. *Water, Air, & Soil Pollution,* 224**,** 1719.

DELTAMETHRIN, E. 2001. Pesticide information project of cooperative extension offices of Cornell University, Michigan State University, Oregon State University, and the University of California.

DEMETRIS, A. & SCHIFF, M. 2008. Functional anatomy of the normal liver.

DIGGS, L. W., STURM, D. & BELL, A. 1954. The morphology of blood cells in Wright stained smears of peripheral blood and bone marrow. *What's new,* 185**,** 13.

DINNING, J. S. 1962. Nutritional requirements for blood cell formation in experimental animals. *Physiological reviews,* 42**,** 169-180.

DOBB, G. J. & EDIS, R. H. 1984. Coma and neuropathy after ingestion of herbal laxative containing podophyllin. *Medical journal of Australia,* 140**,** 495-496.

EBAID, H., DKHIL, M. A., DANFOUR, M. A., TOHAMY, A. & GABRY, M. S. 2007. Piroxicam-induced hepatic and renal histopathological changes in mice. *Libyan Journal of Medicine,* 2**,** 82-89.

EDDY, A. A. & FOGO, A. B. 2006. Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *Journal of the American Society of Nephrology,* 17**,** 2999-3012.

ELVIN-LEWIS, M. 2001. Should we be concerned about herbal remedies. *Journal of ethnopharmacology,* 75**,** 141-164.

ENDESHAW, H. 2003. Promoting the miracle tree of hope. *Ethiopian Herald,* 19.

ERNST, E. 2003. Herbal medicines put into context. British Medical Journal Publishing Group.

ESHETU, N., AFEWORK, M., MAKONNEN, E., DEBELLA, A., ERGETE, W. & TOLESSSA, T. 2016. Evaluation of the acute and sub-chronic toxic effects of aqueous leaf extracts of Artemisia afra on liver, kidney and some blood parameters in Wistar rats. *Advances in Bioscience and Bioengineering,* 1**,** 1-9.

ETUK, E., UGWAH, M., AJAGBONNA, O. & ONYEYILI, P. 2009. Ethnobotanical survey and preliminary evaluation of medicinal plants with antidiarrhoea properties in Sokoto state, Nigeria. *Journal of Medicinal Plants Research,* 3**,** 763-766.

FABRICANT, D. S. & FARNSWORTH, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives,* 109**,** 69-75.
FERNANDES, I. C. & VARGAS, V. M. F. 1999. Mutagenicity of medicinal plant extracts in Salmonella/microsome assay. *Journal of Phytotherapy Research,* 13**,** 397-400.
FUGLIE, L. J. 1999. The miracle tree: Moringa oleifera, natural nutrition for the tropics.
FURNHARM, A. 1996. Why do people choose and use complementary therapies? In complementary medicine an objective appraisal. Edited by: Ernst E. Oxford: Butterworth-Heinemann.
GARCIA, J. F. 1957. Changes in blood, plasma and red cell volume in the male rat, as a function of age. *American Journal of Physiology-Legacy Content,* 190**,** 19-24.
GARTNER, L. P. & HIATT, J. L. 2007. Color Textbook of Histology. 3rd. . Philadelphia: Ann Arbor, WB. Saunders Co.
GATEW, S. & MERSHA, W. 2013. Tannery waste water treatment using Moringa stenopetala seed powder extract. *Wyno Academic Journal of Physical Science,* 1**,** 1-8.
GERSHBEIN, L. L. & ELIAS, H. 1954. Observations on the anatomy of the rat liver. *The Anatomical Record,* 120**,** 85-98.
GILANI, A. H. 2005. Trends in ethnopharmacology. *Journal of ethnopharmacology,* 100**,** 43-49.
GUILHERMINO, L., SOARES, A. M., CARVALHO, A. P. & LOPES, M. C. 1998. Acute effects of 3, 4-dichloroaniline on blood of male Wistar rats. *Chemosphere,* 37**,** 619-632.
GUYTON, A. C. & HALL, J. E. 2006. Textbook of medical physiology. 11th. Philadelphia: WB Sounders Company.
HALL, R. L. 2013. Principles of clinical pathology. *Toxicologic Pathology: Nonclinical Safety Assessment***,** 133-173.
HELLSING, M. S., KWAAMBWA, H. M., NERMARK, F. M., NKOANE, B. B., JACKSON, A. J., WASBROUGH, M. J., BERTS, I., PORCAR, L. & RENNIE, A. R. 2014. Structure of flocs of latex particles formed by addition of protein from Moringa seeds. *Colloids Surfaces A: Physicochemical Engineering Aspects,* 460**,** 460-467.
HIAWATHA, B. 2010. All things moringa. The story of an amazing tree of life.
HIGASHIMOTO, M., PURINTRAPIBAN, J., KATAOKA, K., KINOUCHI, T., VINITKETKUMNUEN, U., AKIMOTO, S., MATSUMOTO, H. & OHNISHI, Y. 1993. Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. *Mutation Research Letters,* 303**,** 135-142.
JAHN, S. A. A. 1991. The traditional domestication of a multipurpose tree Moringa stenopetala (Bak. f.) Cuf. in the Ethiopian Rift Valley. *Ambio***,** 244-247.
JESCHKE, M. G., LOPEZ, O. N. & FINNERTY, C. C. 2018. The hepatic response to thermal injury. *Total Burn Care.* Elsevier.
JIRU, D., SONDER, K., ALEMAYEHU, L., MEKONEN, Y. & ANJULO, A. 2006. Leaf yield and nutritive value of Moringa stenopetala and Moringa oleifera accessions: Its potential role in food security in constrained dry farming agroforestry system. *Proceedings of the Moringa other highly nutritious plant resources: Strategies, standards markets for a better impact on nutrition in Africa, Accra, Ghan.*
JOSHP, P., BOSE, M. & HARISH, D. 2002. Changes in certain haematological parameters in a siluroid cat fish Clarias batrachus (Linn) exposed to cadmium chloride. *Pollution Research,* 21**,** 129-131.
KASSIE, F., PARZEFALL, W., MUSK, S., JOHNSON, I., LAMPRECHT, G., SONTAG, G. & KNASMÜLLER, S. 1996. Genotoxic effects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. *Chemico-Biological Interactions,* 102**,** 1-16.
KOGURE, K., ISHIZAKI, M., NEMOTO, M., KUWANO, H. & MAKUUCHI, M. 1999. A comparative study of the anatomy of rat and human livers. *Journal of hepato-biliary-pancreatic surgery,* 6**,** 171-175.
KONNO, B. 2004. Integration of traditional medicine with modern medicine, EHNRI, Addis Ababa.

KUMSSA, D. B., JOY, E. J., YOUNG, S. D., ODEE, D. W., ANDER, E. L. & BROADLEY, M. R. 2017. Variation in the mineral element concentration of Moringa oleifera Lam. and M. stenopetala (Bak. f.) Cuf.: Role in human nutrition. *PloS one,* 12**,** e0175503.

LALAS, S., GORTZI, O. & TSAKNIS, J. 2006. Frying stability of Moringa stenopetala seed oil. *Plant foods for human nutrition,* 61**,** 93-102.

LANDAU R. 1980. *Essential of Human Anatomy and Physiology.,* U.S.A, Scott and Foresman Company.

LASKIN, D. L. 1996. Sinusoidal lining cells and hepatotoxicity. *Toxicologic Pathology,* 24**,** 112-8.

LAWAL, O. & BANJO, A. 2007. Survey for the usage of arthropods in traditional medicine in southwestern Nigeria. *Journal of Entomol,* 4**,** 104-12.

LÓPEZ PANQUEVA, R. D. P. 2016. Useful algorithms for histopathological diagnosis of liver disease based on patterns of liver damage. *Revista Colombiana de Gastroenterologia,* 31**,** 443-457.

MALARKEY, D. E., JOHNSON, K., RYAN, L., BOORMAN, G. & MARONPOT, R. R. 2005. New insights into functional aspects of liver morphology. *Toxicologic pathology,* 33**,** 27-34.

MARCELA, E., SILVIA, B., JAIME, C. & PILAR, P. 2001. Effect of Solanun nudum extracts on the liver of mice infected with Plasmodium berghei. *The American journal of Chinese medicine,* 29**,** 477-484.

MARTINS, P. N. A. & NEUHAUS, P. 2007. Surgical anatomy of the liver, hepatic vasculature and bile ducts in the rat. *Liver International,* 27**,** 384-392.

MATAKA, L., HENRY, E., MASAMBA, W. & SAJIDU, S. 2006. Lead remediation of contaminated water using Moringa Stenopetala and Moringa oleifera seed powder. *International Journal of Environmental Science Technology,* 3**,** 131-139.

MATAKA, L., SALIDU, S., MASAMBA, W. & MWATSETEZA, J. 2010. Cadmium sorption by Moingastenopetala and Moringaoleiferaseed powder. *International Journal of Environmental Science Technology,* 3**,** 131-139.

MAYER, F. & STELZ, E. 1993. Moringa stenopetala provides food and low-cost water purification. *Agroforestry today,* 5**,** 16-18.

MDHLULI, M. 2003. Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (Chlorocebus aethiops). *University of the Western Cape, Bellville, South Africa***,** 34-38.

MEGERSA, M., BEYENE, A., AMBELU, A., ALEBACHEW, Z. & TRIEST, L. 2015. Ethnomedicinal knowledge on water purification in selected rural areas of Ethiopia. *Ethnobotany Research Applications,* 14**,** 393-403.

MEKONNEN, Y. 2003. The multi-purpose Moringa tree: Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

MEKONNEN, Y. & DRÄGER, B. 2003. Glucosinolates in Moringa stenopetala. *Planta medica,* 69**,** 380-382.

MEKONNEN, Y. & GESSESSE, A. 1998. Documentation on the uses of Moringa stenopetala and its possible antileishmanial and antifertility effects. *SINET: Ethiopian Journal of Science,* 21**,** 287-295.

MELESSE, A., BULANG, M. & KLUTH, H. 2009. Evaluating the nutritive values and in vitro degradability characteristics of leaves, seeds and seedpods from Moringa stenopetala. *Journal of the Science of Food Agriculture,* 89**,** 281-287.

MELESSE, A., TIRUNEH, W. & NEGESSE, T. 2011. Effects of feeding Moringa stenopetala leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. *Tropical Subtropical Agroecosystems,* 14**,** 485-492.

MESCHER, A. L. 2013. Organs Associated with the Digestive Tract, Blood, The Urinary System. *Junqueira's basic histology: text and atlas.* 13th ed. New York: McGraw-hill.

MILLER, S. & HARLEY, J. 1996. Zoology 3 rd ed. Wm. C. Dubuque (Iowa): Brown Publishers.

MOH 1995. Health sector strategy. Addis Ababa, Ethiopia: Ministry of Health.

MOORE, K. L., DALLEY, A. F. & AGUR, A. M. 2013. Abdomen. *Clinically oriented anatomy.* Philadelphia: Lippincott Williams & Wilkins.

NIBRET, E. & WINK, M. 2010. Trypanocidal and antileukaemic effects of the essential oils of Hagenia abyssinica, Leonotis ocymifolia, Moringa stenopetala, and their main individual constituents. *Phytomedicine,* 17**,** 911-920.

NIGATU, T. A., AFEWORK, M., URGA, K., ERGETE, W. & MAKONNEN, E. 2017. Toxicological investigation of acute and chronic treatment with Gnidia stenophylla Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC research notes,* 10**,** 625.

NWANJO, H. 2007. Studies on the effect of aqueous extract of Phyllanthus niruri on plasma glucose level and some hepatospecific markers in diabetic Wistar rats. *International Journal of Laboratory Medicine,* 2**,** 1-18.

ODUOLA, T., BELLO, I., ADEOSUN, G., ADEMOSUN, A.-W., RAHEEM, G. & AVWIORO, G. 2010. Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. *North American Journal of Medical Sciences,* 2**,** 230.

OECD 2001. OECD GUIDELINE FOR TESTING OF CHEMICALS. *Acute Oral Toxicity – Fixed Dose Procedure.* France.

OECD 2018. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. *Repeated dose 90-day oral toxicity study in rodents.*

OFEM, O., ANI, E. & ENO, A. 2012. Effect of aqueous leaves extract of Ocimum gratissimum on hematological parameters in rats. *International Journal of Applied and Basic Medical Research,* 2**,** 38.

OKIGBO, R. & MMEKA, E. 2006. An appraisal of phytomedicine in Africa. *KMITL Science and Technology Journal,* 6**,** 83-94.

OLUDURO, A. & ADERIYE, B. 2007. Impact of Moringa seed extract on the physicochemical properties of surface and underground water. *International Journal of Biological Chemistry,* 1**,** 244-249.

ONYEANUSI, B., ADENIYI, A., ONYEANUSI, C., AYO, J. & IBE, C. 2009. A study of the kidney of the Wistar Rat in Northern Guinea Savannah zone: the morphometric aspect. *Pakistan Journal of Nutrition,* 8**,** 1040-1042.

OTITOJU, O., NWAMARAH, J., OTITOJU, G., OKORIE, A., STEVENS, C. & BAIYERI, K. 2014. Effect of Moringa oleifera aqueous leaf extract on some haematological indices in Wistar rats. *Chemical and Process Engineering Research,* 18**,** 26-30.

OWUMI, B. 2002. The political economy of maternal and child health in Africa. Malthouse Press Limited.

OYEWOLE, O. & MASSAQUOI, H. 2008. Oral administration of Rauwolfia vomitoria extract has no untoward effect on kidney and liver functions in rats. *African Journal of Biotechnology,* 7.

PASS, D. & FREETH, G. 1993. The rat. *ANZCCART news,* 6**,** 1-4.

PATWARDHAN, B. 2005. Ethnopharmacology and drug discovery. *Journal of ethnopharmacology,* 100**,** 50-52.

PIPER, M., TREUTING, D. & SUZANNE, M. 2012. *Comparative Anatomy and Histology.*

POPAT, A. 2001. *Investigating the hepatotoxicity of Callilepis laureola, Impila, a South African traditional herbal medicine.* National Library of Canada= Bibliothèque nationale du Canada.

POPAT, A., SHEAR, N. H., MALKIEWICZ, I., STEWART, M. J., STEENKAMP, V., THOMSON, S. & NEUMAN, M. G. 2001. The toxicity of Callilepis laureola, a South African traditional herbal medicine. *Clinical Biochemistry,* 34**,** 229-236.

POPESKO, P., RAJTOVA, V. & HORAK, J. 1990. Anatomic atlas of small laboratory animals I. *Priroda, Bratislava*.

PRASANTH, K. M., SUBA, V., RAMIREDDY, B. & SRINIVASA, B. P. 2015. Acute and subchronic oral toxicity assessment of the ethanolic extract of the root of Oncoba spinosa (Flacourtiaceae) in rodents. *Tropical Journal of Pharmaceutical Research,* 14**,** 1849-1855.

RAM, J. 1994. Moringa a highly nutritious vegetable Tree, Tropical Rural and Island/atoll Development Experimental station (TRIADES). *Technical Bulletin,* 2.

ROKOS, L. 1969. Eye complications in poisoning caused by" kosso"(Hagenia abyssinicd). *Ethiopian Medical Journal,* 7**,** 11-16.

ROSS, M. & PAWLINA, W. 2011. Histology: a text and atlas. . published by Lippincott Williams & Wilkins: Wolters Kluwer, ISBN-13.

RYTAND, D. A. 1938. The number and size of mammalian glomeruli as related to kidney and to body weight, with methods for their enumeration and measurement. *American Journal of Anatomy,* 62**,** 507-520.

SAAD, B., AZAIZEH, H., ABU-HIJLEH, G. & SAID, O. 2006. Safety of traditional Arab herbal medicine. *Evidence-Based Complementary Alternative Medicine,* 3**,** 433-439.

SAMRA, M. & ABCAR, A. C. 2012. False estimates of elevated creatinine. *The Permanente Journal,* 16**,** 51.

SATO, A. 2012. Revealing the popularity of traditional medicine in light of multiple recourses and outcome measurements from a user's perspective in Ghana. *Health policy planning,* 27**,** 625-637.

SATYAPAL, U. S., KADAM, V. J. & GHOSH, R. 2008. Hepatoprotective activity of livobond a polyherbal formulation against CCl4 induced hepatotoxicity in rats. *International Journal of Pharmacology,* 4**,** 472-476.

SAVAGE, A. & HUTCHINGS, A. 1987. Poisoned by herbs. *British medical journal,* 295**,** 1650.

SCHIMMER, O., HÄFELE, F. & KRÜGER, A. 1988. The mutagenic potencies of plant extracts containing quercetin in Salmonella typhimurium TA98 and TA100. *Journal of Mutation Research/Genetic Toxicology,* 206**,** 201-208.

SCHNEEMANN, J. 2011. Moringa (stenopelata) production and use for water purification in Ethiopia: Final Report Commissioned by ICCO Fair Climate Program in December 2011.

SEIFU, E. 2012. Physicochemical properties of Moringa stenopetala (Haleko) seeds. *Journal of Biological Sciences,* 12**,** 197-201.

SEIFU, E. 2015. Actual and potential applications of Moringa stenopetala, underutilized indigenous vegetable of Southern Ethiopia: a review. *International Journal of Agricultural and Food Research,* 3.

SHARMA, U., PAL, D. & PRASAD, R. 2014. Alkaline phosphatase: an overview. *Indian Journal of Clinical Biochemistry,* 29**,** 269-278.

SHARP, P. & VILLANO, J. S. 2012. *The laboratory rat*, CRC press.

SINGH, A., BHAT, T. K. & SHARMA, O. P. 2011. Clinical biochemistry of hepatotoxicity. *Jornal of Clinical Toxicology,* 4**,** 1-9.

SOFOWORA, A. 1996. Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative Complementary Medicine,* 2**,** 365-372.

SOWASH, J. R. 2009. Dissection of the rat. *Biomedical Scientific Publication***,** 1-5.

STEWART, M. J. & STEENKAMP, V. 2000. The biochemistry and toxicity of atractyloside: a review. *Therapeutic drug monitoring,* 22**,** 641-649.

SUTHERLAND, J., FOLKARD, G., MTAWALI, M. & GRANT, W. 1994. Moringa oleifera as a natural coagulant. Affordable Water Supply and Sanitation: Proceedings of the 20th WEDC Conference, WEDC. Colombo, Sri Lanka.

TADELE, A. 2018. Publication of Traditional and Modern Medicine Research Directorate.

TAYLOR, D., GREEN, N. & STOUT, G. 1997. *Biological Science (Cambridge low price editions),* India, Kundli, Replika Press Pvt. Ltd.

TEO, S., STIRLING, D., THOMAS, S., HOBERMAN, A., KIORPES, A. & KHETANI, V. 2002. A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Sprague–Dawley rats. *Toxicology,* 179**,** 183-196.

TESEMA, T., MIRUTSE, G. & NIGUSU, A. 2002. National Biodiversity strategy and action plan project: Resource base of medicinal plants of Ethiopia, first phase report, Addis Ababa, Ethiopia.
TESHOME, K., GEBRE-MARIAM, T., ASRES, K., PERRY, F. & ENGIDAWORK, E. 2008. Toxicity studies on dermal application of plant extract of Plumbago zeylanica used in Ethiopian traditional medicine. *Journal of ethnopharmacology,* 117**,** 236-248.
THOOLEN, B., MARONPOT, R. R., HARADA, T., NYSKA, A., ROUSSEAUX, C., NOLTE, T., MALARKEY, D. E., KAUFMANN, W., KÜTTLER, K. & DESCHL, U. 2010. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicologic pathology,* 38**,** 5S-81S.
THORNE, S., PATERSON, B., RUSSELL, C. & SCHULTZ, A. 2002. Complementary/alternative medicine in chronic illness as informed self-care decision making. *International journal of nursing studies,* 39**,** 671-683.
TIETZ, N., RINKER, A. & SHAW, L. 1983. International Federation of Clinical Chemistry (IFCC) 2. *Journal of Clinical Chemistry and Clinical Biochemistry,* 21**,** 731-748.
TOMA, A., MAKONNEN, E., DEBELLA, A. & TESFAYE, B. 2012. Antihyperglycemic effect on chronic administration of butanol fraction of ethanol extract of moringa stenopetala leaves in alloxan induced diabetic mice. *Asian Pacific Journal of Tropical Biomedicine,* 2**,** S1606-S1610.
TORTORA, G. J. & DERRICKSON, B. H. 2018. The Blood, The Digestive System, The Urinary System. *Principles of anatomy and physiology.* 15th ed. ed. USA: John Wiley & Sons.
UN 1997. Glossary of environment statistics, studies in methods. United Nations New York.
WALTER, A., SAMUEL, W., PETER, A. & JOSEPH, O. 2011. Antibacterial activity of Moringa oleifera and Moringa stenopetala methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *African Journal of Microbiology Research,* 5**,** 153-157.
WAMBURU, R., KARERU, P., MBARIA, J., NJONGE, F., NYAGA, G. & RECHAB, S. 2013. Acute and sub-acute toxicological evaluation of ethanolic leaves extract of Prosopis juliflora (Fabaceae). *Journal of Natural Sciences Research,* 3**,** 8-15.
WASAN, K. M., NAJAFI, S., WONG, J., KWONG, M. & PRITCHARD, P. H. 2001. Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound, FM-VP4, to gerbils. *Journal of Pharmaceutical Sciences,* 4**,** 228-34.
WEBSTER, S., LILJEGREN, E. & ZIMMER, D. 1947. Organ; body weight ratios for liver, kidneys and spleen of laboratory animals; albino rat. *The American journal of anatomy,* 81**,** 477.
WETZEL, M. S., EISENBERG, D. M. & KAPTCHUK, T. J. 1998. Courses involving complementary and alternative medicine at US medical schools. *Jama,* 280**,** 784-787.
WHO 2002. Traditional medicine strategy: 2002-2005. Geneva: World Health Organization.
WHO 2003. Traditional medicine. Fact sheet Number 134.: World Health Organization.
WHO 2008. Traditional medicine fact sheet. Geneva:1-7.: World Health Organization.
WHO 2013. WHO traditional medicine strategy: 2014-2023. Geneva: World Health Organization.
YAKUBU, A., ADUA, M. & ADAMUDE, H. Welfare and haematological indices of weaner rabbits as affected by stocking density. Proceedings of the 9th World Rabbit Congress, Verona, Italy, 10-13 June 2008, 2008. World Rabbit Science Association, 1269-1274.
YIRGA, G. & ZERABURK, S. 2011. Ethnobotanical study of traditional medicinal plants in Gindeberet District, western Ethiopia. *Mediterranean Journal of Social Sciences,* 2**,** 49-54.
YISEHAK, K., SOLOMON, M. & TADELLE, M. 2011. Contribution of Moringa (Moringa stenopetala, Bac.), a highly nutritious vegetable tree, for food security in South Ethiopia: a review. *Asian Journal of Applied Sciences,* 4**,** 477-488.

APPENDICES

Appendix I: Preparation of working solutions

1. 10% Neutral Buffered Formalin

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

2. Harris's Hematoxylin (H)

- Hematoxylin crystals …….2.5 gm
- Absolute alcohol ……25 ml
- Potassium alum …….50 gm
- Distilled water ……...500 ml
- Sodium iodate ……...……0.5 gm
- Glacial acetic acid …….....20 ml

3. **1% Alcoholic Eosin (E)**

- Eosin Y (Yellow), water soluble (CI 45380) ……....1 gm
- 95% Ethanol ……..…100 ml
- Glacial acetic acid ……..…0.5 ml

4. 1% Acidic alcohol

- 70% alcohol ……..……500 ml
- Hydrochloric acid (concentrated) …….……5 ml

5. Bluing solution

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

Appendix II: Tissue processing procedures

1. Fixation
   - 10% Neutral Buffered Formalin ……...24 hrs
2. Washing
   - Running tap water several times
3. Dehydration
   - 70% Ethanol …….2 hrs
   - 90% Ethanol …….2 hrs
   - Absolute alcohol I ……1 & 1/2hrs
   - Absolute alcohol II …….....1 & 1/2hrs
   - Absolute alcohol III ……..1 & 1/2hrs
   - Absolute alcohol IV ……..overnight
4. Clearing
   - Xylene I ……....1 & 1/2hrs
   - Xylene II ……...……2 & 1/2hrs
5. Infiltration
   - Paraffin wax I ……...…2 & 1/2hrs
   - Paraffin wax II ……..overnight

Appendix III: Hematoxylin and Eosin (H & E) Staining Procedure

1. Deparaffinization
   - Xylene I ……5 min
   - Xylene II ……...5 min
2. Rehydration
   - Absolute alcohol I ……4 min
   - Absolute alcohol II ……...4 min
   - 95% Ethanol ……3 min
   - 70% Ethanol ……3 min
3. Rinse in distilled water .....…..5 min
4. Stain in Hematoxylin ……....15 min
5. Rinse in running tap water …….....5 min
6. Decolorize in acid alcohol …….....1-3 sec
7. Rinse in running tap water ……....5 min
8. Immerse in Sodium bicarbonate solution …….....3-6 sec
9. Rinse in running tap water ……....5 min
10. Counterstain in Eosin ……..1 min
11. Dehydration
    - 70% Ethanol ……..2 min
    - 95% Ethanol ……..2 min
    - Absolute alcohol II ……3 min
    - Absolute alcohol I ……3 min
12. Clearing
    - Xylene II ……...4 min
    - Xylene I ……4 min