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Effects of Chronic Administration of Aqueous Leaf Extracts of *T. schimperi* on Blood Parameters and Histology of Liver and Kidney in Wistar Rats

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DECLARATION

This is to certify that the thesis prepared by Abebe Fisseha, entitled: **Effects of chronic administration of aqueous leaf extracts of *T. schimperi* on blood parameters and histology of liver and kidney in Wistar rat s**at Addis Ababa University in year 2016/2017and submitted in partial fulfillment of the requirements for the degree of Master of science(MSc)in Human Anatomy, complies with the regulations of the University and meets the accepted standards with respect to originality and quality. This thesis has not been presented for any degree in any other university, and all sources of materials used for the thesis have been duly acknowledged.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ALB	Albumin
ALP	Alanine phosphatase
ALT	Alanine amino transferase
ANOVA	Analysis of Variance
AST	Aspartate amino transferase
BASO	Basophiles
BUR	Blood Urea
CREAT	Creatinine
DPPH	Diphenyl-Picryl hydrazyl
EPHI	Ethiopia Health Institution
G	Gram
HGB	Hemoglobin
km	Kilo meters
LYMPH	Lymphocytes
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligrams
MONO	Monocytes
NUET	Neutrophils
OECD	Organization for Economic Co-Operation and Development
PLT	Platelets
RBC	Red Blood Cells
SEM	Standard Error of mean
SPSS	Statistical package for social sciences
T. Schimperi	*Thymus Schimperi*
TM	Traditional Medicine
WBC	White Blood Celle
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Back ground: Since ancient time, plants have been used as indispensable sources of medicine and condiments. Nowadays, many traditional medicinal plants are incorporated in to modern medicine in many developed countries in the western world. However, in the developing countries like Ethiopia, traditional medicinal plants are still used in traditional manner without considering their effect, efficacy and dosage. *T. schimperi* is one of the traditional medicinal plants used for disease alleviation and food spice. This chronic study of toxicity was focused on evaluating the effects of chronic administration of aqueous leaf extracts of *T. schympri* on blood parameters and histology of liver and kidney in Wistar Rats.

Methods and materials: Leaves of *T. schimperi* were collected from Bale Mountain, Oromia region and dried in the shade area at room temperature. The plant leaves then were powdered by pestle and mortar and macerated by aqueous water and shacked by orbital shaker. Finally, the macerated extract was filtered and freezed in deep freeze then dried in lyophilizer vacuum. For this study total of 40 rats, 20 male and 20 female rats, were used. Rats of each sex were randomly grouped into three experimental and one control group with 10 rats in each group 5 male and 5 females in separate cage. The experimental groups, II, III, and IV, were administered with the aqueous leaf extract at doses of 500, 1000, and 2000mg/kg, respectively for 180 days and then finally the rats were sacrificed humanly and liver and kidney and blood were taken for histopathology, hematology and biochemistry assessment.

Results: The general behavior of the rats in treatment group was as normal as the control group. No statistical significant changes were seen between the mean bodyweight, liver and kidney weight and Blood parameters of experimental and control group of Wistar rats, however, considerable variations were observed between these parameters and mild focal mononuclear infiltration have been seen in the treatment of female rats treated with 1000and male rats treated with 2000mg/kg *T. schimperi*.

Conclusion: Based on the findings of present chronic toxicity study it is difficult to draw conclusion with negative results because small sample size of animals was used rather it provides a baseline for future studies.

Keywords: *T. schimperi*, chronic toxicity, medicinal plants and Wistar rats

1. INTRODUCTION

1.1. Background

Traditional medicine has been defined as health practices, approaches, knowledge and beliefs, incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises to prevent, diagnose and treat physical and mental illness, or generally to maintain wellbeing of the societies (WHO, 2001). Since ancient times, traditional medicine has been indispensable sources of both preventive and curative mechanism for human and livestock diseases (Yirga, 2010; Mohammed *et al*., 2015). Historical accounts of traditionally used medicinal plants depict that traditional medicine was in use as early as 5000 to 4000 BC in China and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians (Yirga, 2010; Ashafa *et al*., 2011; Kasa, 2016). The terms complementary/alternative/non-conventional medicine are used interchangeably with traditional medicine in some countries (WHO, 2005). According to (WHO, 2000), practices of traditional medicine vary greatly from country to country, and from region to region, as they are influenced by factors such as culture, history, personal attitudes and philosophy of societies.

Almost 65% of the world's populations have incorporated traditional medicine (mainly herbs) into their primary modality of health care (Fabricant and Farnsworth, 2001). Many people in developing countries, particularly those in rural areas, have more access to traditional than modern medicines and use them more frequently for primary health care (Assefa and Abebe, 2014). African Traditional Medicine (ATM) is the main sources of primary health care for the majority of those in the rural areas and up to 80% of the African population use traditional medicine for primary health care (WHO, 2011). For instance, it is estimated that 80 to 90 % of Ethiopians use herbal medicine as primary form of health care (Mesfin *et al*., 2014; Assefa and Abebe, 2014). Despite the significant recent improvements in modern healthcare, many rural communities continue to have limited access to modern health care and facilities. More than 80% of the people in Ethiopia use traditional medicine to meet their primary health care needs, as do 70% of people in Benin, India, and Tanzania (Patwardhan, 2005). In the African Region, TM/CAM knowledge and practices have been passed on orally among traditional health practitioners for many generations. In recent years, some countries have strengthened training programs to develop the knowledge of health practitioners. Furthermore, in some countries TM is included in university

curricula for health professionals. For instance, various universities in the Economic Community of West African States, Democratic Republic of Congo, South Africa and Tanzania include TM in the curricula for pharmacy and medical students (WHO, 2011).

1.2. Medicinal plants/Medical herbalism

Medical herbalism, or simply, herbalism, is "the study of herbs and their medicinal uses" (Ameh *et al*., 2010). This definition can be extended to include the cultivation, collection, or dispensing of aromatic plants, especially those considered to have medicinal properties. Other terms substituted for medical herbalism, include: herbal or botanical medicine, previously defined as "the use of plant materials to prevent and treat ill health or promote wellness" (Ameh *et al*., 2010). The term of medicinal plants includes various types of plants used in herbalist and some of these plants have a medicinal activity and they are considered as rich resources of ingredients which can be used in drug development and synthesis (WHO, 2011).

Plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Singh, 2015). In addition, these plants play a critical role in the development of human cultures around the whole world (Rasool Hassan, 2012). It is reported that more than 3.5 billion people rely on plants for the treatment of both human and livestock diseases (Sharad *et al*.,2011). As elsewhere in Africa, indigenous people in Ethiopia, by large employed plant based traditional medicine to get cured from diseases arising from worms, fungi, virus and protozoa (Yirga, 2010). Over 80% of the Ethiopian population relies on medicinal plants for the fight against various diseases (Dawit *et al*., 2003). Plant remedies still remain to be the most important and sometimes the only source of therapeutics for 85%and above of the population, in contemporary to Ethiopia (Hayelom *et al*., 2012). TM plants practices have been developed within different cultures in different regions. Therefore, there has been no parallel development of standards and methods either national or international for evaluating their safety, toxicity and efficacy (Adeniyi, 2007). Plants commonly used in traditional medicines are assumed to be safe and their safety is based on their long history usage in the treatment of diseases according to knowledge accumulated over centuries by the traditional healers. However, many recent scientific researchers have shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic because plants

have toxic substance naturally that helps them in maintaining their normal activities (WHO, 20011). The general guidelines of WHO (2001) suggests that in the assessment of safety of herbal medicines toxicological studies, if available, should be part of the assessment (Abebe *et al*., 2001). The period of administration of the test substance to animals will depend on the expected period of clinical use (Fernandes and Vargas, 2003). It also recommends that in long term toxicity studies groups receiving at least three different dose levels should be used (Fernandes and Vargas, 2003). Safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems (Adeniyi, 2007), but in Ethiopia, traditional medicinal plants have been used by many folk medicine practitioners without taking into consideration their side effects on different parts of the body (Abebe *et al*., 2001).

Side effects are caused from biologically active constituents from herbs, contaminants, and herb–drug interactions (Azaizeh, 2003). Medicinal plants typically contain several different pharmacological active compounds that may act individually, additively or synergistically to improve health (Azaizeh, 2003). Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature (Bent and Ko, 2004). Recently, concerns have been raised over the lack of quality control and scientific evidence for the efficacy and safety of medicinal plants (Rousseaux and Schachter, 2003; Firenzuoli and Gori, 2007). Several warnings have been issued regarding the potential adverse effects of herbal remedies including hepato- toxicity and nephron- toxicity (Wojcikowski *et al*., 2004; Stickel *et al*., 2005).

1.3. Thymus schimperi botanical description and its distribution in Ethiopia

Thymus schimperi is belonging to Lamiaceae/*Labiatae*, a large plant family represented by about 236 genera and above 7200 species (WHO, 2011). This family is much diverse in terms of ethno-medicine owing to its diverse chemical composition such as flavonoids, phenolic acid, terpenes,saponins, polyphenols, tannins, iridoids, and quinines (Damtie and Mekonnen, 2015; Nabavi *et al*.,2015). *T. schimperi*, one of the representative species of the genus thymus, is indigenous plant of Ethiopia, locally named as "Tosign " in Amharic (Jaafari *et al*.,2007). *T. Schimperi* is widely used in Ethiopian cooking and traditional healing practices (Demissew and Asfaw, 1994). *T. Schimperi*, a small perennial herb, woody at the base and 5 to 40cm high with prostrate stems, sometimes erect in younger parts, quadrangular, green to purplish and blue green or pale green leaf blade (Figure 1), is endemic to the Ethiopian highlands growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 meters above sea level altitudes (Asressu, 2013; Tesfamaryam *et al*., 2015). *T. Schimperi* is comparatively widespread in Central, Eastern and Northern Ethiopia (Damtie and Mekonnen, 2015). Bale, Shewa, Gonder and Wollo are the major growing areas in Ethiopia (Demissew andAsfaw, 1994*).* Wild thyme of *T. schimperi*is harvested and dried by people living close to the town of, Debresina,Dinsho and near Menz, put in plastic bags and sold to travelers on bus (Hailemariam and Emire, 2013).

Figure1: Photograph of *T. schimperi*, source Tesfamaryam *et al*.,2015

1.4. Chemical composition and uses of *Thymus schimperi*

1.4.1. Chemical composition of *Thymus schimperi*

The essential oil is a pale-yellow liquid with a rich aromatic, warming, herbaceous odor and the main constituents of the essential oils are p-cymene, gammaterpinene, thymol and carvacrol (Dessalegn *et al*., 2015). The main uses of *T. schimperi* in culinary and food processing are defined by the properties of thyme chemical components, the thymol and carvacrol, present in thyme essence, as well as the flavonoids and other polyphenols, are considered to be involved in the aroma, flavor, antioxidant and antimicrobial activities (Hailemariam and Emire, 2013; Nabavi *et al*., 2015).

1.4.2. Uses of *Thymus schimperi*

Medicinal uses

The volatile oil from *T. schimperi* was found to contain *p*-cymene, *γ*-terpine, carvacrol, rosmarinic acid, carvacrol and thymol (Parvez and Yadav, 2010). The volatile oil not only has carminative action, but also has antiseptic, antimicrobial and antifungal activities (Hailemariam and Emire, 2013; Chewaka*et al.*, 2015). *T.scimperi* oil shows an anti-spasmodic effect on the smooth muscle and a contracture involving a direct action on skeletal muscle by an unknown mechanism (Dawit *et al*.,2003).*T. schimperi* is prepared as infusion to treat spasmodic cough, laryngitis, bronchitis, urinary infections, renal diseases, hypertension and *Tineacapitis*(Parvez and Yadav,2010). It is also used as a decongestant, to reduce flatulence and to fight parasites. External uses of *T. schimperi* include preparations to wash skin wounds or infections (Asfaw *et al*., 2000; Dawit *et al*., 2003*).*

In the Ethiopian TM, this plant has many medicinal applications. Some of the reported applications are for the treatment of gonorrhea, cough, inflammation, spasm, thrombosis, mental illness, eye disease, toothache, urinary retention, stomach problems, leprosy, lung TB, acne and ascariasis (Abebe *et al*., 2001; Asressu, 2013). A tea made by adding *T.schimperi* in water has been recommended as a medicinal remedy for respiratory problems, gastrointestinal disorders and liver disease (Asressu, 2013). It is checked to have anti-helminths (Asfaw *et al*.,2000; Hussien *et al*.,2011); antibacterial and fungicidal activities (Nasir *et al*., 2015). Generally, the medicinal uses of T. *schimperi* can be summarized as follows.

A. Antimicrobial activity

Antimicrobial properties have been reported more frequently in a wide range of plant extracts and essential oils and natural products in an attempt to discover new chemical classes of antifungal and antibacterial drugs that could resolve strains expressing resistance to the available antifungal and antibacterial drugs (Chewaka *et al.*,2015; Nasir *et al*.,2015).The components of *T.schimperi* plant, especially its phenol components, thymol and carvacrol, show anti-bacterial activity against gram-positive and gram negative bacteria, mainly due to their effects on the bacteria membrane (Nzeako *et al*.,2006). Since thymol and carvacrol are eliminated via the respiratory tract, these compounds have respiratory antiseptic action. Because of its anti-bacterial activity, ***T. schimperi*** is also useful as an antiseptic for the urinary tract, mouth and skin wounds (Bekele *et al*.,2015).

B. Anti-inflammatory activity

Inflammation is considered as a primary physiological defense mechanism and is associated with the protection of the body against burns, infections, toxic chemicals, allergens and other harmful stimuli (Maheen *et al*., 2015). The in-vivo tests of *T. schimperi* ethanol extract on rats show anti-inflammatory and analgesic activities (Maheen *et al*., 2015). These activities could be related to carvacrol and thymol, which showed inhibitory effects on the enzyme cycloxygenase in animal models, as well as inhibitory effects on the complement and on nitric oxide synthesis (Reddy *et al*., 2002). Carvacrol has inhibitory action on prostaglandin synthesis. This action supports the use of thyme in ointments to treat muscle and joint pain. The rosmarinic acid in this extract also has anti- inflammatory activity. Topical applications of other thyme essential oil are rubefacient and generate analgesia, beneficial in cases for bruises or sprains (Reddy *et al*., 2014; Chewaka *et al.*,2015). Anti-inflammatory action has been reported for the phenol compounds in *T. schimperi* (Ahmed *et al*.,2011; Reddy *et al*., 2014).

Non- medicinal uses

A. Food spices

The fresh or dried leaves of *T.schimperi* are locally used as condiments in the preparation of stew, bread and tea (Damtie and Mekonnen, 2015). Thyme is often used to flavor meats, soups and

stews. It has a particular affinity to and is often used as a primary flavor with lamb, tomatoes and eggs (Asressu, 2013; Hailemariam and Emire, 2013; Bakhtiar *et al*., 2016).

B. Antioxidant activity

The thymol and carvacrol, present in *T. schimperi* essence, as well as the flavonoids and other plyphenols are considered to be involved in the antioxidant activity (Hailemariam and Emire, 2013). Rosmarinicacid, hydroxycinnamic derivatives and flavonoid compounds showed important in vitroantioxidant activity by inhibiting iron-induced superoxide anion formation and lipid per oxidation in microsomal and mitochondrial systems (Hailemariam and Emire, 2013). Furthermore, the thymol present in the essential oil showed in vitroantioxidant activity by neutralizing the DPPH (diphenyl-picryl hydrazyl) radical (Basch., 2004).

1.4.3. Safety and toxicity of *Thymus schimperi*

Findings of an acutetoxicity testingof *T.scimperi* done on rats showed that no clinical signs of toxicity, mortality as well as behavioral changes were observed at the oral limit dose of 5000 mg/kg of *T. schimperi* (Haji *et al*., 2016). In addition to the above study, acute toxicity study done by Debelo *et al*., (2016) has shown that the extract did not reveal any signs of toxicity; hence the LD50 was suggested to be higher than 10,000 mg/kg of *T. schimperi*. There was no significant change ($p< 0,05$) in the gain mean body weight and most of evaluated hematological and biochemical parameters after 90 days of sub-chronic treatment at 200 and 600 mg/kg. The kidneys and liver of treatment group appear normal in their texture, shape, size or color compared to the control group in gross and histopathological examination. However, the light microscopic examination reveals that there was localized mononuclear lymphocytic infiltration and mild blood congestion within the hepatic portal and central veins in liver at higher dose (600 mg/kg) (Debelo *et al*., 2016).

1.5. Blood

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients, oxygen and transports of waste products away from those of same cells (Savithri *et al.*, 2010). Blood is the most important body fluid that governs vital functions of the body like

respiration, circulation, excretion, osmotic balance and the transport of metabolic substance. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs (Savithri *et al.*, 2010). It is a specialized connective tissue that consists of fluid, known as plasma and formed elements, including white blood cells (WBC) or leukocytes, red blood cells(RBC) or erythrocytes and platelets or thrombocytes (Health J.W,1993). Plasma comprises about 55% of the total blood volume. It is an aqueous solution, containing <1% of the electrolytes and ions, such as, calcium, sodium, potassium and bicarbonate; about 7% of proteins, namely, albumins, globulins and fibrinogen; and organic compounds as varied amino-acids, lipids, vitamins, hormones and co-factors (Gartner and Hiatt, 2006). The blood volume of the rat is estimated to account 7% of its body weight; usually the younger rats have a larger blood volume relative to their body weight than older rats (Garcia, 1997). The red blood cells that have biconcave disks like shape which luck nucleus and under normal conditions they never leave the circulatory system and their main function is carrying oxygen no their hemoglobin (Ross and Pawlina, 2006). Red blood cell count is 7–13 million/µl. Rat erythrocytes are 4–7µm in diameter and appear similar in morphology to those in humans (Gartner and Hiatt, 2006). The average life span of erythrocytes in human is 120 days and only 61 days in rat (Gartner and Hiatt, 2006). Leucocytes are complete cells, each containing a nucleus and cell organelles. They include the granulocytes, lymphocytes and monocytes. The granulocytes are divided into three types according to the staining properties of the granules; these are called as the neutrophils, eosinophils and basophiles and constitute 55-65%, 1-3% and 0.3-0.5% of the white blood cells, respectively. The lymphocytes and monocytes also constitute 20-30% and 3-8% of the white blood cells, respectively (Porth, 2011). Leucocytes participate in the body's defense against infections (Porth, 2011). Platelets are small, discoid, non-nucleate structures containing red-purple granules. Platelets play a vital role in the control of bleeding (homeostasis) by plugging defects in the blood vessel walls and contributing to the activation of the blood clotting cascade (Health J.W, 1993).

Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition (Hymavathi *et al.*, 2000). The importance of hematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that, any alterations of WBCS, RBCS, platelet

counts, RBC indices and WBC or leukocyte components may be used as valuable indicators of disease or stress in animals (Savithri *et al.*, 2010).

1.6. Liver structure and function

The rat liver is the most cranial structure on the right side of the abdominal cavity, and it becomes in intimate contact with the diaphragm and it is a multi-lobulated organ (Vdoviakova *et al*., 2015). The mass of the rat liver accounts for about 5- 6% of the total body weight while the weight of the rat liver is averagely 15.5 g with total volume of 22. 6ml.The transverse and dorso - ventral diameter of the rat liver are around 8.5-9cm and 4.8-5.2 cm respectively while its cranio-caudal diameter is 3.2-3.5cm (Vdoviakova *et al*., 2015). The liver has thousands of vital functions including the efficient uptake of amino acids, carbohydrates, bile acids, cholesterol, proteins, lipids and vitamins for storage and metabolism subsequent to release into bile and/or blood (Vdoviakova *et al*., 2015).

As stated by (Vdoviakova *et al*., 2015), when opened the rat abdominal cavity through the lineaalba, the rat liver has generally two surfaces: diaphragmatic and visceral surfaces. Because the rat liver is a multi-lobulated organ, it has about the same surfaces as lobes positioned flat against each other. The diaphragmatic convex surface is in contact with the diaphragm and right abdominal wall. The visceral concave surface is very rugged, because it is in relation to the guts (stomach, descending duodenum, right colic flexure, jejunum, spleen, pancreas, right kidney, and suprarenal gland. The rat liver lobes, like the human liver, are named after the portal branches that supply them, as among mammals, the portal system is the most constant anatomical reference (Lorente *et al*., 1995). Like in other mammals, the rat liver is multi- lobulated. Rats liverhas lobes: median (or middle), left, right, and caudate and all, except the left, are further sub divided in to 2 or more parts (Kogure *et al*., 1999; Zanchet, 2002; Malarkey*et al*., 2005) (Figure-2). Mice and humans have a gall bladder, but not the rat. Human liver lobes have traditionally been designated as right, left, quadrate, and caudate, but recently it has been proposed that the liver can be subdivided into 8 segments based on the vascular and ductal branching patterns to the right and left sides (Kogure *et al*., 1999; MacSween *et al*., 2002). The rat liver lobes are equivalent to the human liver segments (Kogure *et al*., 1999). SegmentI is equivalent to CL, whereas segment II is

equivalent to LL and segments III, IV, and VIII are equivalent to ML while segments VI and VII are analogous to RL (Kogure *et al*., 1999).

The middle (ML) is the largest, accounting for majority of the liver weight. It has a trapezoidal shape and is fixed in the diaphragm and abdominal wall by the falciform ligament. It is in continuity with the left lateral lobe (LLL) and is subdivided by a vertical fissure (main fissure or umbilical fissure) into a large right medial lobe (RML) and a smaller left middle lobe (LML) **(**Rowe *et al*. 2011). The RML has both left and right hepatic vascular components (Kongure *et al*., 1999).

The right lobe (RL) is located on the right of the vena cava and posteriorly in the right hypochondria and is almost completely covered by the medial lobe. It is divided by horizontal fissure into two pyramidal-shaped lobules: the superior (SRL, also called the right posterior lobe) and inferior (inferior right lobe, IRL, also called the right anterior lobe) (Rowe *et al*., 2011).

The left lateral lobe (LLL) has a rhomboid shape, is flattened and situated in the epigastria 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 parts (Kogure *et al*., 1999)

The caudate lobe (CL) is situated behind the LLL and on the left of the vena porta and inferior cava. It is divided into two portions: the paracaval portion (caudate process), which encircles the inferior vena cava and bridges the CL and the right lateral lobe, and the Spiegel lobe, which has an anterior (superior) and a posterior (inferior) portion in the form of discs **(**Rowe *et al*., 2011). The anterior part of the CL is located anterior to the esophagus and stomach and its pedicle lies superior, while the posterior is located behind these structures and its pedicle **(**Rowe *et al*., 2011).

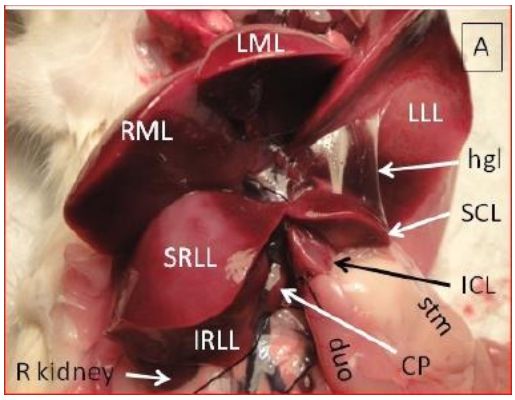

Figure 2: Lobes of rat liver. LML=Left median lobe, RML=right median lobe. SRLL =superior right lateral lobe, IRLL=inferior lateral lobe, LLL=left lateral lobe, SCL= superior caudate lobe, ICL= inferior cuadte lobe. (Rowe *et al.*, 2011). Comparative Hepathology)

The blood supply to the liver parenchyma flows from the portal areas to the central veins. Accordingly, the hepatic parenchyma of liver lobule is divided into three metabolic zones (Figure- 3). Zone 1 or the peri-portal area, is closest to the arterial or portal blood supply and hence bears the brunt of all toxic injury (Geerts *et al*., 1997). Zone 3 or the Centro-lobular area surrounds the central vein and is most remote from the blood supply and thus suffers from the effects of hypoxic condition and affected by toxic agents. Zone 2 is the intermediate mid-zonal area (Geerts *et al*., 1997).

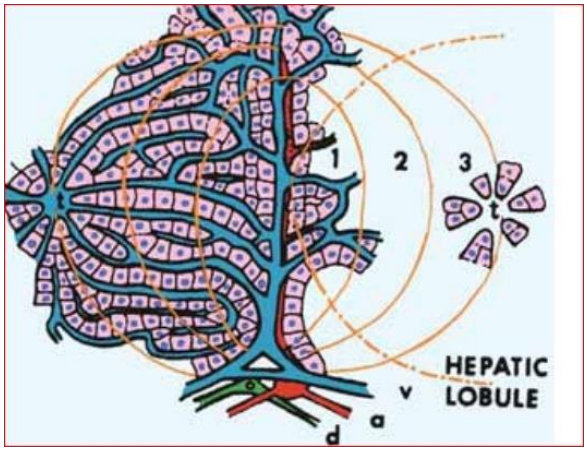

Figure 3: Diagram comparing the hepatic acinus with zones 1, 2, and 3 to the hepatic lobule (dash-dotted line). Portal tract contains portal venule (v), hepatic arteriole (a), and hepatic duct (d). t, terminal hepatic venule. (Source: Histology for Pathologists 4th edition)

According to (Kmiec, 2001; Malik *et al*., 2002), at least 15 different cell types can be found in normal liver. Hepatocytes are the most numerous and comprise 60% of the total cells and 80% of the volume of liver. Sinusoidal endothelial cells, Kupfer cells, hepatic stellate cells, and biliary epithelium make up a significant number (3–20% each) of the remaining biologically important cells. Hepatocytes are arranged in plates or laminae of cords one cell thick that branch and anastomose in a continuous labyrinth with limiting plates being at the capsule and portal regions (MacSween *et al*., 2002). The 6 or more surfaces of the hepatocyte either abut adjacent parenchyma cells, border bile canaliculi, or are exposed to the peri sinusoidal space (this surface being covered by microvilli). Being the workhorses of the liver, hepatocytes contain the machinery necessary to carry out the thousands of vital functions. Normally, about 15% of the cell volume is composed of smooth and rough endoplasmic reticulum and there are about 30 lysosomes and 500 peroxisomes per cell (MacSween *et al*., 2002). The number of mitochondria account about 1000 per hepatocyte and there is numerous free ribosome, Golgi complex, cytoskeleton elements (such as microfilaments intermediate filaments, and microtubules), and varying levels of cytoplasmic lipid and glycogen (Malik *et al*., 2002).

Biliary epithelium cells primarily act as a lining of the conduit for bile flow but it also modifies canalicular bile and concentrates bile in the gall bladder. Biliary epithelia are "effective communicators" with neighboring cells in producing mediators that are involved in cell growth and response to injury (Malarkey *et al*., 2005).

The sinusoidal endothelial cells are the primary barrier between blood and hepatocytes and they act to filter fluids, solutes, and particles between the blood and space of Disse (Braet and Wisse, 2002) and represent up to 20% of the liver cells.

Kupffer cells represent 15% of the liver cells (30% of sinusoidal cells) and are derived from circulating monocyte (MacSween *et al*., 2002; Bykov *et al*., 2004). They can proliferate locally, and are the major producers of cytokines as mediators of inflammation and provide "cross-talk" with other cells. Hepatic stellate cells also called Ito or fat-storing cells comprise about 5% of liver cell. The liver parenchyma or hepatocytes have the critical function of maintaining the body's metabolic homeostasis both anabolic and catabolic. This includes the processing of dietary amino acids carbohydrates, lipids and vitamins; removal of microbes and toxins in visceral blood

enterto the systemic circulation; synthesis of many plasma proteins; and detoxification and excretion into bile of endogenous waste products and pollutant (Geerts *et al*.,1997).Some of the patterns of hepatic injury that might be observed in the liver parenchyma are necrosis, inflammation, fibrosis occurred as a result of direct toxic insult to the liver, neoplastic processes and degeneration of the liver tissue (Geerts *et al*., 1997).

1.7. Kidney structure and function

The kidney is a complex organ with many functions, including filtration of the blood; preservation of fluid, electrolyte, and acid-base balance, and regulation of blood pressure (Verlander, 1998). Rat kidneys are paired, bean-shaped organs situated in the retroperitoneal position on posterior aspect of the abdominal cavity, on either side of the vertebral column and each kidney is enclosed in a fibrous capsule (Reilly*et al.*, 2007). The renal artery and nerves enter and the renal vein, lymphatic vessel and ureter leave, the kidney through the hilum. The right kidney of the rat is larger, heavier, and located more anterior and cranial than that of the left kidney (Appel *et al*., 2008). The medulla in the human kidney is divided into 8 to 18 renal pyramids. However, in rodents the medullary tissue of each kidney typically forms a simple coherent structure that contains just one renal pyramid and is termed uni-appellate (Appel *et al*., 2009).). Each kidney is composed of more than 1 million nephrons and each nephron has corpuscles and tubules (Guyton and Hall, 2006). Like in other mammalian kidney the kidney parenchyma of rats is divided into 2 easily recognized regions, the cortex and the medulla (Verlander, 1998). The cortex is the outer portion of the parenchyma and spans the area between the kidney capsule and the arcuate vessels and it contains the glomeruli and a heterogeneous population of renal tubule segments while the medulla is located deep to the cortex and extends from the arcuate vessels to the papillary tip (Verlander, 1998).Glomeruli are absent and convoluted tubule segments are largely absent, although some areas of convoluted tubule segments extend slightly into the outer medulla near the cortico-medullary junction (Schrier,2007). The medulla is divided into outer and inner regions, defined by the presence of profiles of the thick ascending limb of Henle's loop in the outer medulla and their absence in the inner medulla (Schrier, 2007). The glomeruli are arterial capillary tufts, located throughout the renal cortex, that are responsible for filtration of the blood (Allen and Tisher, 1996). The capillary tufts are encased by fibrous structure known as Bowman's capsule that is lined with a single squamous epithelial cell layer known as parietal epithelial (Reilly and

Ellison, 2000). The capillary loops are formed of 3main layers, basement membrane that is lined with fenestrated endothelium on the blood side and covered with visceral epithelial cells on the filtrate side which create the filtration barrier.

At the urinary pole of the glomerulus the squamous epithelium of Bowman's capsule makes a dramatic transition to the cuboidal proximal tubule epithelium that is characterized by a prominent, lush, apical brush border (Nortier *et al*.,2000). The function of the proximal tubule segments is primarily that of bulk re-absorption of filtered substances, such as water, electrolytes, glucose, low molecular weight proteins, peptides, and amino acids (Onyeanusi *et al*.,2009). At the distal end of the straight segment of proximal tubule the there is an abrupt transition to the flat epithelium of the thin descending followed by thin ascending Limb of the loop of Henle whose function is urine concentration (Reilly and Ellison, 2000). The thin ascending limb traverses the inner medulla until it makes an abrupt transition at the border between the outer and inner medulla to the thick ascending limb lined by cuboidal epithelium (Verlander,1998). Distal portion of the cortical thick ascending limb contains the macula densa the specialized epithelial cells that constitute one element of the juxtaglomerular apparatus that play role in establishing a feedback mechanism that allows auto regulation of renal blood flow and keeps the rate of glomerular filtration relatively constant (Verlander,1998; Reillyand Ellison, 2000).

Distal to the macula densa, the cortical thick ascending limb makes atransition to the distal tubule and the distal tubule consists of distal convoluted tubule, connecting segment, and initial collecting tubule (Clapp *et al*.,1994). All those structures are lined by cuboidal epithelial cells. The distal convoluted tubule like thick ascending tubule involves in active transport of sodium –potassium ions (Clapp *et al*.,1994). The initial collecting tubules merge with the cortical collecting duct in the medullary ray (Loffing *et al*.,2004). From this point the collecting ducts travel directly to the papillary tip, where the tubule fluid empties into the renal pelvis (Loffing *et al*.,2004)

1.8. Statement of the problem

The use of plants as source of medicine is not limited to any region of the world (Ashafa *et al*., 2011). It has been practiced since long time ago in various parts of the world for both preventive and curative purposes (Yirga, 2010). Dependence on herbs as medicine in the treatment of diseases is still much practiced by a large proportion of the rural populace because of its ready availability and affordability (Sani *et al*., 2009). Herbal medicines are widely perceived by the public as being natural, healthful and free from side effects. Most people believe that herbal medicines have no side effects or any potential risks due to their natural origins and are often considered as food supplements and not drugs. Medicinal herbs are usually self-prescribed by the consumers and there is a lack of control and review in terms of dose, manner, and frequency of administration (Arsad *et al*., 2014). The chemicals in medicinal herbs may be natural to the plant, but may not be natural to the human body and any compound with therapeutic effect has the potential to be incorrectly prescribed or overdosed (Arsad *et al*., 2014). Medicinal plants typically contain several different pharmacological active compounds that may act individually, additively or synergistically to improve health (Azaizeh, 2003). Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature (Ashafa *et al*., 2011; El Nur *et al*., 2016). With the upsurge of interests in medicinal plants, there are needs for thorough scientific investigations on their efficacy, safety and potential toxicity (El Nur*et al*., 2016). The administration of herbal preparations without any standard dosage coupled with non- availability of adequate scientific studies on their safety has raised concerns on their toxicity (Abebe *et al*., 2001; Ashafa *et al*., 2011). In Ethiopia, above 80% of human population and 90% of livestock rely on traditional medicine. Ethiopian plants have shown very effective medicinal value for various health problems of humans and domestic animals, and in addition used for food spices as flavoring agents (Yirga, 2010). In Ethiopia, *T.schimperi* is one of the widely practiced wild traditional medicinal plants used for disease alleviation and sources of condiments; however, there is little acute and sub chronic toxicity research regarding its safety and toxicity.

1.9. Significance of the study

Different researches have been done regarding the efficacy of *T. schimperi* and there is a little information on acute and sub chronic safety and toxicity investigation of this plant, however, there is no information regarding its chronic toxicity and safety study. Thus, the rationale of conducting this chronic toxicity study is to investigate the safety and toxicity of *T. schimperi*. As a result, the findings of this research may provide a baseline for further studies on this area and ascertain its safety and toxicity.

2. OBJECTIVES OF THE STUDY

2.1. General objective

- To evaluate the effect of chronic administration of aqueous leaf extracts of *T. schimperi* on blood parameters and histopathology of liver and kidney in Wistar rats.

2.2. Specific objectives

- To assess the effects of the aqueous leaf extract of *T.schimperi* on general behavior and gross lesion of liver and kidney of Wistar rats.
- To determine effects of aqueous leaf extracts *T.schymperi* on body and organ weights of Wistar rats
- To evaluate the effects of the aqueous leaf extract of *T. schimperi* on the hematological and biochemical parameters of Wistar rat.
- To assess the effects of the aqueous leaf extract of *T. schimperi* on the histopathology of the liver and kidney of Wistar rat.
- To generate a baseline data for future detailed study

3. MATERIALS AND METHODS

3.1. Study design

Comparative experimental based study

3.2. Study place

The study was conducted at Department of Anatomy (Histology Laboratory), College of Health Science, School of Medicine, Addis Ababa University, and Department of Traditional Medicine and Modern Drug Research, Ethiopian Public Health Institute Laboratory

3.3. Study period

The study was conducted from Jan. 2016-JUL.2017

3.4. Collection and extraction of plant material

Based on the identifications described by botanist, the fresh leaves of *T. schimperi* were collected based on ethno-botanical description from its' natural habitats, Densho (Bale Mountains National park, Oromia Region), which is 370 k.ms away from Addis Ababa through Assela. The collected fresh leaves were cleaned from extraneous materials, dried under shade at room temperature, and grinded by pestle and mortar to obtain fine powder particles. The powdered leaves (300gm. of *T. schimperi*) were macerated with 3000ml.distilled water and shacked at 172 rotations per minute for 90 minutes/day for 3 consecutive days with intermittent agitation by orbital shaker (DS-500). Then, the supernatant part of agitated materials was decanted and filtered with 0.1 mm^2 mesh gauze followed by nylon from the non-dissolved portion of the plants. The filtrates of the plants were freeze-dried at lower temperature and reduced pressure, and then lyophilized to form crude extract. An average yield of 27.3gm of *T. schimperi* was obtained from 300gm of dry powder. Then it was kept in desiccators at room temperature until the time of administration.

3.5. Selection and preparation of experimental animals

For this study, a total of 40 healthy young male and female Wistar rats were employed. The basis for recruitment of these animals was the fact that in recent years the rats have become the model organism of choice for the study of human disease; partly as a consequence of the low cost, easiness for handling, physiologic and genomic similarities (OECD, 2008*).* Healthy young animals with average age of 8 weeks weighing 137-146g were used for this study. Females were nulliparous and non-pregnant. All animals were obtained from Ethiopian Public Health Institute (EPHI). Animals of the same sex were grouped into experimental and control groups for aqueous extracts of *T. schimperi*. These animals were kept under standard conditions (at a temperature of 20°C (± 2°C), with natural 12hrs light / 12hrs dark cycle) with humidity of 30%-70% (OECD, 2008). For feeding, conventional rodent laboratory diets were used with an unlimited supply of drinking water using ad libitum. The animals received 110-115g of diets/day/group of rats. Then the animals were acclimatized to laboratory conditions for one week prior to the experiment to alleviate any non-specific stress *(*OECD, 2008). Finally, the animals were randomly grouped into three experimental and one control group consists of total of 10 rats 5 female and 5 males in each group and housed in a separate cage.

3.6. Grouping of animals

Healthy animals, which have been acclimated to laboratory conditions for 7 days and have not been subjected to any previous experimental procedure, were randomly assigned to 3 experimental and 1control group and each group consists 10 rats, 5 males and 5 females, housed in separated cage.

3.7. Dose selection and administration

Dose levels were selected based on results of acute toxicity studies.2000mg /kg was used as the highest dose level, and using two-fold interval frequency test performance, the other two descending doses levels, that is 1000 and 500mg/kg, were obtain. The test substance was administered through oral route using gavage based on the predominant route and method of exposure of humans.

3.8. Method of extract administration

To study this chronic toxicity of *T. schimperi*, the animals in the experimental group (II, III, and IV) were given the aqueous leaf extract for 180consecutive days via oral route using gavage. Animals in the control group (I) were given distilled water. The experimental groups received the leaf extract at different doses. that is, group(II) 500, group(III)1000, and group (IV) 2000mg/kg /body weight, respectively while the control group(I) received distilled water. All equipment used were cleaned and placed in an oven after each administration to prevent any contamination. All experimental animals were observed closely for toxicity signs like morbidity, mortality and other behavioral changes such as changes in skin, fur, eye, occurrences of secretions and excretions, gait, posture and response to handling, the presence of clonic or tonic movements, repetitive circling, mutilation, proprioception and assessments of grip and motor activity.

3.9. Data collection techniques

Both qualitative and quantitative data were collected from the experimental and control group of Wistar rats before and after they were sacrificed.

3.9.1. Body weight measurement

Before commencing the first oral administration and then weekly till last day of oral administration of the aqueous extract, body weight of all ratsin the experimental and control groups was taken using digital electronic balance after calibrating it by pressing the tare button to avoid any cofounder.

3.9.2. Cage side observations

Animals were observed for morbidity and behavioral changes such as changes in skin, fur, eye, occurrences of secretions and excretions, gait, posture and response to handling, presence of clonic or tonic movements, repetitive circling, mutilation, proprioception and assessments of grip and motor activity daily before and immediately after administration of aqueous leaf extract of *T. schimperi*

3.9.3. Hematological parameters and biochemical analysis

At the end of experiment, the animals were fasted overnight and sacrificed humanly by using cervical dislocation then blood samples were collected via sterile syringe by cardiac puncture into test tubes with anti-coagulants for hematology and into a tube without anti-coagulant for blood chemistry. Blood samples in test tubes containing anti-coagulant were immediately processed for hematological parameters using automated hematological analyzer (SYSMEX XT-1800i, SYSMEXCORPORATION, Japan). White blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLC) were determined. For biochemical analysis, the blood samples in the plain test tubes were allowed to stand for 3 hours for complete clotting and then centrifuged at 5000 rotations per minute for 15 minutes using a bench top centrifuge (HUMAX-K, HUMAN-GmbH, Germany). The plasma was withdrawn and transferred into other clean vials. The sera were kept at -20°C until analysis for clinical biochemistry measurements. The concentrations of ALT, AST, ALP, urea, albumin and creatinine were automatically determined using COBAS INTEGRA 400 plus Analyzer (ROCHDIAGNOSTICS, Japan).

3.9.4. Organs weight measurements and tissue sample taking procedure

At the end of 180^{th} day of treatment, both the experimental and control groups of animals were humanly sacrificed using cervical dislocation method. Then the animals were dissected and the target organs, liver and kidney, for the study were taken from the body and kept in 10% normal saline for a few minutes to clean off any extraneous tissues, and the organs were weighted with a calibrated electronic digital balance and then tissue samples were taken from Liver and Kidney**.** Sample tissues were kept in a test tube which contain 10% buffered formalin for 24hrs and thoroughly rinsed over running tap water overnight.

3.9.5. Tissue processing

The dissected liver and kidney tissue were immersed and fixed in 10% buffered neutral formalin overnight at room temperature. After overnight fixation with10%buffered neutral formalin solution, the tissues were rinsed in tap water, then the tissues were dehydrated with

increased concentration of ethanol, 70%, 90%, Absolute-I and II alcohol for one and half hours, absolute alcohol-III for two and half-hours. Then, the tissues were cleared with xylene: Xylene-I for one and half hours, xylene-II for two and half hours. Then the tissues were impregnated in paraffin wax for one and half hours. Then, tissue blocks were prepared by embedding with paraffin wax in square metal plate area of 3cm×3cm. All tissue blocks were labeled and sectioned at a thickness of 5µm using calibrated Leica rotary microtome (LEICA RM 2125 RT, Germany). Separate blades were employed for trimming and final sectioning. The ribbons of the final sections were picked with forceps and floated on the surface of a warm water bath at temperature of 30-40^{0C}. The smooth, unwrinkled and non-distorted floated sections were selected and mounted on pre-cleaned slides coated with egg albumin to assure reliable adhesion. The slides then were dried in the air and labeled using lead pencil. Immediately before starting the process of staining, the batch of tissue samples (slides)containing paraffin wax, arranged within slide holder, were placed in an oven with temperature of 20-40^{0c} overnight to facilitate the fixation of the specimens onto the glass slides. The specimen was cooled for half an hour and stained with routine Harris hematoxylin and 0.5% alcoholic eosin. Two series of Coplin jars, a down series for paraffin removal and hydration, and an up series for dehydration and clearing were prepared. In a down series of jars, sections were placed in Xylene-I and xylene-II for 5 minutes each to deparaffinize the tissues, and hydrated with decreased alcohol concentrations i.e., absolute alcohol twice, 95%, 70% and 50% alcohol each for 3minutes. After rinsing in tap water for 5 minutes, the sections were then stained regressively with Harris hematoxylin for 7-10 minutes and rinsed under a running tap water for 5 minutes to wash off the surplus stain. Over-staining was controlled by decolorizing the tissue by immersing (one to two dipping) in 0.1% acid alcohol and followed by immersion in 70% alcohol for one minute. To regain the blue color, the slides were immersed in sodium bicarbonate solution (1g/L). The slides were rinsed in tap water and then immersed in 95% alcohol and counterstained in0.5% alcoholic eosin for three minutes respectively. After dipping in tap water, the sections were dehydrated with increased alcohol concentrations i.e., in a 95% alcohol and absolute alcohol twice for 3 minutes each and cleared in xylene I and xylene II for five minutes each and then kept in xylene during mounting. Finally, well stained and non-opaque sections were mounted on microscopic slides permanently using DPX (Dibutyl Phthalate in xylene) and cover slips.

3.9.6. Light microscopy and photomicrography

Stained tissue sections of the liver and kidney were carefully examined under binocular compound light microscope (OLYMPUS CX41, Japan). Tissue sections from the treated groups were examined for any evidence of histopathologic changes with respect to those of the controls groups. After examination, photomicrograph of selected slides, those which were suspected for any change and not distorted during sectioning, were taken from the treated groups under a magnification of x20 objective by using (EVOS XI, China) automated built-in digital photo camera.

3.10. Data analysis

All quantitative data such as body, liver and kidney weight and hematological parameter were packed and analyzed by SPSS statistical software version 23. All values of parameters were expressed as mean ±SEM (standard error of mean). Treatments over time were compared between control and treated groups by using one-way ANOVA followed by Tukey to determine level of significance of their differences. The values $p<0.05$ levels were regarded as statistically significant.

3.11. Ethical consideration

The study was conducted after the recommendation letter has been sent to Ethiopian Public Health Institute from Addis Ababa University, College of Health Science, School of Medicine, and Department of Anatomy. Ethical clearance letter was obtained from Ethiopian Public Health Institute. Animals used in this study were kept from any unnecessary painful and terrifying situations. To keep the pain and suffering minimal during any surgical intervention (i.e. blood collection), all animals were given appropriate care; all procedures were carried out by well-trained person. Animals were protected from any pathogens and placed in appropriate environment.

4. RESULTS

4.1. Cage side observations for changes in general behavior and gross lesions of liver and kidney

During the study period cage side observation was included in order to assess for physical signs of toxicity. In reference to the (OECD,2008) guideline, changes of clinical and functional observations like changes in skin, fur, eye, occurrences of secretions and excretions ,gait ,posture and response to handling , the presence of clonic or tonic movements, repetitive circling ,mutilation, proprioception and assessments of grip and motor activity were taken into consideration to observe physical signs of toxicity in groups treated with the aqueous leaf extract of *T. schimperi* throughout the study period that is 180 days or 24 weeks. Based on those criteria the treated groups did not show any of the above listed behaviors during the study period as compared with the control groups. The gross observation of the liver and kidney of the animals' treated with the aqueous leaf extract of *T. schimperi* also did not show any gross abnormal and pathologic signs of toxicity like changes in texture, shape, size, lesion and they had gross similar appearances as compared with the liver and kidney of rats in the control group. At the same time, no morbidity or mortality was occurred throughout the duration of the study.

4.2. Effects of aqueous leaf extracts of *T. schimperi* on the general body weight of male and female rats

The research was conducted for 180 days or 24 weeks respectively to evaluate the toxicity effect of *T. schimperi* on rats' liver, kidney and blood parameter. Both the female and male rats in each respective treatment group which orally received repeated doses of plant leaf extract of *T. schimperi* at doses 500mg/kg, 1000mg /kg and 2000mg /kg/body weight during the study period. Tables 1 and 2 show the effect of aqueous leaf extract of *T. schimperi* on the general body weight of both male and female rats respectively during the period of chronic study. As displayed in the respective Tables 1 and 2 below, there was parallel general increment of body weight of both the treated groups and control groups throughout the entire study period. Although general body weight increment of the control groups was slightly higher than the treated groups, there were no observable significant differences between the mean body weight of the control group and treated

groups at all dose levels. As indicated in Table-1, the mean body of male rat in group II treated with 500mg/kg increased form the initial weight of 139.67±0.88 g to 354.74±0.89g. The mean body weight of male rats in group III (treated with 1000mg/kg) increases from 138.67±1.2g to 352.33±2.3g. The average body weight of the male rats in group IV (with 2000mg/kg of aqueous leaf extract of *T. schimperi*) increased from 140.3±0.33g to 356.83±1. 85g.The mean body weight of male rats in the control group, increased from initial dosing weight of 139.67±0.67g to 359.67±2.02g at the end of 24thweek. When increment of the mean body weight of all treated groups was compared to the mean body weight of the control group, the increment of mean body weight was more or less similar and the difference between the control and treated groups was insignificant at all dose levels. That indicates the gain body weight was not dose dependent rather it was normal and physiological increment. Figure4 illustrates the body weight patterns of the male rat body weight during the 24 weeks study period of time.

Table1: Effects of chronic administration of aqueous leaf extract of *T.schimperi* on the body weight of male rats

Weeks	Groups			
	I(Control)	II(500mg/kg)	III(1000mg/kg)	IV(2000mg/kg)
0	139.67±0.87	139.7±0.88(1)	138.67±1.2(0.83)	140.33±0.33(0.94)
1	145.3±0.33	143.15±0.71(0.09)	142.83±0.72(0.052)	144.6±0.331(0.78)
2	163.67±3.92	159.73±7.99(0.95)	162.0±5.51(0.99)	159.67±2.90(0.95)
3	171.67±2.84	165.0±6.80(0.67)	164.67±1.20(0.64)	171.00±3.51(0.99)
4	176.00±1.15	170.33±2.90(0.31)	174.33±1.86(0.94)	173.67±2.33(0.87)
5	183.00±1.53	178.33±3.76(0.63)	181.33±2.73(0.97)	181.33±2.40(0.97)
6	198.33±1.76	193.33±1.67(0.28)	195.0±2.65(0.59)	195.0±0.58(0.59)
7	226.±0.45	222±3.51(0.59)	222.67±2.40(0.71)	226.67±0.88(0.71)
8	245.67±6.06	236.67±4.41(0.83)	236.67±3.52(0.83)	241.0±12.34(0.83)
9	267.±1.52	253.±3.05(0.38)	246.67±5.55(0.14)	250.33±9.61(0.25)
10	280.33±2.60	269.33±4.40(0.25)	259.50±6.25(0.25)	263.0±10.96(0.34)
11	285.33±2.18	270.67±3.33(0.38)	263±6.55(0.15)	265.67±10.49(0.22)
12	294.7±1.45	279.07±4.87(0.34)	268.40±7.63(0.06)	276.33±8 (0.22)
13	297.33±7.75	289.2±2.88(0.99)	274.33±8.68(0.58)	277.±7.50(0.74)
14	305.67±8.95	291.28±1.61(0.61)	276.67±8.52(0.13)	291.85±10.30(0.64)
15	310.33±5.36	294.18±2.41(0.52)	285.98±4.71(0.21)	293.63±14.05(0.40)
16	313.33±3.48	295.01±1.32(0.28)	287.52±4.38(0.11)	295.18±12.70(0.32)
17	314.67±4.09	296.09±1.42(0.34)	290.04±3.56(0.15)	297.17±13.40(0.38)
18	322±3.05	297.63±.96(0.13)	293.55±4.50(0.06)	300.33±12.32(0.18)
19	324.33±2.96	312.53±1.584(0.09)	314.47±2.38(0.18)	316.67±4.49(0.35)
20	329.67±4.25	317.67±3.84(0.26)	318.33±6.22(0.30)	323.67±0.67(0.95)
21	333.33±3.33	326.±0.57(0.23)	323.33±2.90(0.07)	326.67±2.027(0.08)
22	347±1.00	345.33±1.76(0.91)	345.48±2.45(0.93)	346.43±1.67(0.99)
23	354.33±0.88	353.52±.29(0.94)	352±1.73(0.43)	353.23±0.67(0.90)
24	359.67±2.02	354.74±0.89(0.31)	352.33±2.33(0.25)	356.83±1.85(0.25)

Values of the mean body weight are expressed as Mean ± S.E.M and figures in bracket show the *p*-value
Number of rats in each group=5

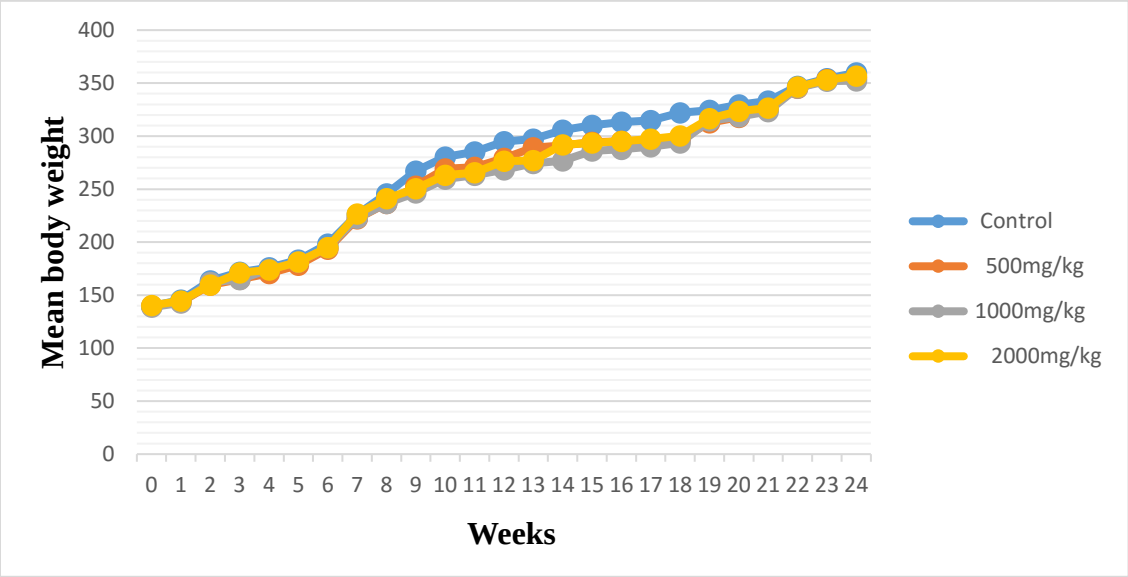

Figure 4: Graphical Comparison of mean body weight between the treatment (at the doses of 500,1000and 2000mg/kg, respectively and the control groups of male Wistar rats . Number of rats in each group =5

As displayed in Table 2, the mean body weight of female rats in group II (treated with 500mg/kg) increases their mean body weight form the initial 143.33±0.88g to 281±3.78. The mean body weight of female rats in group III (treated with 1000mg/kg) increased from 141.34±1.76g to 284.12±2. 41g.The average body weight of the female rats in group IV (treated with 2000mg/kg of aqueous leaf extract of *T. schimperi*) increased from 143±2.4g to 279.33±1.2g. The mean body weight of male rats in the control group increased from the initial dosing weight of 142.67±2.02g to 285.57±3.18g at the end of 24thweek. When the increment of the mean body weight of the treated groups was compared to that of the control group, the increment of was more or less similar and the difference of the mean body weight between the control and treatment groups was insignificant at all dose levels. This concomitant bodyweight increment indicates, the gain of body weight was not dose dependent rather it was normal and physiological increment. Figure5 illustrates the graphical comparison of general body weight gain patterns of the treatment and control groups of female rats during the study period of 24 weeks.

Table 2: The effect of chronic administration of aqueous leaf extracts *T.* schimperi on the body weight of female rats

Weeks	Groups			
	I(control)	II(500mg/kg)	III(1000mg/kg)	IV(2000mg/kg)
0	142.67±2.02	143.33±0.88(0.99)	141.34±1.76(0.95)	143.7±2.4(0.98)
1	151.43±0.72	150.33±0.33(0.67)	149.67±0.88(0.32)	152.66±0.67(0.59)
2	164.33±2.33	165.00±1.52(0.99)	165.0±1.120(0.99)	166.67±3.52(0.88)
3	168.52±2.22	166.33±1.67(0.90)	167.33±1.45(0.99)	170.67±1.76(0.70
4	174±2.51	169.67±2.60(0.83)	169.83±4.08(0.84)	173.17±4.62(0.87)
5	177.33±1.76	176±5.29(0.98)	173.33±4.37(0.86)	176.33±1.47(0.13)
6	185.33±2.90	179.67±4.91(0.69)	183.67±4.33(0.98)	179.33±0.88(0.06)
7	194±2.65	188.33±2.33(0.68)	187.54±4.58(0.29)	181±2.40(0.08)
8	201±3.60	199±3.05(0.98)	191.67±6.69(0.47)	188±3.05(0.23)
9	208±3.78	204±5.03(0.97)	198.67±5.92(0.98)	192.67±5.04(0.31)
10	211.33±1.76	209.67±2.72(0.99)	205.67±5.48(0.72)	196.07±4.07(0.72)
11	220.33±3.17	214±3.51(0.68)	210.37±6.03(0.35)	206.33±2.18(0.35)
12	226±3.78	222.±4.51(0.94)	211.67±4.91(0.26)	209.40±6.50(0.17)
13	229.67±2.60	223.33±4.10(0.63)	217±3.214(0.14)	217.33±4.37(0.16)
14	234±3.21	225.17±3.65(0.30)	220.33±1.45(0.07)	222.07±4.179(0.12)
15	236.67±2.40	227±2.78(0.34)	223.57±1.75(0.11)	221.85±4.37(0.06)
16	238.33±3.33	229.23±1.92(0.73)	229.97±3.06(0.83)	229.71±2.60(0.79)
17	240.67±1.76	233.47±1.66(0.17)	232.37±3.61(0.11)	236.55±0.72(0.57)
18	244.67±.33	242±3.31(0.73)	245.98±0.98(0.95)	239.17±1.09(0.22)
19	248±0.57	246.63±3.09(0.24)	246.70±0.95(0.96	245.43±1.59(0.75)
20	256±0.58	251.73±0.32(0.14)	251.55±1.79(0.12)	252.76±1.53(0.31)
21	269.00±8.38	255.48±0.80(0.19)	256.65±0.72(0.25)	261.61±1.14(0.63)
22	275.33±3.52	269.15±2.42(0.50)	266.68±3.92(0.25)	267.70±1.48(0.34)
23	282.13±4.85	273.59±2.42(0.21)	276.72±0.90(0.55)	273.23±1.02(0.19)
24	285.57±3.18	281±3.78(0.65)	284.12±2.41(0.97)	279.33±1.20(0.12)

Values of the mean body weight are expressed as Mean ± S.E.M and figures in bracket show the *p*-value. Number of rats in each group=5

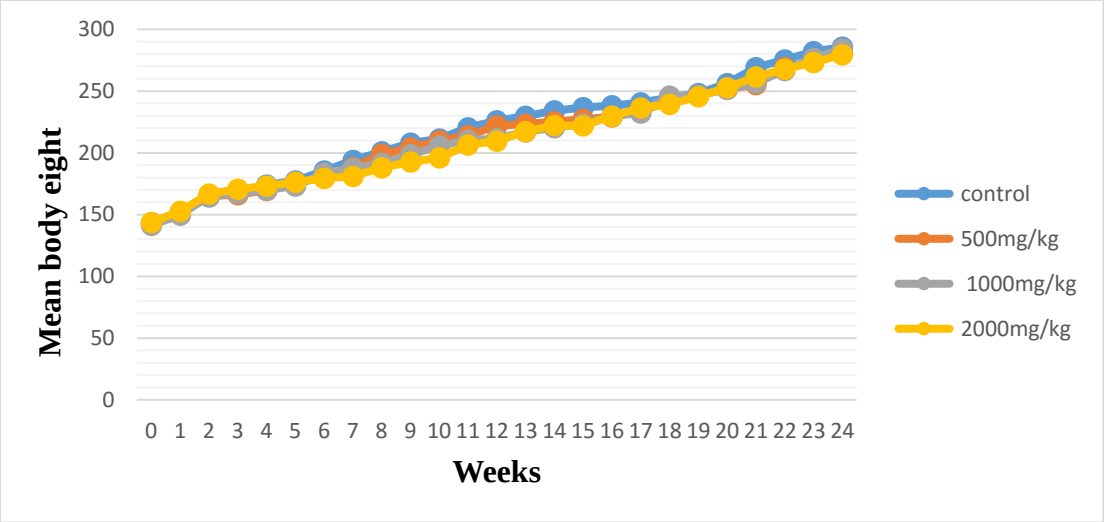

Figure5: Comparison of mean body weight between the treatment groups (at doses of 500,1000and 2000mg/kg respectively) and the control groups in female Wistar rats . Number of rats in each group=5

4.3. Effect of aqueous leaf extract of *T. schimperi* on the weight of liver and kidney in male and female rats

As shown below in Tables3and 4 respectively, the aqueous extract of *T. schimperi* did not bring any effect on the mean weight of liver and kidney of both male and female rats at all dose levels when compared with the mean body of the rats in the control group. The difference in mean weights between the mean weight of liver and kidney weight of rats in control groups and treatment groups was insignificant.

Table3: Effects of chronic administration of aqueous leaf extracts of *T. schimperi* on the weight of liver and kidney in male Wistar rats

Organs	Groups			
	I (Control)	II (500mg/kg)	III (1000mg/kg)	IV (2000mg/kg)
Liver	14.31±2.13	13.72±.91(0.24)	11.83±.24(0.05)	12.54±0.79(0.09)
Kidney	1.24±0.08	1.1967±0.06(0.93)	1.1±0.03(0.39)	1.07±0.03(0.21)

Values are expressed as Mean ±S.E.M. Figure in bracket are p-value**.** Number of rats in each group=5

Table4: Effects of chronic administration of aqueous leaf extract of T. *schimperi* on the weights of liver and kidney in female Wistar rats

Organs	Groups			
	I(Control)	II(500mg/kg)	III(1000mg/kg)	IV(2000mg/kg)
Liver	10.64±0.83	11.55±0.82(0.79)	9.43±0.62(0.62)	8.09±.39(0.11)
Kidney	0.89±0.04	0.87±0.01(0.99)	0.78±0.03(0.18)	0.81±0.04(0.44)

Values are expressed as Mean ±S.E.M. Figures in bracket are p-value. Number of rats in each group=5

4.4. Effects of aqueous leaf extract of *T. schimperi* on the hematology of male and female rats

Table5 below displays the effect of chronic administration of *T. schimperi* on the hematological parameters of male rats. The result of the chronic study indicated that mean difference of the hematological parameters of the treatment groups were statistically insignificant at all dose levels in comparison with the control groups. Even though there were decrements of WBC counts at all dose levels of treatment groups of male rats, the differences were statistically insignificant. WBC count of the treatment groups showed slight decrements in groups of male rats which were orally administered with the plant extract at doses of 500,1000,2000mg/kg /body weight /day respectively, but this decrement was statistically insignificant .Concurrently, the RBC count were decreased in groups of male rats that were administered by plant extract at 500,and 2000mg/kg/body weight while there was increments of RBC count in the groups of male rats

which received 1000mg/kg/body weight of plant extracts ,however, both the decrements and increments of the RBC count were statistically insignificant . At the same time although the changes were statistically insignificant, the platelet counts showed that there was decrement of number of platelets in the groups of male rats orally received plant extracts at doses of 500, and 1000mg /kg/body weight and increments of platelet in the groups of male rats which received plant extracts at dose of 2000mg/kg/body weight. Generally, in the RBC indices, HGB, HCT, HCV, MCH, and MCHC, statistically insignificant mean differences have been observed between the treatment and control groups as shown in Table 5 below. Similarly, there were statistically insignificant differences of the mean value of the leukocyte components.

Table 5: Effects of chronic administration of aqueous leaf extracts of *T. schimperi* on the hematology of male rats

Hematological parameters	Groups			
	I(control)	II(500mg/kg)	III(1000mg/kg)	IV(2000mg/kg)
WBC(x10^3/µL)	6.99±0.49	4.97±1.5(0.64)	5.96±1.71(0.93)	4.65±0.42(0.54)
RBC(x10^6/µL)	9.07±.17	8.05±.57(0.27)	9.63±.34(0.71)	8.88±.25(0.98)
HGB(g/dL)	17.23±.37	15.33±1.3(0.35)	18.60±0.55(0.60)	17.20±0.35(1)
HCT (%)	51.43±0.95	47.47±3.17(.56)	56.27±1.89(.41)	54.73±1.71(0.69)
MCV (fL)	56.73±.03	58.03±.87(0.69)	58.40±.55(.52)	58.60±1.3(0.43)
MCH(pg)	19±0.10	19.03±0.3(1.0)	19.40±1.20(0.96)	19.37±0.15(0.97)
MCHC(g/dl)	33.5 ±0.20	32.23±0.64(0.86)	33.17±2.08(0.99)	31.43±0.73(0.61)
PLT(x10^3/µL)	669.33±1.1	464.33±1.8(0.42)	531±1.44(0.88)	810.00±.85(0.87)
NEUT(x10^3/µL)	0.77±1.22	0.82±0.25(0.99)	1.39±.35(0.44)	1.22±0.34(0.68)
LYMP(x10^3/µL)	5.67±0.56	4.05±1.53(0.73)	3.78±1.48(0.64)	3.11±.13(0.41)
MON(x10^3/µL)	0.35±0.04	0.19±0.06(0.73)	0.43±0.17(0.95)	0.24±0.12(0.87)
EOS(x10^3/µL)	0.11±0.06	0.13±0.01(0.99)	0.33±0.11(0.18)	0.06±0.02(0.95)
BASO(x10^3/µL)	0.03±0.006	0.023±.008(0.86)	0.03±0.01(1)	0.02±0.003(0.87)

All measurements are expressed as Mean ±S.E.M and figures in bracket indicate p-value. Number of rat in each group=5

As depicted in Table6, the chronic oral administration of aqueous extract of *T.schimperi* did not revealed any statistical significant differences in all hematological parameters as compared the groups of female rats that have been orally administered with plant extract at all dose levels and the control groups .The WBC count in the treatment groups of female rats which received plant extract at doses of 500,1000, and 2000gm/kg /body weight, respectively was slightly higher in comparison with the control group, but that increment of WBCs count was statistically insignificant. Similarly, there was statistically insignificant difference of HGB, HCT, RBCS and platelet counts between the treatment and control groups of female rats as illustrated in Table 6. The RBC indices (HCV, MCH, and MCHC) and WBC (leukocyte) components of the rats in the treatment groups at all dose levels also did not reveal any statistically significant difference as compared with the RBC indices and leukocyte components of rats in control groups.

Table 6: Effects of chronic administration of aqueous leaf extracts of *T. schimperi* on the hematology of female rats

Hematology parameters	Groups			
	I(control)	II(500mg/kg)	III(1000mg/kg)	IV(2000mg/kg)
WBC ($x10^3$/µL	4.47±1.11	6.28±0.80(0.84)	5.11±1.44(0.99)	6.87±2.29(0.68)
RBC($x10^6$/µL)	8.66±1.38	9.89±0.33(0.75)	7.71±0.47 (0.85	8.94±0.82(0.99)
HGB(g/dL)	16.13±1.60	17.73±0.47(0.6)	14.73±0.73(0.72)	17.8±0.31(0.60)
HCT (%)	45.50±6.76	56.97±1.29(0.25)	47.37±3.31(0.98)	52.03±2.32(0.67)
MCV (fL)	52.76±0.74	57.63±0.64(0.34)	59.97±1.85(0.11)	58.77±3.17(0.19)
MCH(pg)	19.46±1.83	17.93±0.17(0.89)	19.13±0.23(0.99)	20.50±2.5(0.96)
MCHC(g/dL)	36.78±2.87	31.13±0.23(0.17)	31.20±0.61(0.18)	34.10±1.81(0.70)
PLT($x10^3$/µL)	443.67±101	679.±59.18(0.66)	380.33±17.8(0.8)	711.67±19.2(0.6)
NEUT ($x10^3$/µL	0.97±0.35	1.70±0.21(0.29)	0.81±0.19(0.97)	1.21±0.27(0.92
LYMPH($x10^3$/µL)	2.92±0.59	3.78±0.64(0.79)	2.51±0.66(0.97)	3.04±0.71(0.99)
MONO($x10^3$/µL)	0.37±0.12	0.60±0.07 (0.79)	0.21±0.11(0.91	0.21±0.29(0.98)
EOS($x10^3$/µL)	0.18±0.10	0.17±0.08 (1)	0.11±0.07(0.98)	0.33±0.22(0.84)
BASO($x10^3$/µL)	0.023±0.006	0.03±0.02(1)	0.08±0.05(0.78)	0.17±0.07(0.19)

All measurements are expressed as Mean ±S.E.M and figures in bracket indicates p-value. Number of rats in each group =5

4.5. Effects of aqueous leaf extract of *T. schimperi* on the biochemical parametersin male and female rats

Table 7: Effects of aqueous leaf extract of *T. schimperi* on biochemical parameters in male Wistar rats

Groups	Biochemical parameters					
	ALP (U/L)	ALT (U/L)	AST (U/L)	UREA (mg/dl	CREA (mg/dl)	ALB (mg/ dl)
I (control)	77.7±17.2	88.7±14.6	176.9±31.5	44.7±3.3	0.3±0.03	4.9±0.4
II (500mg/kg)	75.7±11.8 (0.9)	167.83±29 (0.4)	138±16 (0.3)	51.7±2.3 (0.4)	0.2±0.006 (0.2)	4.5±0.2 (0.7)
III(1000mg/kg)	122.7±3.9 (0.1)	111.5±9.9 (0.9)	172±32.6 (0.9)	41.7±3.6 (0.9)	0.3±0.007 (0.9)	4.9±0.2 (0.9)
III(2000mg/kg)	95.7±10.7 (0.7)	136±54.9 (0.7)	153.4±25(0 .8)	49±2.2 (0.7)	0.2±0.04 (0.5)	5±0.3 (0.9)

All measurements are expressed as Mean ±S.E.M. figures in bracket indicate p-value. Number of rats in each group =5

As shown in the above Table 7, the enzyme of liver function test like ALP, ALT and AST of the male rats treated with aqueous leaf extracts of *T. schimperi* reveal statistical insignificant changes as compared to the liver function test enzymes of male rats of the control groups. The concentrations ALB protein in the blood serum of the treated male rats also shows statistically insignificant changes when compared with the rats in the control groups and similarly the diagnostic biomarkers for kidney dysfunction, like creatinine and urea concentrations in the blood serum of the treatment group of male rats did not bring any statistically significant changes as compared to the male rats in the control group. However, the differences in mean of liver function enzyme were statistically in significant there was an elevation of ALP, ALT. Similarly, there was an elevation of UREA in the treatment groups.

Table 8: Effects of aqueous leaf extract of *T. schimperi* on biochemical parameters in female Wistar rats

Groups	Biochemical Parameters					
	ALP (U/L)	ALT (U/L)	AST (U/L)	UREA (mg/dl	CREA (mg/dl)	ALB (mg/dl)
I(control)	133.67±24.7	61.73±23.2	84.56±19.9	51.53±3.49	0.2±0.04	4.34±0.22
II(500mg/kg)	93±2.30(0.3)	120±22.67(0.67)	76.5±16.5 (0.99)	44.9±2 (0.44)	0.19±0.1 (0.99)	3.88±0.42 (0.7)
III(1000 mg/kg)	104.3±12.34(0.55)	152±22.23 (0.99)	106.9±27(0.48)	49.23±3.46 (0.94)	0.12±0.02 (0.17)	3.89±0.23 (0.7)
III(2000 mg/kg)	95.67±12.7(0.35)	86.3±27.54 (0.19)	96.43±12 (0.79)	47.1±2.7 (0.73)	0.04±0.02 (0.007)	3.76±0.26 (0.5)

All measurements are expressed as Mean± S.E.M. figures in bracket indicate p-value. Number of rats in each group =5

Table8 illustrates chronic toxicity effects of aqueous leaf extract of *T. schimperi* on the biochemical parameters of female Wistar rats. As depicted in this table the aqueous leaf extract of *T. schimperi* did not result in any statistical significant changes in liver function enzymes test and protein (ALP, ALT, AST, and ALB), however, there was an elevation of ALT and AST. At the same time, the plant leaf extract did not show statistically significant changes in UREA and CREA which are used to diagnose kidney dysfunction; however, there was significant decrement of creatinine in the group of female rats treated with 2000mg/kg/body weight.

4.6. Effects of aqueous leaf extract of *T. schimperi* on the histology of liver

Results of Microscopic examinations of liver sections of both male and female rats, from the control group (A&B) and treatment groups(C&D) Figure 6 treated with 500mg/kg, (E and F) Figure 7 treated with 1000mg/kg and (G and H) Figure 8 treated with2000mg/kg dose levels, illustrated normal architectural arrangements of the hepatocyte lobules delimitated by sinusoids. Hepatocytes radiated normally from the central vein without any distortion of hepatocyte cords. No pyknotic nucleus, cell necrosis, diffused cytoplasmic inclusions and mononuclear and lymphocytic infiltrations have been observed. In overall appearances, the microscopic examinations of liver histological sections of the treated groups at all dose levels did not show significant differences comparing to the treatment groups. However, mild blood congestion in

central and portal vein has been seen at all dose levels. Mild focal mono nuclear infiltrations also have been observed in the treatment groups of female rats administered with 1000mg/kg and male rats administered with dose of 2000mg/kg.

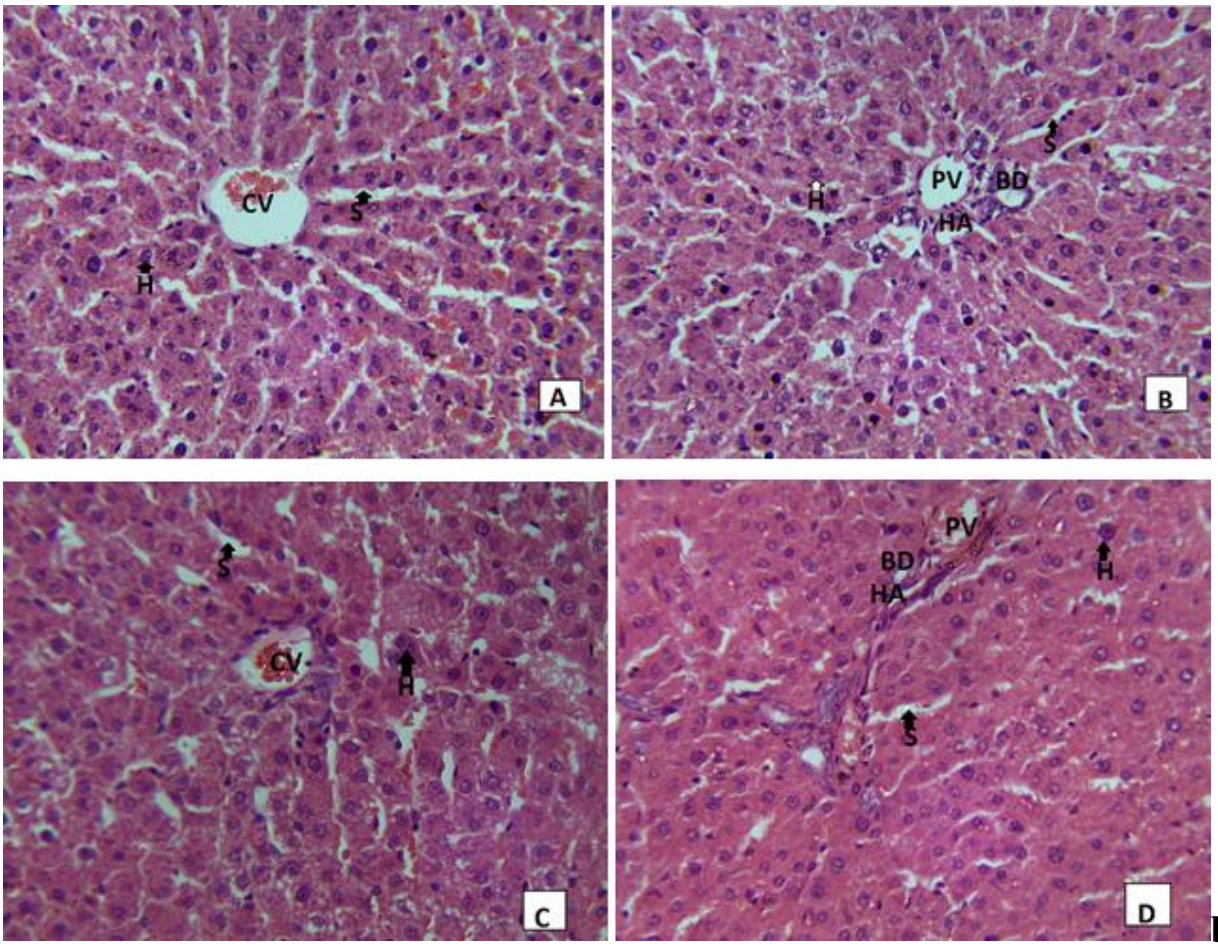

Figure 6: Photomicrograph of rats' liver sections of the control groups (A&B) and treatment groups treated with 500mg/kg (C&D) .CV=Central vein, PV=Portal vein, S=Sinusoids, H=Hepatocytes, BD=Bileducts, (H&E Stains), X200 magnification

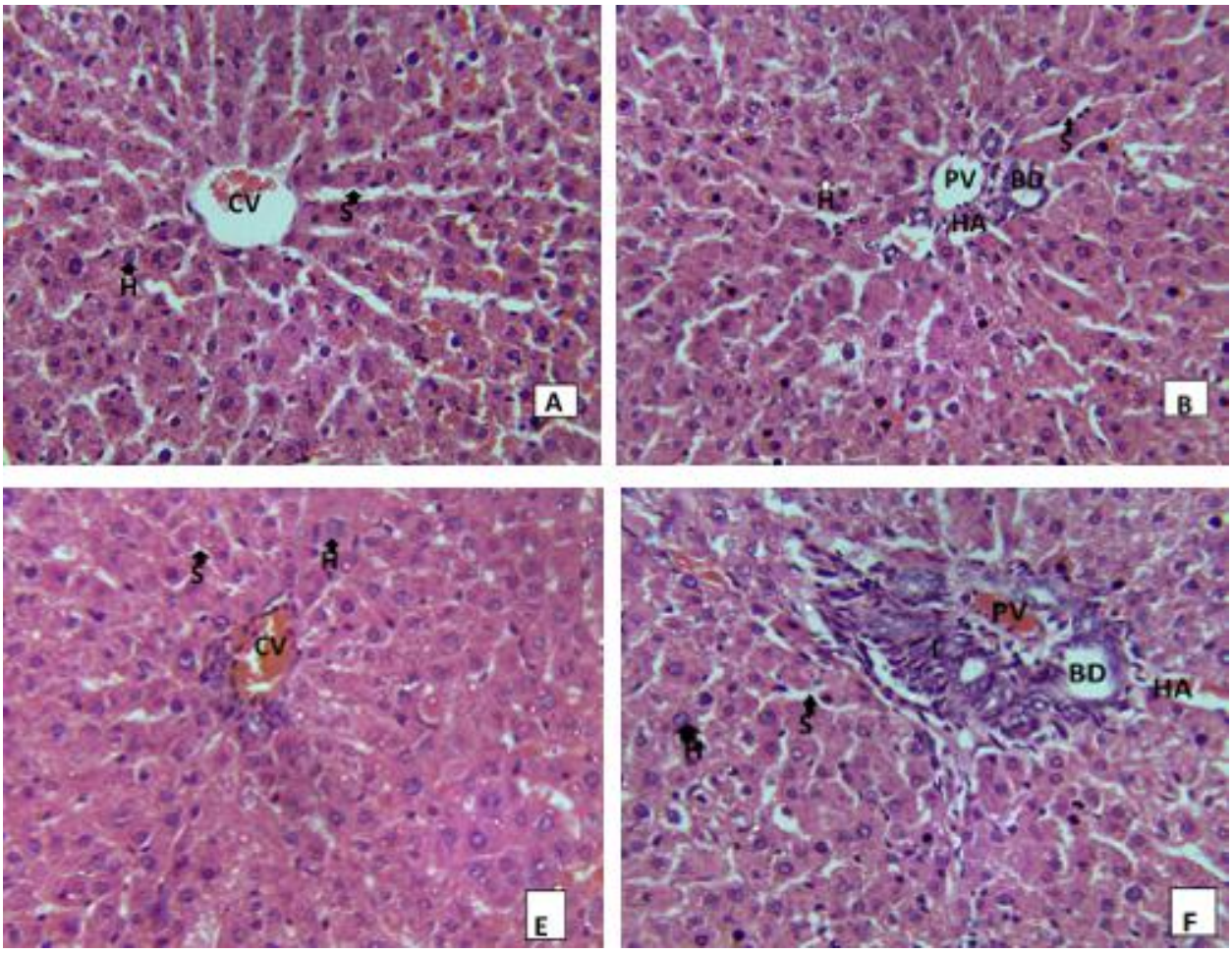

Figure7: Photomicrograph of rats' liver sections from control groups (A&B) and treatment groups (E&F) treated with 1000mg/kg. (CV=central vein, PV=Portal vein, S=Sinusoids, H=Hepatocytes, BD=Bileducts, I= =Infiltration of leukocytes (H&E Stains), X200 magnification

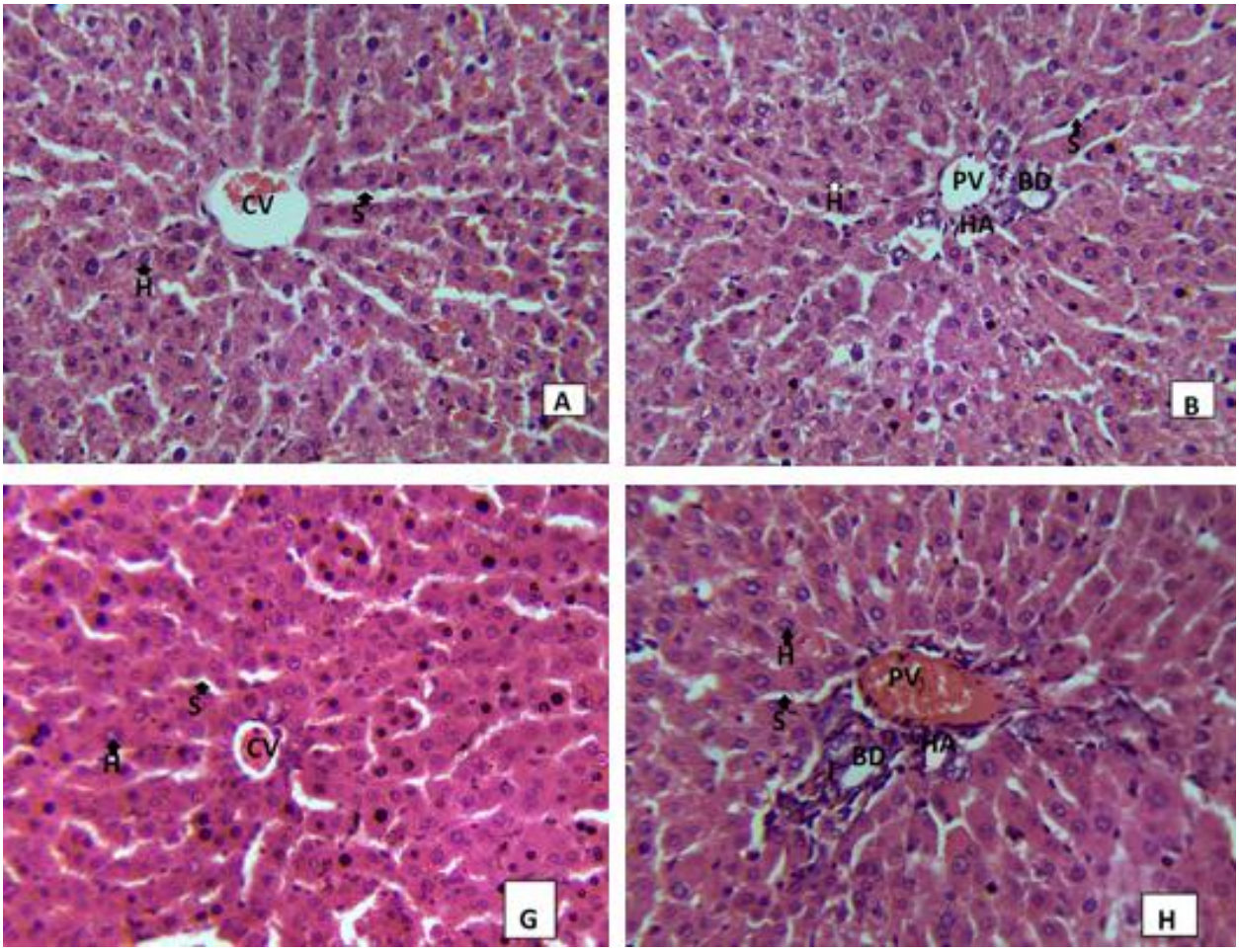

Figure 8: Photo micrograph of rats' liver sections from control groups(A&B) and treatment groups(G&H) treated with 2000mg/kg.CV=central vein, PV=Portal vein, S=Sinusoids, H=Hepatocytes, BD=Bile ducts, I= =Infiltration of leukocytes (H&E Stains), X200 magnification

4.7. Effects of aqueous leaf extract of *T. schimperi* on the histology of kidney

Findings of Microscopic examinations of kidney histological sections of both male and female rats, control groups (A&B) and treatment groups (C&D) Figure 9 treated with500mg/kg, (E&F) Figure10treated with 1000mg/kg) and(G&H) Figure 11 treated with 2000mg/kg, did not bring any abnormalities in the histological appearance of the kidney as compared with histological architecture of the kidney of the control groups(A and B).Microscopic architecture of the kidney in the treated rats of both sexes showed similar rearrangements of the cellular structures like that of the control groups. The renal corpuscles maintain their normal size without any congestion and dilatation of the urinary space. The tubular structures also were normal without any hyaline globule

or protein casts. The overall histological structural appearance of the kidney of the rat in the treated groups of rats at all dose levels did not show any significant difference when compared with the control groups.

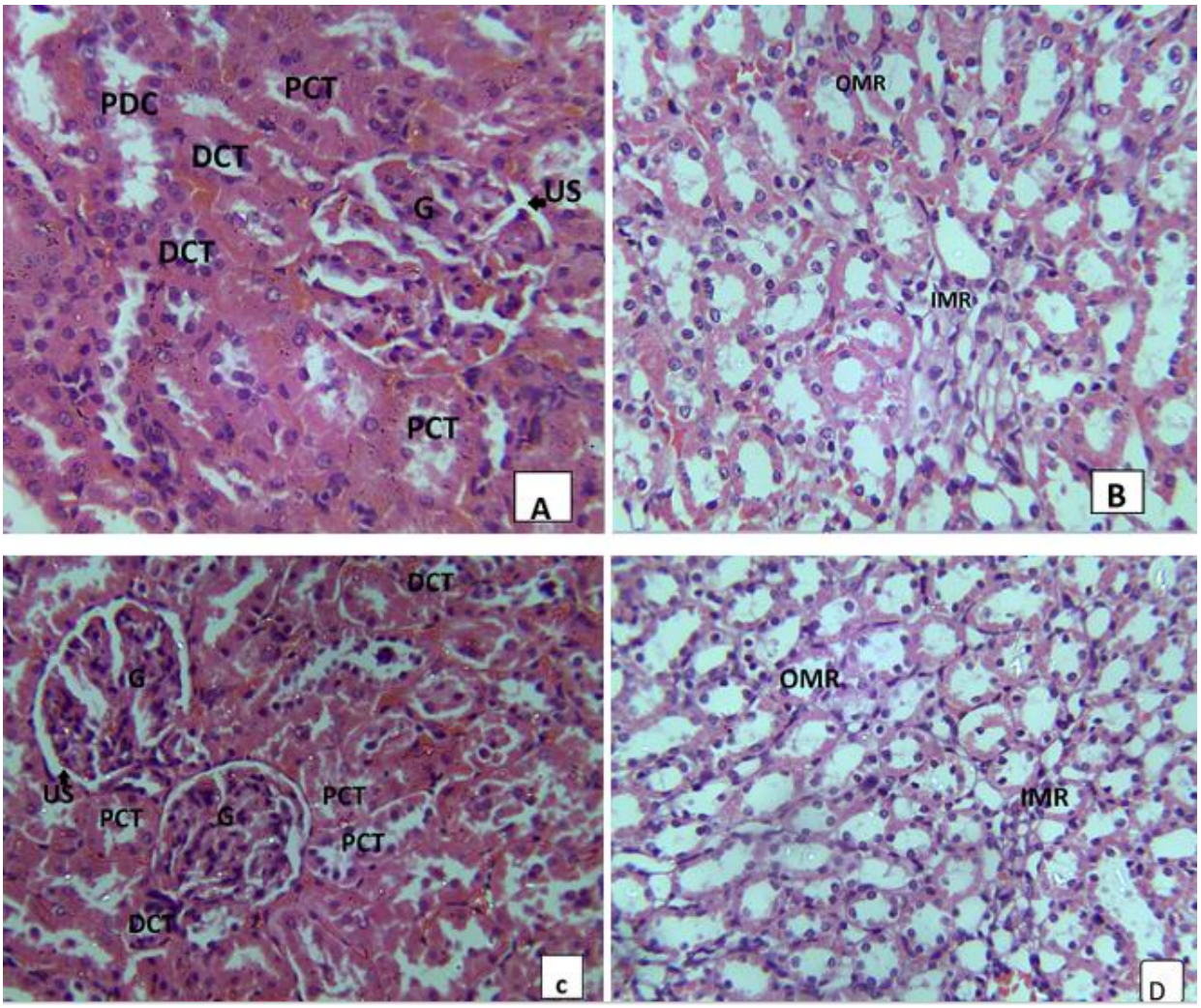

Figure 9: Photo micrograph of rats' kidney sections from control groups (A&B) and treated groups(C&D) treated with 500 mg/kg. G=Glomeruli, PCT=Proximal convoluted tubules, DCT=Distal convoluted tubules US=Urinary space, OMA=Out medullar region and IMR=Inner medullar region. (H & E Stains) X200 magnifications, BV =blood vessel.

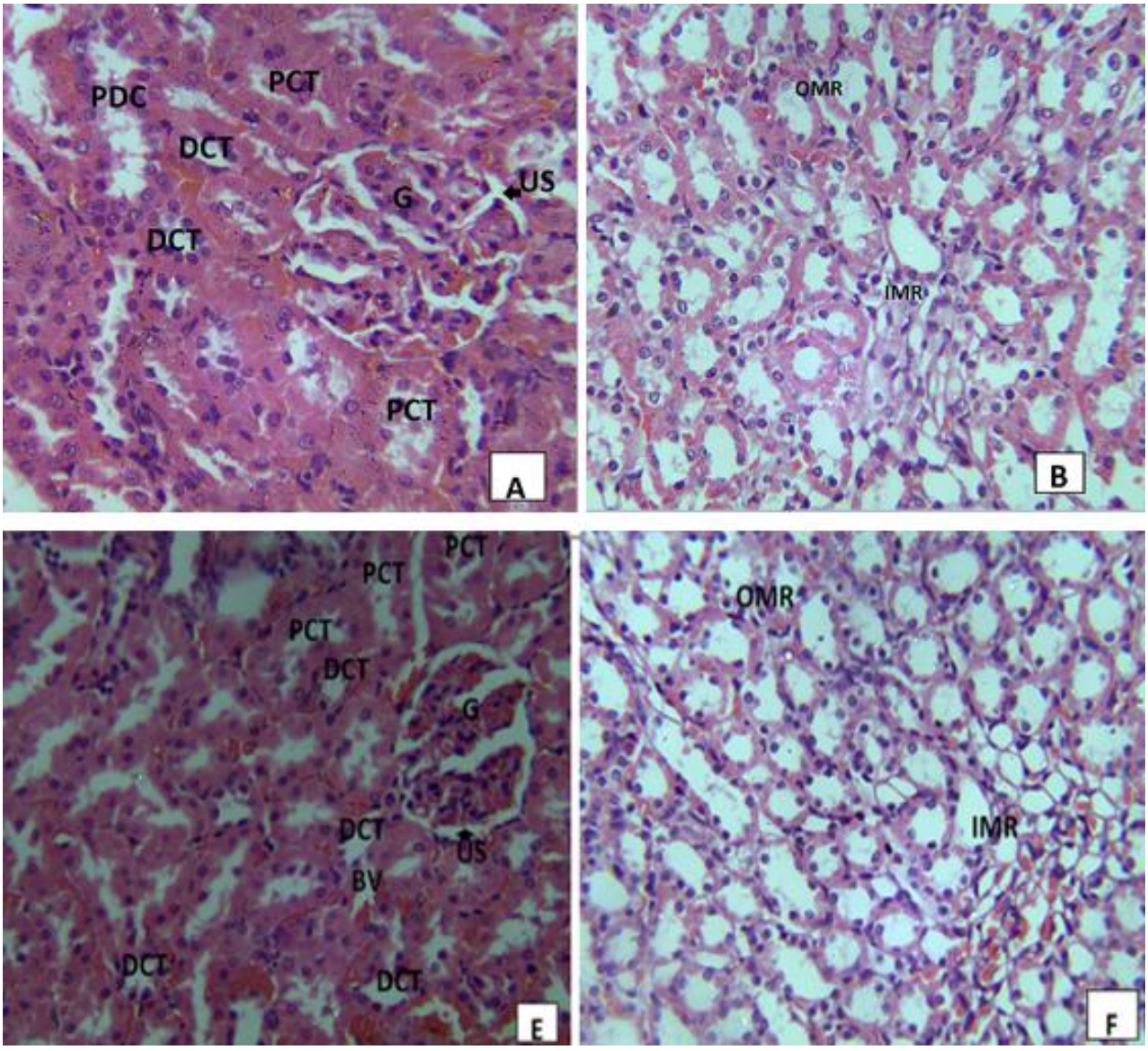

Figure 10: Photo micrograph of rats' kidney sections from control groups(A&B) and treatment groups treated with 1000mg/kg (E&F). G=Glomeruli, PCT=Proximal convoluted tubules, DCT=Distal convoluted tubules US=Urinary space, OMR=Out medullar region and IMR=Inner medullar region, BV =Blood vessel. (H & E Stains) X200 magnifications.

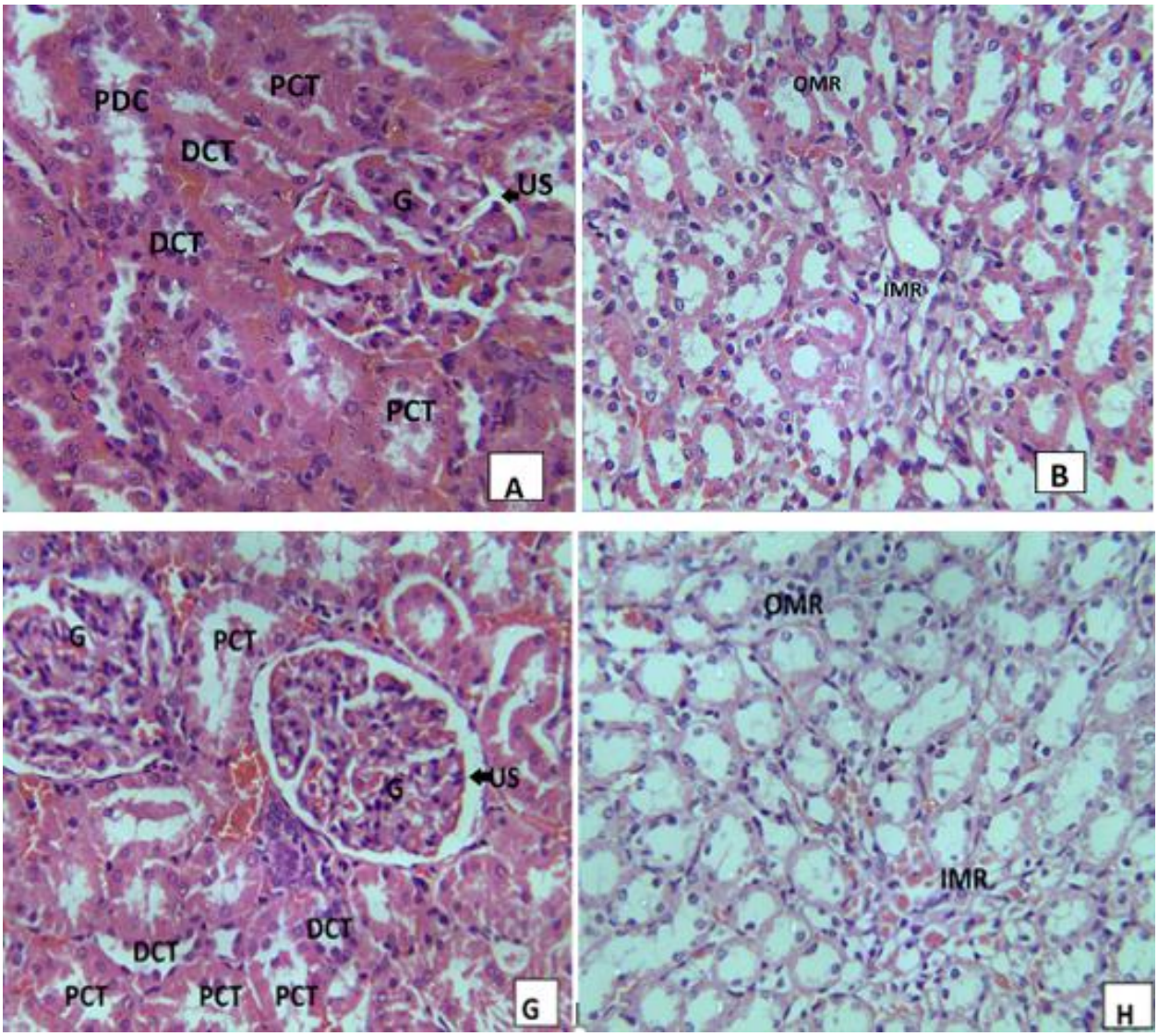

Figure 11: Photo micrograph of rats' kidney sections from control groups(A&B) and treatment groups treated with 2000mg/kg (G&H). G=Glomeruli, PCT=Proximal convoluted tubules, DCT=Distal convoluted tubules US=Urinary space, OMR=Out medullar region and IMR=Inner medullar region, BV =Blood vessel. (H & E Stains) X200 magnifications.

5. DISCUSSION

Herbal medicines are widely perceived by the public as being natural, healthful and free from side effects due to their natural origins and are often considered as food supplements and not drugs (Arsad *et al*., 2014; Abebe *et al*., 2001). They are usually self-prescribed by the consumers and there is a lack of control and review in terms of dose, manner, and frequency of administration. The chemicals substances in medicinal herbs may be natural to the plant, but they are not natural to the human body and one of the stated problem of using plants as medicine is that in many cases no definite doses are prescribed often resulting in over dose and toxicity (Arsad *et al*., 2014). Thus, the administration of herbal preparations without any standard dosage along with non- availability of adequate scientific studies on their safety has raised concerns on their toxicity. *T. schimperi*, an indigenous plant of Ethiopia mostly confined to the afro alpine regions, is used by the people in the country as condiments and flavoring agent, anti-helminths, anti-bacteria, anti-fungus, anti-oxidant, and anti-inflammatory, anti–diabetic, for urinary, oral infection and gastro intestinal spasm remedy, and so on. However, there is no available information on its chronic toxicity effect on liver, kidney and blood parameters. Therefore, this study was aimed at investigating the chronic toxicity effect of aqueous leaf extract of this plant.

Weight alteration is one of the most sensitive indicators of drug toxicity and this maybe an indication of impairment in the normal functioning of the body organs. During consumption of toxic substances, organ weight may indicate organ atrophy or hypertrophy (Amresh *et al*., 2008; Ashafa *et al*.,2011). In the present study as shown in Table 1 and 2 there was no discernable and statistically significant differences in the mean body weight between the treatment groups treated with the aqueous leaf extract of *T.schimper* and the control groups, however, the mean body weight of the control groups was somewhat higher than that of the treatment groups. Concurrently no hypertrophy or atrophy was seen in the liver and kidney of the treated groups of rats in both male and female sexes at all does levels. The aqueous leaf extract of *T. schimperi* did not bring any statistically significant changes in the absolute weight of liver and kidney as indicated in Table 3 and 4. This may suggest that the plant may not have adverse effect. This finding is in agreement with the findings of several authors. Debelo *et al*., 2016 reported similar findings after conducting sub chronic toxicity study on mice and rats using *Thymus serolatus* and *T. schimperi* aqueous leaf extracts, respectively. In agreement with the present chronic toxicity study his finding showed that

there were no significant differences between the organ and body weight of mice treated with aqueous extract of *Thymus serolatu.* and control groups. However, there was no significant changes between the mean body weight of the rats treated with aqueous leaf extract of *T. schimperi* and control groups in most of the weeks, there was significant increment of body weight of the male rats administered with low dose (200mg /kg) during weeks 6,7,8,9and12and similarly significant changes were observed in female rats administered with low dose (200mg/g) in weeks 9 and 10.(Cross *et al*.(2002); Demir *et al*. (2008) reported similar findings after feeding growing broiler chicken for 42 days using wheat based supplementary diet of *Thymus vulgaris* at dose of 400mg/kg. Saadat Shad *et al*. (2016) also reported similar results after feeding the broiler chickens with doses of 300and 500mg/kg diet prepared from Thymus *vulgaris* essential oils.

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood parameters (Modaresi*et al*., 2011; Agbaje *et al*.,2009). Such laboratory investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational, disease diagnosis, prevention and treatment (Yakubu *et al*., 2003; Agbaje *et al.,* 2009). Toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies (Sunmonu and Oloyede, 2014; Ashafa *et al*., 2011). Anemia, defined clinically as a decrease in HCT or HGB concentration, may be caused by blood loss, excessive hemolytic condition, or deficient erythropoietin and HGB synthesis (Jee*et al*., 2005; Savithri*et al*., 2010). Decrement in HCT is attributable to the reduction in RBC count caused by either destruction or reduction in size (Savithri*et al*., 2010). MCH, MCHC and MCV relates to individual red blood cells while HGB, RBC and HCT are associated with the total population of red blood cells (Agbaje *et al.,* 2009).Increase in total WBC count has been suggested to be due to stimulated mainly lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue (Agbaje *et al*.,2009;Princewill-Ogbnna *et al*., 2015) .Such lymphocyte response might be due to the presence of toxic substances may be associated with the toxicity induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of lymphocytes and in contrast the number of neutrophils decrease if the toxicity is sever since neutrophils are involving in phagocytosis and engulfing damaged cells and they ruptured and die (Savithri *et al*., 2010). On the other hand, reduction of total WBC count, platelets indicates that

plant extracts and other drugs might have adverse effects on the granulocyte-macrophage colony stimulating factor, such as interleukins IL-2, IL-4 and IL-5 that regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells (Agbaje *et al*.,2009) and platelets (Ashafa *et al*, .2011). Taking this in to account, the present study measures different hematological parameters like RBC, HCT and HGB count, RBC indices (MCV, MCH and MCHC), WBC count, leukocyte components (lymphocyte, basophilsneutrophils, eosinophils and monocyte) and platelet count.

As shown from Table 5and6, in this study there was no significant difference between the RBC count and RBC indices of the treatment groups of rats of both sexes which received aqueous extract of *T. schimperi* and control groups. The insignificant difference between RBC and HGB counts of the treatment and control group could mean that the rate of production of RBC (erythropoietin) and the amount of oxygen carrying proteins (HGB) in the blood were not affected by the plant extract that indicated no toxic molecules were incorporated into the blood and site where the RBC and HGB are produced. On the other hand the insignificant difference of RBC indices between the treated and control groups revealed that the percentage blood (HCT) consists of RBC, the average size of RBC (MCV), average amount of oxygen carrying hemoglobin in side RBC (MCH), and average percentage of hemoglobin (MCHC) were not affected negatively and this suggests that the aqueous leaf extracts of *T. schimperi* did not possess any potential of inducing macrocytic or microcytic and hyper-chromic or hypo-chromic anemia. As displayed in Table 5and 6, there is no statistically significant difference between the total WBC count, leukocyte components,(lymphocytes, basophiles, neutrophils , eosinophils and monocytes)and platelets between the treatment and control groups of rats at all dose levels .This infers the aqueous leaf extract of *T.schimperi* did not impose toxicity which resulted in leucopenia or leukocytosis and thrombocytopenia or thrombocytosis in the blood of rats which received the plant extracts , therefore, this may suggest that the plant is relatively safe to the consumers even they intake it for prolong period time .

Many authors such as, Demir *et al*. (2008); Saadat Shad *et al*.(2016) conducted a research on broiler birds using *Thymus vulgaris*,Debelo *et al*., 2016 who conducted research on mice and rat using aqueous leaf extracts of *T. serolatus* and *T.schimperi* respectively reported similar findings on most of the hematological parameters, however there was significant increment of WBC count

and decrement of monocyte in the of the female rats treated at low dose(200mg/kg) and reduction of basophils of the male rats treated at higher dose (600) of *Thumus serolatus* and similarly there was significant reduction of basophils in female rats treated at low dos and male rats at both 200 and 600mg/kg doses of *T*. schimperi.

Liver is one of the most important organs in the body and is responsible for breaking down all 'poisons' that enter the body and therefore it is highly vulnerable to toxic substances and drugs (Ojiako *et al*., 2014). Liver function tests conducted through blood assays give information about the state of the liver, describing its functionality (Agbaje *et al*.,2009). Transaminase enzymes like ALT, AST and ALP and albumin protein are most commonly used serum liver chemistry tests (Agbaje *et al*., 2009). ALT is a cytoplasm enzyme found only in liver cell and an increment of ALT in plasma is an indication of injuries caused by drugs to the liver and characterized as hepatocellular when there is predominant elevation of the ALT, while AST is a mitochondria enzyme whose increment in plasma reflects severe tissue injuries (Awe and Banjoko, 2013). The increment of AST in plasma could be linked to the fact that, AST increase in the serum is in a wide variety of conditions particularly, cardiac, liver and skeletal muscles disease due to cellular necrosis allowing enzyme leakage into the circulation (Usman *et al*., 2014). However, concurrent increment in ALT and AST is suggestive for liver cell necrosis. This could be linked to the fact that liver toxicity is ascertained if there is an increase in ALT three times its level at the beginning of study (Ojiako *et al*.,2014; Usman*et al*.,2014; Manal*etal*.,2016).ALP is a non-plasma specific enzyme that is released from the sinusoidal surface of the liver cell and thus is present in the serum at low levels in the absence of liver damage and it helps to evaluate damage to hepatobiliary routes and integrity of plasma membrane (Sunmonu and Oloyede,2010; Alex *et al*.,2016).The concentration of albumin, in the serum is one of the important protein parameter that can be used to assess the health status of the liver and can be used to ascertain different types of liver damage because during liver damage albumin synthesis is affected and low amount of albumin is found in the blood serum (Yakubu *et al*., 2003;Sunmonu *et al*.,2014). Kidney is the second target organ to be affected by xenobiotic and toxic substances that circulatein the blood which can disrupt its function. Creatinine and urea are removed from the plasma by glomerular filtration and are then excreted in the urine without being reabsorbed by the tubules to any significant extent. Analysis of plasma creatinine and urea levels is a tool to evaluate kidney's function and if any elevation of

blood plasma creatinine and urea concentrations in the blood serum are diagnostic of renal dysfunction and pathologic conditions of kidney (Owoyele *etal*., 2011; Sunmonu *et al*., 2014; Usman *et al*., 2014).Taking this in to consideration the present study evaluates the biochemical markers, like ALP, ALT, AST and ALB protein for liver damage and blood urea and creatinine, for kidney damage, respectively.

In the current chronic toxicity study, treatment of rats with T. schimperi aqueous leaf extract did not result in statistically significant changes, either in the liver enzymes function test, ALP, ALT, AST or the ALB protein in the blood plasma of the rats of both male and female sexes treated with *T. schimperi* aqueous leaf extract as compared to that of the control groups. However, the mean difference of the liver enzyme function test was insignificant the was considerable elevation of ALP in the male rats treated with 1000 and 2000mg/kg and ALT in the rats treated with 500 and 2000mg/kg of *T. schimperi*. The ALT and AST values of treated female rats also showed considerable elevations as compared to that of the control groups.

The insignificant differences of biochemistry between the treatment and control groups may be due to small sample size or consumption of *T. schimperi* aqueous leaf extract may not adverse effect on hepatocytes and biliary routes in the liver parenchyma cells. Therefore, plant may not adversely affect the liver. Similar findings reported by Debelo *et al*. (2016) who conducted sub chronic toxicity research in mice and rats using aqueous extract of *Thymus serolatus* and *T. schimperi*, respectively, showed that most parameters of liver function test in the treated groups were as normal as these of the control groups, but significant reduction of AST at both doses (200and 600mg/kg) of T. *schimperi* was reported, but this does not have an implication for toxicity. Another author (Kovacs *et al*.,2016) also reported similar finding after feeding developing rabbits using3%*Thymus vulgaris* powder along with their normal diet for 42 days. The finding of this chronic toxicity study conducted in male and female rats did not cause any significant elevations of urea and creatinine in the blood plasma and the changes of biomarkers of kidney between treatment and control groups were statistically insignificant, however elevation of urea in the animals treated with 500 and 2000mg/kg of *T. schimperi* aqueous leaf extract.This may indicate the aqueous leaf extract of plant did not cause adverse glomerular damage, as a result, filtration process was not disrupted.

This also in line with the findings of sub chronic research conducted in mice and rats using aqueous extract of *T. Serolatus* and *T. schimperi* by Debelo *et al*., 2016, however, there was significant reduction in the female rats treated with T. *serolatus* at both low and high doses (200 and 600mg/kg.

Histological analysis can be used to examine the morphological changes in organs to reflect possible effect of xenobiotic. These changes are a late manifestation of chemical, physical, mechanical or inflammatory assault on the affected tissue and usually complement enzyme study (sunmonu *et al*., 2014; Manal *et al*.,2016). Evidence of liver damage, usually manifest as a result of architectural disarray, vascular congestion, hepatocytes cell necrosis, apoptosis, diffused leukocyte cell infiltration in either acute or chronic condition (Katsayal *et al*.,2008).

In this chronic study the aqueous leaf extract of *T. schimperi* did not cause either of these liver damage manifests such as disarray of hepatocyte plate, cell necrosis, pyknosis and diffused leukocyte infiltration, lipid cytoplasm inclusion and fluid accumulation. However, mild focal inflammatory cell infiltrations have been observed around the portal area and blood congestion in central vein and portal vein of the liver in female and male rats treated at doses of 1000and 2000 mg /kg ,respectively, but this mild focal infiltration and blood congestion have no an implication for toxicity since the liver function test enzymes and proteins showed statistically insignificant changes. The blood congestion may be due to blood coagulation during fixation and the mild mononuclear cell infiltration may be response for other infection or the plant extract may cause sub cellular cell damage. This finding is in agreement with the findings reported by (Debelo *et al*., 2016).

Damages to kidney caused either by chemical agents or drugs could be manifested as vascular congestion (glomerulus), inflammatory cell infiltration, disintegration interstitial space and hyaline globule (protein cast) in collecting tubules (Katsayal *et al*., 2008). All these features were not observed in the kidney sections of treatment groups on this study. This finding was in line with the findings reported by (Debelo *et al*., 2016)

6. CONCLUSION

The Wistar rats treated by the aqueous leaf extract of *T*. schimeri at 500, 1000 and 2000mg/kg did not show significance in terms of morbidity or mortality, behavioral changes and gross appearance of the liver and kidney. *T. schimperi* appears to be non-toxic at lower doses (500mg/kg) in Wistar rats. However, because of the small sample size used in the present chronic toxicity study ((5/sex instead of at least 20 per group per sex required for chronic toxicity study), in addition signs of toxicity like mononuclear leucocytes infiltration were observed at higher doses, and moreover data was obtained only from chronic toxicity study of only the liver and kidney . in the present study ,although there were not statistically significant differences between control values for body weight ,for example, the body weight of animals from the treatment groups were constantly smaller compared to control groups.And also absence of reproductive toxicity data were one of the limilations of the present study . because of these limitations, the results of the present study may not be conclusive. However, The results of this chronic toxicity study provide a baseline for future detailed studies .

7. LIMITATION OF THE STUDY

- The small sample size used may affect the accuracy of the results.
- No rigorous chronic toxicity study has been done regarding to toxicity of this plant, so comparison of the results was not possible.
- Data were obtained only from chronic toxicity study
- Data were obtained only from rat model
- Data were obtained only from two organs

8. RECOMMENDATION

- Conducting similar research and investigation on organs other than liver and kidney is recommended.
- Conducting similar chronic toxicity investigation on same organ using increased number of animal in both rodents and non-rodents is recommended.
- Conducting reproductive toxicity study using pregnant animals is recommended
- Conducting acute and sub chronic toxicity study is recommended

9. REFERENCES

Abebe, D., Urga, K., Debella, A., Ambaye, C. and Dejene, A., 2001. Survey of poisonous plants in southern Ethiopia. *Ethiopian Journal of Health Development, 15* (3), pp.209-221.

Adeniyi, F.A.A. and Ogunyemi, E.O., 2007. Toxicity studies on an unripe Carica papaya aqueous extract: biochemical and haematological effects in wistar albino rats. *Journal of Medicinal Plants Research, 1*(1), pp.001-004.

Agbaje, E.O., Adeneye, A.A. and Daramola, A.O., 2009. Biochemical and toxicological studies of aqueous extract of Syzigium aromaticum (L.) Merr. & Perry (Myrtaceae) in rodents. *African Journal of Traditional, Complementary and Alternative Medicines, 6*(3).

Ahmed, S.B.H., Sghaier, R.M., Guesmi, F., Kaabi, B., Mejri, M., Attia, H., Laouini, D. and Smaali, I., 2011. Evaluation of antileishmanial, cytotoxic and antioxidant activities of essential oils extracted from plants issued from the leishmaniasis-endemic region of Sned (Tunisia). *Natural Product Research, 25*(12), pp.1195-1201.

Alex, O.D., Lawrence, O.A., Ephraim, I.C. and Otuchristian, G., 2016. Phytochemical screening and hematological studies of Parquetina nigrescens ethanol and chloroform leaves extracts in normal albino rats. *African Journal of Pharmacy and Pharmacology, 10*(10), pp.164-169.

Allen, F. and Tisher, C.C., 1996. Morphology of the ascending thick limb of Henle. *Kidney international, 9*(1), pp.8-22.

Ameh, S.J., Obodozie, O.O., Abubakar, M.S. and Garba, M., 2010. Current phytotherapy an inter-regional perspective on policy, research and development of herbal medicine. *Journal of Medicinal Plants Research, 4*(15), pp.1508-1516.

Amresh, G., Singh, P.N. and Rao, C.V., 2008. Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. *Journal of ethno pharmacology, 11*6(3), pp.454-460.

Appel, D., Kershaw, D.B., Smeets, B., Yuan, G., Fuss, A., Frye, B., Elger, M., Kriz, W., Floege, J. and Moeller, M.J., 2009. Recruitment of podocytes from glomerular parietal epithelial cells. *Journal of the American Society of Nephrology, 20*(2), pp.333-343.

Arsad, S.S., Esa, N.M. and Hamzah, H., 2014. Histopathologic changes in liver and kidney tissues from male Sprague Dawley rats treated with Rhaphidophora decursiva (Roxb.) schott extract. *Journal of Cytology & Histology*, (S4), p.1.

Asfaw, N., Storesund, H.J., Skattebøl, L., Tønnesen, F. and Aasen, A.J., 2000. Volatile oil constituents of two Thymus species from Ethiopia. *Flavor and Fragrance Journal, 15*(2), pp.123-125.

Ashafa, A.O.T., Sunmonu, T.O. and Afolayan, A.J., 2011. Effects of leaf and berry extracts of Phytolacca dioica L. on hematological and weight parameters of Wistar rats. *African Journal of Pharmacy and Pharmacology, 5*(2), pp.150-154.

Asressu, K.H., 2013. Antimicrobial activity and phytochemical screening of crude extract of medicinal plants grown in Eastern Ethiopia. *Int. J. Pharm. Biol. Sci, 4*(4), pp.326-333.

Assefa, A. and Abebe, T., 2014. Ethnobotanical Study of Wild Medicinal Trees and Shrubs in Benna Tsemay District, Southern Ethiopia. *Journal of Science and Development, 2*(1).

Awe, E.O. and Banjoko, S.O., 2013. Biochemical and hematological assessment of toxic effects of the leaf ethanol extract of Petroselinum crispum (Mill) Nyman ex AW Hill (Parsley) in rats. *BMC complementary and alternative medicine, 13*(1), p.75.

Azaizeh, H., Fulder, S., Khalil, K. and Said, O., 2003. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. *Fitoterapia, 74*(1), pp.98-108.

Bakhtiar, Z., Mirjalili, M.H. and Sonboli, A., 2016. In vitro callus induction and micropropagation of Thymus persicus (Lamiaceae), an endangered medicinal plant. *Crop Breeding and Applied Biotechnology, 16*(1), pp.48-54.

Basch, E., Ulbricht, C., Hammerness, P., Bevins, A. and Sollars, D., 2004. Thyme (Thymus vulgaris L.), thymol. *Journal of Herbal Pharmacotherapy, 4*(1), pp.49-67.

Bekele, A., Abera, R., Mebratu, T., Dessie, W., Getu, A. and Getnet, B., 2015. Antimicrobial activity of Thymus schimperi Ronninger (Lamiaceae) against standard and clinical isolates of human pathogenic bacteria. *Journal of Medicinal Plants Research, 9*(11), pp.379-384.

Bent, S. and Ko, R., 2004. Commonly used herbal medicines in the United States: a review. *American journal of medicine, 116*(7), pp.478-485.

Braet, F. and Wisse, E., 2002. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. *Comparative hepatology, 1*(1), p.1.

Bykov, I., Ylipaasto, P., Eerola, L. and Lindros, K.O., 2004. Functional differences between periportal and perivenous Kupffer cells isolated by digitonin-collagenase perfusion. *Comparative hepatology, 3*(1), p. S34.

Chewaka, D., Abate, D. and Urga, K., 2015. Growth inhibition of grain spoilage fungi by selected herbs and spices essential oils. *Science, Technology and Arts Research Journal, 3*(4), pp.135-140.,

Clapp, W.L., Madsen, K.M., Verlander, J.W. and Tisher, C.C., 1994. Morphologic heterogeneity along the rat inner medullary collecting duct. *Laboratory Investigation; A Journal of Technical Methods and Pathology, 60*(2), pp.219-230.

Cross, D.E., Acamovic, T., Deans, S.G. and McDevitt, R.M., 2002. The effects of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. *British Poultry Science, 43*, pp. 33-35.

Damtie, D. and Mekonnen, Y., 2015. Thymus species in Ethiopia: Distribution, medicinal value, economic benefit, current status and threatening factors. *Ethiopian Journal of Science and Technology, 8*(2), pp.81-92.

Dawit, A., Asfaw, D. and Kelbessa, U., 2003. Medicinal plants and other useful plants of Ethiopia. *Ethiopian Health and Nutrition Research Institute*, p.312

Debelo, N., Afework, M., Debella, A., Makonnen, E. and Ergete, W., 2016. Assessment of Hematological, Biochemical and Histopathological Effects of Acute and Sub-Chronic Administration of the Aqueous Leaves Extract of Thymus schimperi in Rats. *Journal of Clinical Toxicology, 6*(286), pp.2161-0495.

Demir, E., Kilinc, K., Yildirim, Y., Dincer, F. and Eseceli, H., 2008. Comparative effects of mint, sage, thyme and flavomycin in wheat-based broiler diets. *Archiva Zootechnica, 11*(3), pp.54-63.

Demissew, S. and Asfaw, N., 1994. Some useful indigenous labiates from Ethiopia. *Lamiales Newsletter*, (3), pp.5-6.

Dessalegn, E., Bultosa, G., Haki, G.D. and Rupasinghe, H.V., 2015. Antioxidant and-amylase inhibition activities in vitro of various solvent extracts of Thymus schimperi Ronniger. *Journal of Medicinal Plants Research, 9*(15), pp.515-524.

El Nur, E.EB., Ibrahim, A.M., Ahmed A. Gameel, A.A., Awad, A.M.,2016.Effects of Maerua pseudopetalosa (Gilg and Bened.) De Wolf tuber extracts on haematological parameters of Wistar rats. *Scholars Academic Journal of Pharmacy,5*(8), pp. 333-336.

Fabricant, D.S. and Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives, 109*(1), p.69.

Fernandes, J.B.F. and Vargas, V.M.F., 2003. Mutagenic and anti-mutagenic potential of the medicinal plants M. laevigata and C. xanthocarpa. *Phytotherapy Research, 17*(3), pp.269-273.

Firenzuoli, F. and Gori, L., 2007. Herbal medicine today: clinical and research issues. *Evidence-Based Complementary and Alternative Medicine, 4*(1), pp.37-40.

Garcia, J.F., 1997. Changes in blood, plasma and red cell volume in the male rat, as a function of age. *American Journal of Physiology--Legacy Content, 190*(1), pp.19-24.

Gartner, L.P. and Hiatt, J.L., 2006. Color textbook of histology. Elsevier Health Sciences.

Geerts, W.J., Jonker, A., Boon, L., Meijer, A.J., Charles, R., Van Noorden, C.J. and Lamers, W.H., 1997. In situ measurement of glutamate concentrations in the periportal, intermediate, and pericentral zones of rat liver. *Journal of Histochemistry & Cytochemistry, 45*(9), pp.1217-1229.

Guyton, A. and Hall, J., 2006. Textbook medical physiology 11th edition Elsevier Saunders Company.

Hailemariam, G.A. and Emire, S.A., 2013. Antioxidant activity and preservative effect of thyme (Thymus schimperi R.). *British Journal of Applied Science Technology, 3*(4), pp.1311-1326.

Haji, H., Makonnen, E., Debella, A., Geleta, B.,2016. Evaluation of diuretic and antihypertensive activity of leaf extracts of Thymus schimperi in rats. *BJPT, 7*, pp.1-8.

Hayelom, K., Mekbeb, A., Eyasu, M., Wondwossen, E. and Kelbesa, U., 2012. Methanolic effect of Clerodendrum myricoides root extract on blood, liver and kidney tissues of mice. *African health sciences, 12*(4), pp.489-497.

Heath, J.W., 1993. Wheater's functional histology: a text and colour atlas.*New York, NY: Churchill Livingstone.*

Hussien, J., Teshale, C. and Mohammed, J., 2011. Assessment of the antimicrobial effects of some Ethiopian aromatic spice and herb hydrosols. *International. Journal of. Pharmacology,7*, pp.635-640.

Hymavathi, V. and Rao, L.M., 2000. Effect of sub lethal concentration of lead on the haematology and the biochemical constituence of Channa punctata. Bull. *Pure Applied. Science 19*, pp. 1-5.

Jaafari, A., Mouse, H.A., Rakib, E.M., Tilaoui, M., Benbakhta, C., Boulli, A., Abbad, A. and Zyad, A., 2007. Chemical composition and antitumor activity of different wild varieties of Moroccan thyme. *Revista Brasileira de Farmacognosia, 17*(4), pp.477-491.

Jee, J.H., Masroor, F. and Kang, J.C., 2005. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, Sebastes schlegeli (Hilgendorf). *Aquaculture Research, 36*(9), pp.898-905.

Jeong, S.H., Habeebu, S.S. and Klaassen, C.D., 2000. Cadmium decreases gap junctional intercellular communication in rat liver. *Toxicological Sciences, 57*(1), pp.156-166.

Kasa, T.,2016. Uses of medicinal plants in Ethiopia. *International Journal of Advanced Research, 4*, (7), pp.242-248.

Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G. and 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), pp.1-16.

Katsayal, U.A., Nadabo, Y.A. and Isiorho, V.J., 2008. Effects of methanol extract of Azadirachta indica leaves on the histology of liver and kidney of Wister rats. *Nigerian Journal of Pharmaceutical Sciences, 7*(1), pp.9-14.

Kmieć, Z., 2001. Cooperation of Liver Cells in the Synthesis and Degradation of Eicosanoids. *In Cooperation of Liver Cells in Health and Disease* (pp. 51-59).

Kogure, K., Ishizaki, M., Nemoto, M., Kuwano, H. and Makuuchi, M., 1999. A comparative study of the anatomy of rat and human livers. *Journal of Hepato-Biliary-Pancreatic Sciences, 6*(2), pp.171-175.

Kovács, M., Tuboly, T., Mézes, M., Balogh, K., Gerencsér, Z., Matics, Z., Dal Bosco, A., Szendrő, Z., Tornyos, G., Hafner, D. and Milisits, G., 2016. Effects of dietary supplementation of spirulina (Arthrospira platensis) and thyme (thymus vulgaris) on serum biochemistry, immune response and antioxidant status of rabbits. *Annals of Animal Science, 16*(1), pp.181-195.

Loffing, J., Vallon, V., Loffing-Cueni, D., Aregger, F., Richter, K., Pietri, L., Bloch-Faure, M., Hoenderop, J.G., Shull, G.E., Meneton, P. and Kaissling, B., 2004. Altered renal distal tubule structure and renal Na+ and Ca2+ handling in a rat model for Gitelman's syndrome. *Journal of the American Society of Nephrology, 15*(9), pp.2276-2288.

Lorente, L., Aller, M.A., Rodriguez, G., Duran, M.C., Duran, H.J., Alonso, S. and Arias, J., 1995. Surgical anatomy of the liver in Wistar rats. *Surgical Research Communications, 17*(2), pp.113-122.

MacSween, R.N.M., 2002. Developmental anatomy and normal structure. *Pathology of the Liver,48*, p.69.

Maheen, S., Malik, M.N.H., Ahmad, T., Latif, F., Tabassum, N., Khan, A.Q., Ahsan, H., Khan, W., Javed, I. and Ali, H., 2015. Evaluation of anti-inflammatory, analgesic and antipyretic activities of Thymus serphyllum Linn. in mice. *Acta poloniae pharmaceutica, 72*(1), p.113.

Malarkey, D.E., Johnson, K., Ryan, L., Boorman, G. and Maronpot, R.R., 2005. New insights into functional aspects of liver morphology. *Toxicological pathology, 33*(1), pp.27-34.

Malik, R., Selden, C. and Hodgson, H., 2002. The role of non-parenchymal cells in liver growth. *International Seminars in cell & developmental biology 13*, (6), pp. 425-431).

Manal, A.I., El Bushra, E. and Ahmed, A.G., 2016. Changes in body weight and serum biochemical parameters of wistar rats orally dosed with maerua pseudopetalosa (gilg and bened.) de wolf tuber extracts. *European Journal of Research in Medical Sciences*,*4*(1).

Mesfin, K., Tekle, G. and Tesfay, T., 2013. Ethnobotanical study of traditional medicinal plants used by indigenous people of Gemad District, Northern Ethiopia. *Journal of Medicinal Plants Studies, 1*(4).

Modaresi, M., Pour, M.G., Tabeidian, S.A. and Jalalizand, A., 2011. Study of Histopathologic changes of the effect of Zingiber extract on mice kidneys. *International Conference on Food Engineering and Biotechnology, 9*, pp. 16-20).

Mohammed, S., Danjuma, M.N. and Abdulkarim, I.A.,2015. Ethnobotanical Survey of Medicinal Plants in Metropolitan Kano, Nigeria. *International Journal of Public Health Research, 3*(6), 345-351.

Nabavi, S.M., Marchese, A., Izadi, M., Curti, V., Daglia, M. and Nabavi, S.F., 2015. Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. *Food chemistry, 173*, pp.339-347.

Nasir, M., Tafess, K. and Abate, D., 2015. Antimicrobial potential of the Ethiopian Thymus schimperi essential oil in comparison with others against certain fungal and bacterial species. *BMC complementary and alternative medicine, 15*(1), p.260.

Nortier, J.L., Martinez, M.C.M., Schmeiser, H.H., Arlt, V.M., Bieler, C.A., Petein, M., Depierreux, M.F., De Pauw, L., Abramowicz, D., Vereerstraeten, P. and Vanherweghem, J.L., 2000. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi*). New England Journal of Medicine, 342*(23), pp.1686-1692.

Nzeako, B.C., Al-Kharousi, Z.S. and Al-Mahrooqui, Z., 2006. Antimicrobial activities of clove and thyme extracts. Sultan Qaboos Univ *Medical Journal, 6*, pp.33-39.

OECD, 2008. Chronic toxicity studies.in OECD guidelines for the testing of chemicals.Test Guideline 452: Organization for Economic Cooperation and Development, Paris.

Ojiako, O.A., Chikezie, P.C. and Ogbuji, A.C., 2014. Blood indices/histopathological status of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations. *International Journal of Green Pharmacy*, p.184.

Onyeanusi, B.I., Adeniyi, A.A., Ayo, J.O., Ibe, C.S. and Onyeanusi, C.G., 2009. A comparative study of the urinary system of the African giant rat (Cricetomys gambianus Waterhouse) and the Wistar rat. *Pakistan Journal of Nutrition, 8*(7), pp.1043-1047.

Owoyele, B.V., Oyelowo, O.T., Biliaminu, S.A., Alaran, O.N., Alimi, S.A. and Saliu, R.S., 2011. Hematological and biochemical studies on Parquetina nigrescens root extract in albino rats. *Journal of Applied Pharmaceutical Science, 1*(10), p.176.

Parvez, N. and Yadav, S., 2010. Ethno pharmacology of single herbal preparations of medicinal plants in Asendabo district, Jimma, Ethiopia. *Indian Journal of Traditional Knoulege,9*(4), pp.724-729.

Patwardhan, B. and Partwardhan, A., 2005. Traditional Medicine: Modern Approach for affordable global health.

Porth, C., 2011. Essentials of pathophysiology. *Concepts of altered health states*. Lippincott Williams & Wilkins

Princewill-Ogbonna, I.L., Abagha, O.J. and Ijioma, S.N., 2015. Haematological and Histopathological parameters of rats fed with Aerial yam (Dioscorea bulbifera). *Comprehensive Journal of Food Science and Technology Research, 2*(2), pp.09-16.

Rasool Hassan, B.A., 2012. Medicinal plants (importance and uses). *Pharmaceut Anal Acta, 3*, p.139.

Reddy, P., Kandisa, R.V., Varsha, P.V. and Satyam, S., 2014. Review on Thymus vulgaris traditional uses and pharmacological properties. *Medicinal & Aromatic Plants, 3*, pp.164-166.

Reilly, R.F. and Ellison, D.H., 2000. Mammalian distal tubule: physiology, pathophysiology, and molecular anatomy. *Physiological reviews, 80*(1), pp.277-313.

Reilly, R.F., Bulger, R.E. and Kriz, W., 2007. Structural-functional relationships in the kidney. *Diseases of the Kidney and Urinary Tract, 1*, pp.2-53.

Ross, H. and Pawlina, W., 2006. Histology: A Text and Atlas: with Cell Correlation and Molecular Biology.

Rousseaux, C.G. and Schachter, H., 2003. Regulatory issues concerning the safety, efficacy and quality of herbal remedies. *Birth Defects Research Part B: Developmental and Reproductive Toxicology, 68*(6), pp.505-510.

Rowe, A., Zhang, L., Hussain, A., Braet, F. and Ramzan, I., 2011. Assessment and histological analysis of the IPRL technique for sequential in situ liver biopsy. *Comparative Hepatology, 10*(1), p.7.

Saadat Shad, H., Mazhari, M., Esmaeilipour, O. and Khosravinia, H., 2016. Effects of Thymol and Carvacrol on Productive Performance, Antioxidant Enzyme Activity and Certain Blood Metabolites in Heat Stressed Broilers. *Iranian Journal of Applied Animal Science, 6*(1), pp.195-202.

Sani, D., Sanni, S. and Ngulde, S.I., 2009. Phytochemical and antimicrobial screening of the stem aqueous extract of Anisopus mannii. *Journal of Medicinal Plants Research, 3*(3), pp.112-115.

Savithri, Y., Sekhar, P.R. and Doss, P.J., 2010. Changes in hematological profiles of albino rats under chlorpyrifos toxicity. *International Journal of Pharma and Bio Sciences, 1*(3), pp. 975-1195

Schrier, R.W. ed., 2007. Diseases of the kidney and urinary tract (Vol. 1). Lippincott Williams & Wilkins

Sharad, S., Patel, M., Bhuch, M., Chatterjee, M. and Shrivastava, S., 2011. Regulatory status of traditional medicines in Africa Region. *International Journal of Research in Ayurveda and Pharmacy, 2*(1), pp.103-110.

Singh R. ,2015. Medicinal Plants. *Journal of Plant Sciences,3*, pp.50-55

Stickel, F., Patsenker, E. and Schuppan, D., 2005. Herbal hepatotoxicity. *Journal of Hepatology, 43*(5), pp.901-910.

Sunmonu, T.O. and Oloyede, O.B., 2010. Performance and haematological indices in rats exposed to monocrotophos contamination. *Human & Experimental Toxicology, 29*(10), pp.845-850.

Sunmonu, T.O., Oloyede, O.B., Owolarafe, T.A., Yakubu, M.T. and Dosumu, O.O., 2014. Toxicopathological evaluation of Picralima nitida seed aqueous extract in Wistar rats. *Turkish Journal of Biochemistry-Turk Biyokimya Dergisi, 39*(2), pp.119-125.

Tesfamaryam, G., Tsegaye, B., Eguale, T. and Wubete, A.,2015.In vitro Screening of Antibacterial Activities of Selected Ethiopian Medicinal Plants.*International Journal of Microbiological Research, 6* (1), pp.27-33.

Usman, M.M., Sule, M.S. and Gwarzo, M.Y., 2014. Toxicological studies of aqueous root extract of Euphorbia lateriflora (Schum and Thonn) in rats. *Journal of Medicinal Plants, 2*(2).

Vdoviaková, K., Petrovová, E., Maloveská, M., Krešáková, L., Teleky, J., Elias, M.Z.J. and Petrášová, D., 2015. Surgical anatomy of the gastrointestinal tract and its vasculature in the laboratory rat. *Gastroenterology research and practice, 2016*.

Verlander, J.W., 1998. Normal ultrastructure of the kidney and lower urinary tract. *Toxicologic pathology, 26*(1), pp.1-17.

Wojcikowski, K., Johnson, D.W. and Gobe, G., 2004. Medicinal herbal extracts–renal friend or foe*? Part one: The toxicities of medicinal herbs. Nephrology, 9*(5), pp.313-318.

World Health Organization, 2000. General guidelines for methodologies on research and evaluation of traditional medicine: WHO/EDM/TRM/2000.

World Health Organization, 2001. Legal status of traditional medicine and complementary. WHO/EDM/TRM/2001.

World Health Organization, 2005. *WHO global atlas of traditional, complementary and alternative medicine* (Vol. 2). World Health Organization.

WHO (2011). Progress report on decade of traditional medicine in the African region. Brazzaville, WHO Regional Office for Africa; AFR/RC61/PR/2.

Yakubu, M.T., Bilbis, L.S., Lawal, M. and Akanji, M.A., 2003. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biokemistri,* 15(2), pp.50-56.

Yirga, G., 2010. Assessment of indigenous knowledge of medicinal plants in Central Zone of Tigray, Northern Ethiopia. *African Journal of Plant Science, 4*(1), pp.006-011.

Yirga, G., 2010. Assessment of traditional medicinal plants in Endrta District, South-eastern Tigray, Northern Ethiopia. *African Journal of Plant Science, 4*(7), pp.255-260.

Zanchet, D.J. and Montero, E.F.D.S., 2002. Pig liver sectorization and segmentation and virtual reality depiction. *Acta Cirurgica Brasileira, 17*(6), pp.381-387

10. APPENDICES

10.1. Appendix I: preparation of working solution for tissue fixation

10% Neutral Buffered Formalin

- 40% formaldehyde 100 ml……100ml
- Distilled water 900 ml…….900ml
- Sodium dihydrogen phosphate monohydrate …….....4g
- Disodium hydrogen phosphate anhydrous…….....6.5g

Harris's Hematoxylin (H)

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

1% Alcoholic Eosin (E)

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

1% Acidic alcohol

- ✓ 70% alcohol…….500ml
- ✓ Hydrochloric acid, concentrated……5ml

Bluing solution

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

10.2. Appendix II: Tissue processing procedures

Fixation

- 10% Neutral Buffered Formalin …….....24 hrs.

Washing

- Running tap water…….....several times

Dehydration

- 70% Ethanol…… .....2 hrs.
- 90% Ethanol…….2 hrs.
- Absolute alcohol I …….....11/2hrs
- Absolute alcohol II……...……11/2hrs
- Absolute alcohol III……...11/2hrs
- Absolute alcohol IV…… ……...overnight

Clearing

- Xylene I……11/2hrs
- Xylene II ……...21/2hrs

Infiltration

- Paraffin wax I…….21/2hrs
- wax II……. overnight

10.3. Appendix III: Hematoxylin and Eosin (H&E) staining protocol

Deparaffinization

- Xylene I…….5min
- Xylene II……5min

Rehydration

- Absolute alcohol I…….3 min
- Absolute alcohol II……3 min
- 95% Ethanol…….....3 min
- 70% Ethanol…….3 min

Rinse in distilled water……5 min

Stain in Hematoxylin…….....15min

Rinse in running tap water…….....5min

Decolorize in acid alcohol……1-3 sec.

Rinse in running tap water…….....5min

Immerse in Sodium bicarbonate solution…….....1min

Rinse in running tap water…….....5min

Counter stain in Eosin……1min

Dehydration

- 70% Ethanol…….....3min
- 95% Ethanol …….....3min
- Absolute alcohol II……...3min
- Absolute alcohol I…….3min

Clearing

- Xylene II……5 min
- Xylene I…….....5 min