ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMY

Evaluation of the acute and sub-chronic toxic effects of aqueous leaves extracts of *Artemisia afra* on Liver, Kidney and some Blood parameters in Wistar Rats at Addis Ababa University on year 2014/2015.

A thesis submitted to the Department of Anatomy, School of medicine, College of health science, Addis Ababa University, Addis Ababa, Ethiopia in partial fulfillment of the requirement for the Degree of Master of science (MSc) in anatomy.

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List of Abbreviations

A……Artemisia
ALP……Alkaline Phosphatase
ALT……Alanine Amino transferase
ANOV……Analysis of Variance
AST ……Aspartate Amino transferase
BV ……Blood volume
BW……Body weight
^{0}C: ……Degree Celsius
cm……Centimeter
CAM……Complementary and alternative medicine
DPX. ……Dibutyl phthalate in xylene
EDTA ……Ethylene Diamine Tetra-acetic Acid
HCT……Hematocrit
Mg……Milligram
Kg……Kilogram
g ……Gram
HMs……Herbal medicine
H&E ……Heamatoxylin and Eosin
HGB ……Hemoglobin concentration
LD_{50}……Lethal dose that kills 50 percent of animals
M……Mean
MCH……Mean Corpuscular Hemoglobin
MCHC……Mean Corpuscular Hemoglobin Concentration
MCV……Mean Corpuscular Volume
NK……Natural killer
PCV……Packed Cell Volume
PLT……Platelet count
RBC……Red Blood Cell
Rpm……Revolution per minute
$SE(\bar{x})$ ……Standard Error of the Mean
SPSS……Statistical Package for Social Sciences
TM ……Traditional medicine
US$……Dollar of united state of America
WBC ……White Blood Cell

List of Acronyms

AAU……Addis Ababa University

EPHI ……Ethiopian public Health institution

FAO……Food and agricultural organization

OECD……Organization of Economic Co-operation and Development

WHO ……World Health Organization

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Abstract

Traditional medicine has remained to be the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities and, it is the only means of treatment for such communities.

Plants have been the basis for treatment throughout human history, and they are still widely practiced today as part of traditional medicine. The plant Artemisia afra has been shown to display a wide spectrum of biological and pharmacological activities, which provide experimental support for the empiric ethno-pharmacological use of this plant in traditional medicine. However, despite its widespread use, very little is known about its safety and efficacy.

This Experimental laboratory based study has been carried out to investigate the acute and subchronic toxic effects of leaves of Artemisia afra on liver and kidney; and some blood parameters in rats. The study was carried out in Department of Anatomy, School of Medicine, Addis Ababa University. The experiments were performed on 59 Wister rats (32 for acute and 27 for subchronic study) based on the OECD guideline they were assigned to each group randomly. The study was conducted from January 2014-July 2015. Various doses of aqueous extract of the leaves were employed for single dose toxicity studies, while the effective dose and triple the effective dose were used for repeated toxicity studies.

This study showed that the oral lethal dose (LD_{50s}) is higher than 5000mg/kg. Generally in the acute toxicity study; the general behavior and body weights were not altered in Wister rats administered with doses up to 5000mg/kg. After subchronic study with both doses (600 and 1800mg/kg), there were no significant changes in the overall body weight, the evaluated hematological and most of the biochemical parameters. No death was recorded. In gross observation, the kidneys and liver of treatment groups appear normal in their texture, size or color as compared to the control. Histopathological presentations were generally normal though there were mild mononuclear leukocytic infiltrations around the central venules & portal areas of Wister rats' liver at both 600 and 1800mg/kg dose in addition minor tubulointerstitial leukocytic infiltrations were observed in small areas of kidney sections administered higher dose. Findings of this study revealed that A. afra is relatively safe.

Keywords: *A. afra, Traditional medicine, Toxicological assessment*

1. Introduction

1.1. Background of the study

1.2. Traditional medicine

Man has been able to appreciate through his superior observing and learning capabilities to use and exploit the natural resources, the flora and fauna for his survival and comfort, to alleviate pain and to cure diseases; to constantly improve upon his health and build longevity (WHO, 2008).

Traditional medicine is at the crossroads of two different clusters of competences: values and responsibilities (WHO, 2008). According to the figures provided by WHO in the report on the world medicines' situation in 2011, between 70% and 95% of citizens in many developing countries use traditional medicine as a primary source of health care

In the absence of an efficient primary healthcare system, traditional medicine occupies a central place in the provision of health care, especially among rural communities of developing countries (Tabuti *et al*., 2003). However, traditional Medicine has been given very little attention in modern medical research and development, and less effort has been made to upgrade its role and side effects in many countries including Ethiopia (Abebe, 1996).

Herbal medicines are one component of complementary and alternative medicine, which includes acupuncture, chiropractic manipulation, meditation, homeopathy, and other approaches. Since the introduction of orthodox medicines in Africa, the use of herbal medicine in treating various ailments has existed alongside western medicines (Liang *et al*., 2009). Herbal medicines are playing major roles in the health of thousands of people worldwide and their preparations have been widely used for thousands of years in many oriental countries, such as China, Korea, Japan, etc, and it is currently attracting more and more attention from all over the world (Liang *et al*., 2004). In China, traditional herbal medicine played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS) (WHO, 2003). More than seventy per cent of African populations use some form of traditional herbal medicine, and the worldwide annual market for these products in 2004 was estimated to be US$ 60 billion (Willcox and

Bodeker, 2004). Plants are also origins for the development of several modern drugs. Examples include; digoxin from *Digitalis purpurea*, quinine and quinidine from *Cinchona officinalis*, vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* (Kroll and Winslow, 1998; Rates, 2001).

The use of plants as medicines is as old as human civilization itself (Ahmedulla and Nayer, 1999). The strong historical bond between plants and human health is well substantiated by plant species diversity and related knowledge of their use as herbal medicines (Tabuti *et al*., 2003). In addition, it is attributable to the accessibility and affordability of herbal medicines (Steenkamp, 2003).

The growing dissatisfaction with modern medicine coupled with the misconception that herbal products being natural and may be devoid of adverse and toxic effects have increased their use. (Alli *et al*., 2010). Furthermore, uncontrollable quality of herbal medicine is the obstacle for internationalization and modernization (Liang *et al*., 2009). Traditional herbal medicine research will therefore play a critical role in global health. Indeed, China, India, Nigeria, the United States of America (USA) and WHO have all made substantial research investments on traditional herbal medicines (WHO, 2002).

More than 35,000 plant species are reported as being used across the globe for medicinal purposes (Lewington, 1993). In Africa, more than 2,000 plants have been identified and used as herbal medicines to treat several ailments, but very few of these plants have been screened for their safety (Fennell *et al*., 2004). The current account of medicinal plants of Ethiopia shows about 887 plant species are utilized as traditional medicine in Ethiopia. Among these, about 26 species are endemic (Miruts *et al*., 2003). Such medicinal plants of Ethiopia play major supplementary roles to the limited modern health care available (Desta, 1988; Miruts, 2007). It is reported that a significant proportion of the Ethiopian population still depends on traditional medicine for its health care services (Asres *et al*., 2009).

Most of the traditional medicinal plant preparations are used in fresh form. It is reported that leaves are the most widely used plant part, accounting for 48% of the reported medicinal plant uses, followed by roots 33%, flowers 9%, fruits and seeds 9%, above

ground parts 9%, and the whole plant 9% (Mirutse, 2001). Oral, dermal and nasal are the most common routes of application of remedies. Squeezing, grinding, boiling, chewing, crushing and tying are the methods of remedy application (Gidey, 2011).

In Western and African folk medicine, several species of the Genus, Artemisia are used for their claimed healing properties and the curing of specific ailments. Among those species *Artemisia afra* is one of the widely used herbal medicinal plants (Pappas and Sheppard-Hanger, 2004).

1.3. *Artemisia Afra*

Artemisia afra is known by many names like "African wormwood" in English "Umhlonyane" in Xhosa, "Mhlonyane" in Zulu, "Lanyana" in Sotho, "Lengana" in Tswana, "Wilde als" in Africaans, "Koddoo-adi" & "Chugughee" in Ethiopia (Asres *et al*., 2001). *Artemisia afra* belongs to Domain: Eukaryota, Kingdom: Plantae, Subkingdom: Viridaeplantae, Phylum: Tracheophyta, Subphylum: Euphyllphytina, Infraphylum: Radiatopses, Class: Magnoiopsida, Subclass: Asteridae, Superorder: Asteranae, Order: Asterales, Family: Asteraceae, Subfamily: Asteroideae, Tribe: Anthemideae, Genus: Artemisia, Species: afra-Jacq., Botanical name: *Artemisia afra* (Bremer, 1994). The Asteraceae is one of the most important families of plants in the world. More than 23000 species from about 1300 genera have been identified (Bremer, 1994).The genus Artemisia contains more than 400 species (Mucciarelli and Maffei, 2002)

Artemisia afra is a herb growing in the high land areas of Eastern and Southern Africa with altitudes ranging between 1500 and 3000m where the soils range from volcanic ash, loamy sands, to sandy or calcareous clay loams of volcanic or granitic origin (Van der Walt, 2004). The plant grows in the South and Eastern regions of the continent and has been located in Kenya, Tanzania, Zaire, Zambia, Zimbabwe, Angola, Republic of South Africa and Ethiopia (Iwu, 1993). In Ethiopia, it usually grows in rocky mountainous areas along forest margins and stream sides and its natural distribution extends from Bale mountains National Park (Dinsho) southeastern Ethiopia to northern parts of Ethiopia (Abebe, 1986). It is also predominantly found in Asia, Europe and North America (Mucciarelli and Maffei, 2002).

Artemisia afra is a medium sized multi-stemmed, clump-forming woody perennial shrub, which grows up to 2 meters in height with a leafy, hairy ridged stem (Van der Walt, 2004). As shown in (Fig 1) its soft leaves are finely-divided (like a fern), are silver-grey due to the presence of fine hairs reaching in length up to 80 mm and width up to 40 mm arranged alternately, oval in shape, flowers during (Jun – September) inconspicuous, yellow, borne at the ends of branches in globose capitula±3mm in diameter (Van Wyk *et al*., 2000). The adaxial surface of the leaf is darker compared to its abaxial surface (Gericke *et al*., 1997). The plant has an easily identifiable aromatic odor and smells pungent and sweet after bruising (Liu *et al*., 2009).

Figure 1: Photographs showing leaves of *Artemisia afra* taken from Bale mountain national park, during July 2014.Young leaves (A), growing leaves (B), matured leaves (C)

1.3.1. Ethno medical uses of *Artemisia afra*

Artemisia afra is widely used in many parts of the world either alone or in combination with other plants as herbal remedies for a variety of ailments like simple headache to neurological disorder epilepsy (Mander, 1998). In South Africa, the study of the indigenous knowledge indicates the widespread common use of *A. afra* for numerous ailments:- mainly as a remedy for chest conditions, coughs, colds, heartburns, hemorrhoids, fevers, malaria, asthma, and other conditions (Roberts, 1990; Iwu, 1993; Cunningham *et al*., 1996; Dyson, 1998). In addition, leaves are commonly smoked by some tribes to help release phlegm, ease and soothe a sore throat and coughing at night (Roberts, 1990). In Ethiopia, healers use the plant for Epilepsy (Dhibe Qabana), Evil eye (Buda) and febrile illness (Michi) (Yineger and Yewhalaw, 2007). In recent years, it has gained significant attention from the scientific community. Studies have been conducted either to verify or substantiate the traditional use of this herb. Further, its use is also being investigated in the modern

diseases like diabetes, cardiovascular diseases, cancer and respiratory diseases (Roberts, 1990). Results of investigations conducted on the aqueous extract of *Artemisia afra* have indicated that the plant has bronchodilator activity (Harris, 2002), as well as anti-histaminic and analgesic properties (Cunningham *et al*., 1996). Other studies carried out on the ethanolic extract and dichloromethane extract have shown the plant to have *in vitro* hypotensive and antituberculosis effects, respectively (MRC and Healthinfo, 2004). The plant *A. afra* used also in traditional medicine have in fact been an important source of drugs for the treatment of malaria (Moges *et al.*, 1998).

The merit of the empirical use of *Artemisia Afra* has also been corroborated by the isolation and identification of several active compounds, including flavonoids (apigenin, hesperetin, kaempferol, luteolin and quercetin) and volatile oil, which contain mainly 1, 8- cineole, α-thujone, ß-thujone, camphor and borneol (Gericke *et al*., 2000; Waithaka, 2004). Also present in the plant are terpenoids as well as coumarins and acetylenes, although their contribution to the biological activity of *Artemisia afra* is not yet known (Benavente-Garcia *et al*., 1997).

It is well known that flavonoids have many potential activities, e.g. anti-oxidant, and anti-allergic activities (Harborne and Williams, 2000). Additionally, they have chemo-preventive activity against skin cancer (e.g. apigenin); inhibitory effects on chemically induced mammary gland, urinary bladder and colon carcinogenesis in laboratory animals (e.g. hesperetin); and anti-carcinogenic and platelets anti-aggregator effects (e.g. quercetin) (Erlund, 2002; Waithaka, 2004). Furthermore, the flavonoid luteolin has been shown to exhibit anti-mutagenic and anti-tumorigenic activities (Furugori *et al*., 1998) as well as vasodilatory and potent anti-platelet activities (Harborne and Williams, 2000). It is also a promising agent for use in ophthalmology for the prevention and treatment of cataract and vascular eye disorders (China Great vista chemicals, 2002). However, very little is known about the toxicity of this plant (Gericke *et al*., 2000), which is an issue this study is aimed to investigate.

1.4. Toxicological studies

Toxicity is defined as "the potential of a substance to exert a harmful effect on humans or animals, and a description of the effect and the conditions or concentration under which the effect takes place" (Health and Safety, 2004).

There are few clinical data on safety and efficacy of herbal medicine, and there is also no consensus even among traditional healers as far as which plant preparations and dosages are most effective. Parallel with recent increasing interest in herbal medicine for the prevention and treatment of various human illnesses and animal diseases, there is increasing concern about the safety of medicinal plants. There are general and herb-specific concerns regarding medicinal plants and their ability to produce toxicity and adverse effects (Saad *et al*., 2006).

In general, toxicity testing methods can be divided into two categories: The first category comprises tests that are designed to evaluate the overall effects of compounds on experimental animals. Individual tests in this category differ from each other basically with regard to the duration of the test and the extent to which the animals are evaluated for general toxicity. These tests are classified as acute, prolonged and chronic toxicity tests (Loomis and Hayes, 1996). The second category of tests consists of those that are designed to evaluate specific types of toxicity in detail. The prolonged and chronic tests do not detect all forms of toxicity, but they may reveal some of the specific toxicities and indicate the need for more detailed studies. Thus, this second category of tests has been developed for the determination of effects of compounds on the fetus in a pregnant animal (teratogenic tests), on the reproductive capacity of the animals (reproduction tests), on the genetic system (mutagenic tests) and for the determination of the ability of agents to produce tumors (tumorigenicity and carcinogenicity tests) (Loomis and Hayes, 1996; Timbrell, 2002).

In Ethiopia the potential toxicity of the various traditionally used medicinal plants has not been recognized by the general public or by professional groups. In some cases, adulteration, inappropriate formulation, or lack of understanding of plant components and drug interactions or uses has led to adverse reactions that are sometimes life threatening or lethal (Saad *et al*., 2006). Therefore, scientists advocate for proper toxicological studies in

order to ensure safety in the use of herbal medicines (Akhigbe *et al*., 2006; Oyewole *et al*., 2007). In line with this, the primary concern of this research was to investigate if toxicity exists after acute and subchronic administration of aqueous leaf extract of *Artemisia afra* in Wister rats.

Acute and subchronic toxic effects differ principally from each other with respect to the amount of chemical compound involved and the time intervening before the effect is seen (Timbrell, 2002). Acute effects are normally observed soon after exposure and results from the uptake of large amounts of drugs, generally as a single dose. On the other hand, subchronic effects are often detected over an extended period of time during which exposure may be continuous or intermittent, at levels which are too low to produce an acute effect (Pascoe, 1983; Loomis and Hayes, 1996).

1.5. Liver: Structure and functions

Since the rat plays an important role in experimental liver studies, knowledge concerning the organization of its liver would be invaluable. The rat liver is multilobulated as in other mammals. In rats, the liver mass represents approximately 5% of the total body weight, while in adult humans it represents 2.5%. In rats weighing between 250 and 300 g, the liver mean weight was 13.6 g and the liver transverse diameter measured from 7.5 to 8.0 cm. The superior–inferior diameter measured from 3.8 to 4.2 cm, while the anterior–posterior diameter ranged from 2.2 to 2.5 cm. (Monteiro and Zanchet, 2002)

The rat liver, when the rat is in the decubitus position, has basically three surfaces: superior, inferior and posterior. A sharp, well-defined margin divides the inferior from the superior surface. Different from the human liver, the other margins are also sharp. Although the rat liver is lobulated, it has rather uniform surfaces as lobes lie flat against each other. The only exception to this is the posterior caudate lobe (CL), which is separated from the remainder of the liver by the stomach (Aller *et al*., 1999).

Similar to the human liver, the rat liver is connected to the undersurface of the diaphragm and to the anterior wall of the abdomen by five ligaments: the falciform, the coronary, and the two laterals are peritoneal folds; the fifth, the round ligament, is a fibrous cord, the obliterated umbilical vein. The liver is also attached to the lesser curvature of the stomach

by the hepatogastric ligament and to the duodenum by the hepatoduodenal ligament (Elias and Gershbein, 1994).

The rat liver lobes as shown in Fig. 2, 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 (Aller *et al.*, 1995). The middle or median lobe (ML) is the largest, accounting for approximately 38% 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) (2/3 of the volume of the medial lobe) and a smaller left medial lobe (LML; 1/3 of the volume). The RML has both left and right hepatic vascular components. The right lobe (RL) is located on the right of the vena cava and posteriorly in the right hypochondrium and is almost completely covered by the medial lobe. It comprises about 22% of the liver weight and is divided by a horizontal fissure into two pyramidal-shaped lobules: the superior (SRL, also called the right posterior lobe) and inferior (inferior right lobe, IRL, also called the right anterior lobe) lobules. The left lateral lobe (LLL) has a rhomboid shape, is flattened and situated in the epigastric and left hypochondriac regions over the anterior aspect of the stomach. Its medial portion is covered by the left part of the medial lobe. Its upper surface is slightly convex and is molded on the diaphragm. It has no fissure. The caudate lobe (CL) is situated behind the LLL and on the left of the vena porta and inferior cava vein. It comprises 8–10% of the liver weight (Kongure *et al.*, 1999).

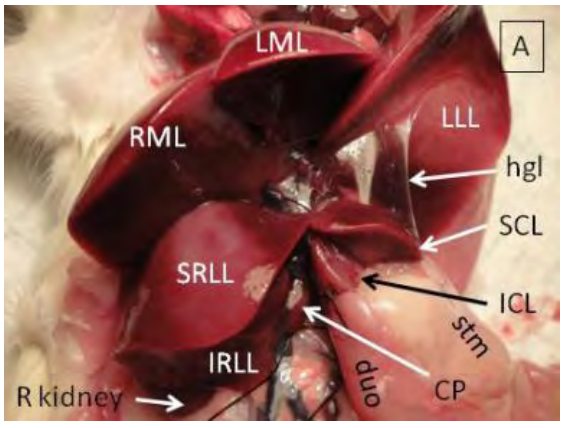


Figure 2**: A picture showing gross anatomical lobes of rat liver insitu, taken from (Aller *et al.*, 1995).**

Despite the gross differences between the liver of human and rat, their microscopic features are more or less similar (Fig. 3). The bulk of the liver is composed of cords of parenchymal cells, the hepatocytes (Gartner and Hiatt, 2000). These epithelial cells are grouped in interconnected plates. The hepatocytes are polyhedral with six or more surfaces having one or two rounded nuclei with one or two nucleoli (Junqueira and Carneiro, 2005). The space between these plates contains dilated capillaries, the liver sinusoids. Resident macrophages, known as Kupffer cells, are associated with the sinusoidal lining cells (Gartner and Hiatt, 2000). In light microscope sections, structural units called liver lobules can be seen. The liver lobule is formed of a polygonal mass of tissue with portal spaces at the periphery and a vein called the central vein in the centre (Junqueira and Carneiro, 2005). The portal areas house branches of the hepatic artery, tributaries of the relatively large portal vein, interlobular bile ducts and lymph vessels (Cook, 2008).

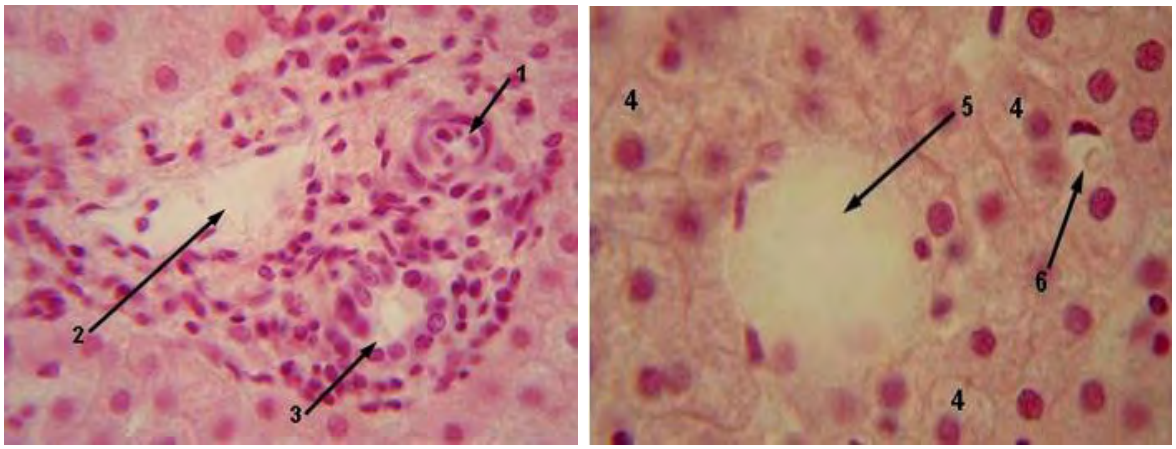


Figure 3: A photograph showing histology of rat liver. 1- hepatic artery, 2 - portal vien, 3 - bile duct, 4 – hepatocytes, 5 - terminal hepatic (centrilobular) venule, 6 - hepatic sinusoid taken from (Piper *et al.,* 2012).

The main function of the liver of rat is more or less similar to that of human and it is used to take up nutrients, store them, and to provide them to the other organs. It also has to take up potentially damaging substances like bacterial products or drugs delivered by the portal blood or microorganisms, which reach the circulation (Ramadori *et al*., 2008). Hepatocytes perform a wide array of metabolic, secretory, and endocrine functions (Tortora and Derrickson, 2009). These cells are involved in protein synthesis, protein storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids.

They are also concerned with detoxification, modification and elimination of exogenous and endogenous substances. The hepatocytes initiate the formation and secretion of bile (Ramadori *et al*., 2008)

The active chemical medium of the liver is well known for its ability to detoxify alcohol and eliminates many drugs (Tortora and Derrickson, 2009). The liver is prone to foreign chemical- induced injuries because of its central role in foreign chemical metabolism, its portal location within the circulation, and its anatomic and physiologic structure (Bello *et al*., 2010). Phytotherapeutics (herbal remedies) are drugs that may cause hepatic damage (Baracho *et al*., 2009).

Some of the patterns of hepatic injury that might be observed in the liver parenchyma are necrosis (usually necrosis of hepatocytes immediately around the terminal hepatic vein), inflammation, fibrosis (formed in response to direct toxic insult to the liver), neoplastic processes and degeneration of the liver tissue (Robbins and Cotran, 2005).

1.6. The Kidneys: Structure and functions

Kidneys are paired retroperitoneal organs situated in the posterior part of the abdomen on each side of the vertebral column (Čukuranović and Vlajković, 2005). In the human, the upper pole of each kidney lies opposite the twelfth thoracic vertebra and the lower pole lies opposite the third lumbar vertebra. The right kidney is usually slightly more caudal in position. The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness. Located on the medial or concave surface of each kidney is a slit, called the hilus, through which the renal pelvis, the renal artery and vein, the lymphatics, and a nerve plexus pass into the sinus of the kidney. The organ is surrounded by a tough fibrous capsule, which is smooth and easily removable under normal conditions (Moore and Dalley, 2006).

The kidneys in rats (Fig. 4) are also paired bean-shaped organs lying retroperitoneally against the dorsal body wall on either side of the vertebral column. The right kidney of the rat is larger, heavier, and located more anteriorly and cranial than that of the left kidney

(Cook, 2008). Male rats have relatively larger kidneys than do females; kidney weight varies between in bred strains (Popesko *et al*., 1990).

The rat kidneys are reddish-brown in color and covered by a thin connective tissue capsule that is adherent to subcapsular connective tissue (El-Beltagy, 2002). The kidney of wister rat also consists of two regions, the outer cortex and the inner medulla same as humans. The rats' kidney is unilobar (unipyramidal) (Fig. 5) with only a single papilla that extends deep into the renal pelvis. The rats' cortex has cone-shaped cortical labyrinths and medullary rays that extend from the outer medulla, which is divided into an outer and inner stripe. The inner portion of the medulla is the renal papilla, which extends into the renal pelvis and ureter (Charmi *et al*., 2009).

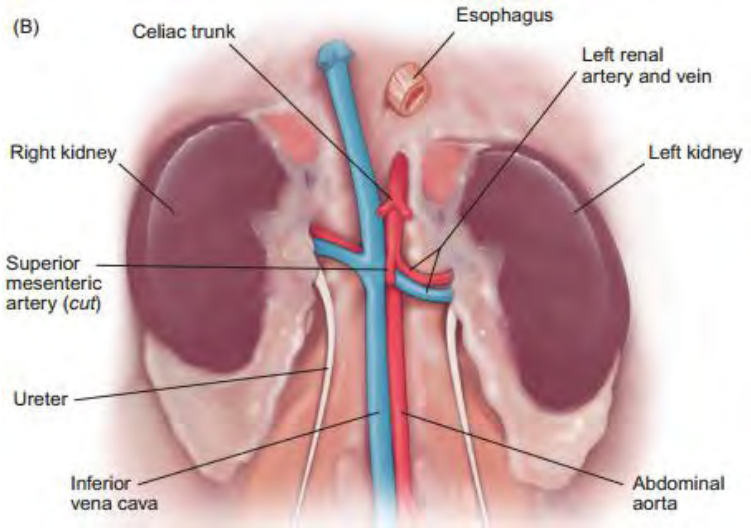


Figure 4: A picture showing gross anatomy of rat kidney, taken from (Piper *et al.,* 2012).

The basic unit of the rat kidney is the nephron. Each nephron can be sub divided into a number of distinct parts in the cortex and medulla. The nephron is composed of renal corpuscle and the first part of nephron which consists glomerulus and Bowman's capsule, the proximal convoluted tubules, loop of Henle which is arranged into ascending and descending limbs and then the distal convoluted tubule (El-Beltagy, 2002). The cortex consists of renal corpuscles, proximal convoluted tubules and distal convoluted tubules of the nephron (Begg and Barclay, 1995).

The renal corpuscle is a rounded or irregular structure which forms the glomerulus that is enveloped by Bowman's capsule. The bowman capsule is formed of two thin cellular layers, the outer parietal layer and inner visceral layer. The parietal layer consists of a flat single layer of squamous epithelium enclosing a narrow space, the urinary space which continuous with the lumen of proximal convoluted tubule. The visceral layer is surrounding the glomerular capillaries (El-Salkh *et al*., 2008).

The proximal convoluted tubule exibit a small uneven lumen and a single layer of cuboidal cells with eosinophilic granular cytoplasm. A brush border lines the cells (Gude *et al.,* 1982). The distal convoluted tubules of the cortex differ from the proximal tubules in that they are lined by cuboidal epithelium with rounded and large nuclei and possess no brush border. The distal convoluted tubules tend to be rather shorter than the proximal convoluted tubules and are therefore fewer in number in the sections of regional cortex. The initial, straight part of the distal tubule makes contact with the vascular pole of the renal corpuscle of its parent nephron and forms part of a specialized structure, the juxtaglomerular apparatus (JGA). Cells of this structure establish a feedback mechanism that allows autoregulation of renal blood flow and keeps the rate of glomerular filtration relatively constant. At the point of contact with the arterioles, the cells of the distal tubule become columnar and more closely packed, with apical nuclei, basal Golgi complexes, and a more elaborate and varied system of ion channels and transporters. This thickened spot of the distal tubule wall is called the macula densa (Basuony, 1997).

The medulla of each kidney is formed from collecting tubules, thick and thin parts of the loops of Henle. The thin limb has a distinct rounded lumen. It could be clearly distinguished from the other parts of the nephron on the bases of its low lining epithelial squamous cells and the cytoplasm is homogenous eosinophilic. The thinner wall of this limb resembles the capillaries or small venules in their lumina. The thick descending portion in medulla is similar to the proximal convoluted tubules in cortex, while the thick ascending portion of medulla is similar to distal convoluted tubules in the cortex. The ascending limb of loop of Henle appears large in size than that of the descending limb and is enclosing a wider lumen (Junqueria, 1998).

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

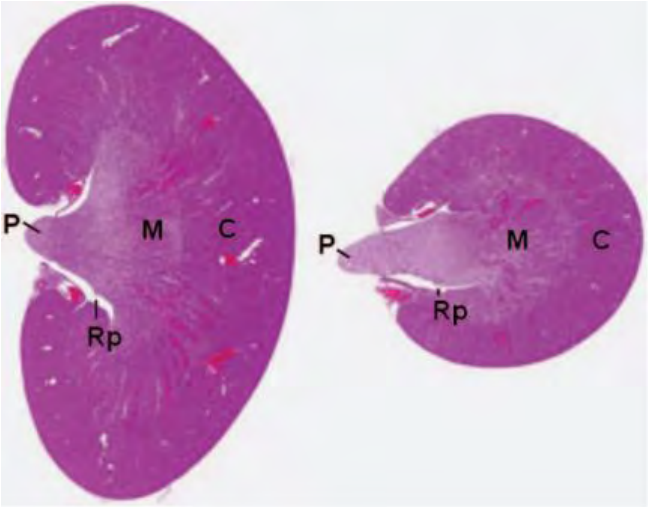


Figure 5: A photograph showing microscopic structure of rat kidney (**P**: papilla, **M**: medulla, **C**: cortex, **Rp**: renal pelvis), taken from (Piper *et al.,* 2012).

The functions of the kidneys include excretion of the final metabolic products and surplus water (filtration of the blood), maintenance of the constant composition of body liquids (preservation of fluid), preservation of electrolytes and the acid-base balance, and endocrinal function manifested as the production and release of erythropoietin, rennin and1,25 dihydroxycholecalciferol (Verlander, 1998).

The kidney is the primary organ for clearance and excretion of foreign chemicals including drugs and drug metabolites, from the body. Damage to the kidney could arise due to the administration of plant extracts (Nwanjo *et al.*, 2007). In Africa, the use of traditional herbal remedies has been implicated in 35% of all the cases of acute kidney failure (Colson and Broe, 2005). Nephrotoxicity can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and,

decreased synthesis of essential hormones (Bello *et al*., 2010). As a result, a study of the effect of drug extracts on the kidney is essential. Therefore, the assessment of the histology of the kidney tissues and the determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys; such metabolites includes urea and creatinine (Nwanjo *et al*., 2007).

1.7. Blood: Composition and functions

Blood is specialized type of connective tissue made of two parts, plasma (a liquid extracellular matrix- 55%) and formed elements (various blood cells suspended in plasma- 45%). The formed elements are red blood cells (RBCs), white blood cells (WBCs), and platelets (Tortora and Derrickson, 2009).

The blood volume (BV) of the rat is estimated to account 7% of its body weight (BW), usually the younger rats have a larger blood volume relative to their body weight than older rats (Garciai, 1997).

The red blood cells (erythrocytes) are biconcave disks with no nucleus and under normal conditions they never leave the circulatory system. Red blood cell count is 7–13 million/µl. On stained smears, they are circular with distinct, smooth margins. Rat erythrocytes are 4–7µm in diameter and appear similar in morphology to those in humans. Human erythrocytes are 6–8 µm in diameter (similar to the size of the nucleus of a resting lymphocyte) and 1.5–2.5 µm thick, with a central pallor area comprising approximately one third of the diameter of the cell *(*Diggs *et al.,* 1975*).*The major function of erythrocytes is to transport hemoglobin, which in turn carries oxygen and carbon dioxide (Baracho *et al*., 2009, Tortora and Derrickson, 2009). An estimate of the volume of packed erythrocytes per unit volume of blood is referred to as hematocrit (Junqueira and Carneiro, 2005). The average life span of erythrocytes in human is 120 days and only 61 days in rat (Bernstein, 1993).

The white blood cells (leukocytes) are the mobile units of the body's defense system and most of them are specifically transported to areas of serious infection and inflammation. When leukocytes reach their destination, they leave the blood stream by migrating between the endothelial cells of the venules and capillaries, enter the connective tissue

spaces, and perform their function generally defending the body against foreign substances (Junqueira and Carneiro, 2005; Guyton and Hall, 2006).

According to the type of granules in their cytoplasm and the shape of their nuclei, leukocytes are divided as granulocytes (possessing specific and azurophilic granules) and agranulocytes (possessing only azurophilic granules but lacking the specific ones). The granulocytes include the neutrophils, eosinophils, and basophils; while the agranulocyte include lymphocytes and monocytes (Junqueira and Carneiro, 2005; Tortora and Derrickson, 2009). Acting together, these cells provide the body with powerful defenses against viral, bacterial, and parasitic infections (Barrett *et al*., 2010).

In humans, neutrophils are the most abundant of the leukocytes, also known as polymorphonuclear neutrophilic granulocytes. They are usually the first to arrive at a site of injury or inflammation and their primary function is to attack and destroy invading bacteria, viruses, and other injurious agents and eliminate tissue debris by way of phagocytosis (Guyton and Hall, 2006; Tortora and Derrickson, 2009). In rat the nuclei of neutrophils display hypersegmentation and it accounts 14-20% of total leucocytes (Pass and Freeth, 1993).

The mature eosinophils are about the same size as neutrophils and contain a characteristic bilobed nucleus. Their main identifying characteristic is the presence of many large and elongated refractile specific granules (Junqueira and Carneiro, 2005). They phagocytize antigen–antibody complexes and are effective against certain parasitic worms (Tortora and Derrickson, 2009). The nucleus of eosinophils in rat is band-shaped and can form a ring. It accounts 1-4% of total leucocytes (Bernstein, 1993).

Basophils are granulocytes that exhibit a segmented nucleus (Ebo *et al.*, 2008). They play an important role in release of histamine and other inflammation mediators followed by the initial exposure to antigen (Miura and MacGlashan, 1998).

Lymphocytes are the major component of blood of rat leucocytes which accounts 69-86% and acts as soldiers in immune system battles (Pass and Freeth, 1993). They have large round nuclei and scanty cytoplasm (Barrett *et al*., 2010). They are mobile cells, continually recirculating between the blood and the tissues via the lymph. In order to maintain

immune surveillance, the majority of lymphocyte traffic occurs through lymph nodes (Young, 1999).

There are 2 principal types of lymphocyte: B- and T-lymphocytes. B-lymphocytes produce immunoglobulins or antibodies, which attach themselves to antigens on the surfaces of bacteria. This starts a process leading to the destruction of the bacteria. The T-lymphocytes comprise 3 main groups of cells: killer (cytotoxic) cells, helper cells, and suppressor cells. The killer T-lymphocytes attach to abnormal cells and release chemicals called lymphokines, which help to destroy the abnormal cells. Helper T-cells enhance the activities of the killer T-cells and the B-cells, and also control other aspects of the immune response. Suppressor T-cells act to "switch off" the immune response. Some lymphocytes do not participate directly in immune responses, but serve as a memory bank for antigens that have been encountered (Guyton and Hall, 2006).

Monocytes have abundant agranular cytoplasm and kidney-shaped nuclei (Barrett *et al*., 2010). The monocytes originate from a bone marrow stem cell. They are then, released into the peripheral blood, where they circulate for 2–3 days in the bloodstream before they migrate and enter into different tissues to replenish the tissue macrophage populations (Gordon and Taylor, 2005).

Blood platelets (thrombocytes) are non-nucleated, disk-like cell fragments that are formed in the bone marrow from megakaryocytes (Guyton and Hall, 2006). Platelets are the primary cells responsible for the control of bleeding and under normal circumstances their activation in response to bleeding triggers the clotting process (Andrews and Berndt, 2003). They constitute the major portion of the mass of the clot and phospholipids in their cell membranes activate the clotting factors in blood plasma that result in threads of fibrin which reinforce the platelet plug. Platelets that attach together in a blood clot also release a chemical called serotonin, which stimulates constriction of blood vessels, reducing the flow of blood to the injured area (Graaf, 2001).

Plasma, the liquid medium in which formed elements are suspended, transports nutrients from their site of absorption or synthesis, distributing them to various areas of the organism. It also transports metabolic residues which are removed from the blood by excretory organs (Junqueria, 1998). Rat plasma is composed of water, proteins and salts

(Bernstein, 1993). The three major plasma proteins include: the albumin, globulins (α, β -, and γ-globulins), and fibrinogen. When blood is removed from the body and placed in a test tube, clotting occurs unless the tube is coated by an anticoagulant such as heparin (Gartner and Hiatt, 2000).

Since blood plays a key role in the delivery of nutrients, hormones, metabolic excretion and immunological processes as well as homeostatic responses (Clark and Wallis, 2003), its parameters are still most highly accurate, sensitive and reliable that investigators use and depend on for the purpose of disease diagnosis, prevention and treatment (Irshaid and Mansi, 2009).

1.8. Significance of the study

It is well known that herbal medicines contain ingredients to maintain health and to cure ailments. However, they may also contain toxic substances which are harmful or even dangerous to health is least known. Therefore, the rationale of conducting this study is to investigate if there is any toxic effect of *A. afra* which is used as traditional medicine for various ailments. The outcome of the study will serve as premise for further investigation on this plant for better utilization of its claimed therapeutic values.

2. Objectives

2.1. General objective

To evaluate the Acute and subchronic toxic effects of aqueous leaf extract of *Artemisia afra* on some blood parameters, histopathology of Liver and Kidney in Wister rats.

2.2. **Specific objectives**:

- To assess the effect of *Artemisia afra* leaves extract on the general behavior and gross pathology and weight of liver and kidney of Wister rat.
- To determine the LD_{50} of aqueous leaf extract of *Artemisia afra*.
- To assess the effect of *Artemisia afra* leaves extract on the body weights of Wister rat in acute and subchronic treatment.
- To determine the effect of the *Artemisia afra* leaves extract on hematological and biochemical parameters of blood in Wister rat after subchronic treatment.
- To assess the effect of leaves extract of *Artemisia afra* on histology of liver and kidney after subchronic treatment in Wister rat.

3. Materials and Methods

3.1. Study design:

Laboratory-based experiment on rats

3.2. Study area*:*

The study was conducted in Addis Ababa at Ethiopian public health institution (EPHI) and Addis Ababa University, College of Health Science, School of Medicine, Departments of Anatomy and Physiology laboratories.

3.3. Study period:

The study was conducted from January 2014-July 2015

3.4. Plant material collection

The fresh leaves of *A. afra* were collected by me with the help of staff of bale national park and Meda Welabu University from Bale National mountain region of Ethiopia based on ethno-botanical description in July 2014. Specimens of the plant were identified by a taxonomist and a few samples were deposited at the National Herbarium in the college of natural and computational sciences, Addis Ababa University (AAU) with a Voucher specimen number (392/NKI/PHARM). Fresh leaves were cleaned from extraneous materials, dried under shade at room T^o, and grinded by manual crusher and obtained fine particles. It has been then processed to obtain the aqueous extract (Fig. 6).

3.5. Aqueous Extraction

The powdered leaves (620gm *Artemisia afra)* were macerated with disteld water for 2hrs with intermittent agitation by orbital shaker. Then, the supernatant part of agitated materials was decanted and filtered with 0.1 mm^2 mesh gauze from the un-dissolved portion of plant. The filtrate was freeze-dried at lower T^o and reduced pressure to form crude extract. A yield of 67.7gm (10.9%) was obtained. This was kept in a desicator at room T^o until used as described by Asfaw Debella (Debella, 2002)

Figure 6:- Photographs showing different steps used in the preparation of the aqueous extracts from the dried plant leaves that has been processed through. (A) Dried powder leaf. (B). On orbital shaker for shaking (C) Freezing, (D) The lyophilizer, (E) The final extract, (F) The desicator.

3.6. Experimental animal preparation

The animals used in this study were bred and reared at the animal house of the EPHI and transported to Physiology Laboratory of Addis Ababa University Health Science College. Experiments were conducted on 59 healthy adult male and female Wister rats aged 8-12 weeks for both acute and subchronic study. 32 rats were used for acute study, all of which were females and arranged in eight groups, each group containing four rats. The remaining 27 rats (12 male and 15 female) were used for subchronic study and grouped in six: three groups (for male) which contained four rats in each group and three groups (for females), each group contained five rats. Each male and female group had their own control groups. The number of rats for each acute and subchronic study were selected and assigned to each group based on OECD,(2008) guideline. Females were nulliparous and non-pregnant. Grouping of rat was done randomly. The animals were kept in separate aluminum cages and provided with bedding of clean paddy husk. All animals had free access to standard pellet diets and drunken tap water. The rats were acclimatized to laboratory conditions for

one week prior to the experimental protocol to minimize any nonspecific stress (Vipul *et al*., 2007). They were maintained at standard conditions of temperature (20 ± 3^0C) and light/dark cycles till the end of the experiment.

3.7. Acute toxicity study and LD-50 determination

Selection of the dose for toxicological investigation were based on the prior efficacy study of the plant by Taofik and Anthony (2013) who, have found the aqoues extraction of the plant at a dose 200mg/kg body weight as effective dose in decreasing the serum glucose level(act as hypoglycemic agent). This effective dose (i.e. 200mg/kg body weight) was selected as the appropriate initial dose (200mg/kg body weight) for acute toxicity test and increased to a higher dose of (5000mg/kg body weight). Each of the doses comprised of *Artemisia afra* leaves extract. A total of eight groups of animals were used (seven experimental and one control) each consisting of 4 adult female Wister rats. To increase the rate of absorption of the extract all groups of Wister rats were fasted overnight prior to administration. At the end of the fasting period, the body weight of each rat was recorded before dosing and the doses were calculated and administered to the rats in the experimental groups based on their body weight. Each experimental group (group 1 to group 7) received designated doses (200mg/kg to 5000mg/kg of the formulation per body weight) spaced by 500mg/kg to produce test groups with a range of toxic effects and mortality rates in 24 hours observation. The control group (Group 8) received distilled water in the same volume. The individual Wister rats were identified by marks on their tails made with different colored permanent ink and through cage markings indicating the group designation and treatment dose. Before the initiation of dosing, the rats were left to acclimatize to laboratory conditions for 7 days. In accordance with (OECD, 2001), the volume of the extract administered at a time was 1ml/100g body weight of rat. Each animal received single dose of extract or vehicle in an adjusted final volume. Handling of animals and general parameters was monitored. The *A. afra* or distilled water was administered orally as follows after having training at Ethiopian public health institution. First, the rat was held very firmly with the skin of the neck and back so that the head was kept immobile and in line with the back. Then, the gavage attached to the 10ml syringe, was introduced into the mouth as far to one side as possible (i.e. not centrally), and after locating the entry to the esophagus, it was pushed gently into the stomach, where the contents of the syringe

was discharged through the gavage, first slowly (to confirm intra-gastric passage) and then fairly rapidly as was described by Waynforth, (1980). The experimented and control groups were observed continuously with a great attention for 4 hours following administration and then every 12 hours for the next 14 days; and any signs of toxicity and mortality were recorded. The presence of toxic signs like piloerection, lethargic, loss of appetite, dizziness, hypoactivity and convulsion were observed during the study. According to OECD, (2008), the body weight of each rat was recorded at the 7^{th} day. The differences in the body weight were also recorded.

At the end of the second week, the final weight of each rat was recorded and then the entire rats were sacrificed with a high dose of anesthetic diethyl ether. All animals in the study were subjected to careful examination of the external surface of the body and abdominal cavities and their contents including the kidneys and liver. Comprehensive gross pathological observations were carried out on these organs to check for any signs of abnormality and presence of lesions.

A lethal dose for fifty percent of the rat (LD_{50}) of the extract was determined after recording deaths of rats. The dose at which 50% of the rats in a group died was considered to be oral LD_{50} (OECD, 2008).

3.8. Subchronic toxicity study

The subchronic toxicity study was carried out through twelve weeks (90 days). In this subchronic study, two doses (600 and 1800mg/kg body weight) were selected. The low dose was selected in reference to efficacy study of *A. afra* for treatment of malaria which is 400mg/kg as effective dose (Moges *et al.*, 1998), but this dose was modified to 600mg/kg based on the findings in acute toxicity study and triple of the low dose (1800mg/kg) taken as the higher dose (OECD, 2008). The main study sample of twenty seven (12 adult male and 15 female rats) weighing 130-270g was housed in groups of 6 in a rat cages, under the same conditions as described previously for the acute study. There were two experimented and one control animal groups for each sex, lower dose (600mg/kg) groups and higher dose (1800mg/kg) groups. The individual rats were identified as stated in section 3.7. Like that of the acute study, the rats were left for 7 days to acclimatize the laboratory conditions before the initiation of dosing.

The animals in the main 90-day study were randomly assigned to 2 treatment groups to receive 600 and 1800mg/kg *A. afra* aqueous extract and one control group (no *A. afra*) for each sex. In accordance with OECD (2001), the volume of the formulation administered at one time was 1ml/100g body weight of rat in 24 hrs interval throughout the twelve weeks (90 days) study period. Each morning, the animals received the treatment dose or vehicle in an adjusted final volume. Handling of animals and general parameters were monitored. The technique of administering *A. afra* or distilled water was similar to that of acute toxicity study described in section 3.7. To determine the effect of the leaf aqueous extract of *A. afra* on the general wellbeing of the rats, all animals were monitored for the first 4 hour with a great attention following administration then every 12 hours periodically for any deviations in normal behavior, coat condition, discharge, movements and mortality on a daily basis during the whole period of study.

The daily food intake of all rats was also monitored. The body-weight (in gram) of each rat was recorded on the 1st day and at weekly intervals throughout the course of the study and the average body-weights for the groups were calculated. The acute and the subchronic administration was done one after the other to use the results of acute toxicity study as a base line for subchronic toxicity study.

3.9. Hematological and biochemical analyses

Twenty-four hours after the last day of extract administration, each animal was anaesthetized by diethyl ether and put on dissecting board in supine position and blood samples were withdrawn by cardiac puncture. Part of the blood samples obtained from each rat were then collected in separate test tubes with an anti-coagulant substance, EDTA (ethylene diamine tetra-acetic acid) and the remaining in plain test tubes with no EDTA. Blood samples from EDTA containing test tubes were immediately processed for hematological parameters using Automated Hematological Analyzer, (SYSMEX RX 21, 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 rpm for 15 minutes using a bench top centrifuge (HUMAX-K, HUMAN-GmbH, Germany). The serum was withdrawn and transferred into other clean vials to analyze the function of liver and kidney then it was kept at -20°C until analysis. The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea, uric acid and creatinine were automatically determined using, (AUTO LAB 18, clinical chemistry analyzer, Italy).

3.10. Gross pathologic observations

After blood collection, each rat was sacrificed by overdose of diethyl ether, the whole liver and both right and left kidneys were immediately excised. The gross pathological observation of these organs was performed by pathologist to check for any gross lesions. Eventually, all the samples were sliced and preserved in 10% neutral buffered formalin fixative solution for 24 hours.

3.11. Histopathological studies

The liver and the kidney tissue samples of the various groups of rat preserved in 10% neutral buffered formalin were withdrawn after 24 hrs fixation and then thoroughly rinsed over several changes of tap water. They were then dehydrated with increasing concentrations of ethanol (70% and 90%) for 2 hours each followed by absolute alcohol I, II, and III for one and half hours, each and absolute alcohol IV overnight.

The tissue samples were then cleared with two changes of xylene: xylene-I for one and half hours and then xylene-II for two and half hours. Next, the tissues were impregnated in paraffin wax: wax-I for two and half hours and wax-II overnight in an oven at a temperature of 40^{o}c as described in the appendix II. Embedding the tissue samples into tissue block was done by putting tissue samples in squares of metal plates and carefully pouring molten paraffin over them. After proper orientation of the specimens all tissue blocks were labeled and allowed to harden at room temperature.

Tissue blocks were then sectioned with a thickness of 5µm using Leica rotary microtome. Ribbons of the tissue sections were gently collected using a piece of brush and laid onto the surface of a water bath heated at 40^{0}C. After the sections were appropriately spread on the water bath, they were mounted on to tissue slides. The slides were then arranged in slide

racks and were placed in an oven with a temperature of 40^0C overnight to facilitate the adhesion of the specimens onto the glass slides. The specimens were allowed to cool and stained using Heamatoxylin & Eosin staining method as mentioned in the appendix 3 and mounted with DPX.

3.12. Light microscopy and photomicrography

Stained tissue sections of the liver and kidney were carefully examined by pathologist under binocular compound light microscope. Tissue sections from the experimental groups were examined for any evidence of histopathological changes with respect to those of the controls. After examination, photomicrographs of selected samples of liver and kidney sections from both the experimental and control rats were taken under a magnification of x20 objective by using (EVOS XL, USA) automated built-in digital photo camera.

3.13. Statistical analysis

All data were organized and analyzed using SPSS version 21 statistical software. The values of body and organ weight changes and difference, hematological and biochemical parameters were analyzed and the results were expressed as M ± SE($\overline{x}$) (standard error of the mean). Differences between the experimental and control groups were compared using one-way analysis of variance (ANOVA), followed by Dunnett's T-test to determine their level of significance. Differences at $p<0.05$ were considered statistically significant.

3.14. Ethical consideration

The study was conducted after having approval by Department of Anatomy Graduate Committee, School of Medicine, College of Health Sciences, Addis Ababa University. Animals used in this study were kept from any unnecessary painful and terrifying situations (OECD, 2008). To make pain and suffering minimal during any surgical intervention (blood collection), the animals were anesthetized using diethyl ether and the procedures were carried out by well-trained persons. Animals were protected from pathogens and placed in appropriate environment.

3.15. Communication of the result

The result of the study was compiled in the form of thesis, and communicated to all concerned institutions including the Department of Anatomy (AAU), Ethiopian Public Health Institute (EPHI) and to advisors and will be published in pear reviewed journal and delivered in scientific conferences. Health education materials in local languages will be also prepared for the public.

4. Results

4.1. Acute toxicity

4.1.1. Effects of acute toxicity of extracts on behavior and body weight

The administration of the aqueous leaf extract of *Artemisia afra* to the experimental rats as shown in Table 1 did not show any mortality of experimental rats with single oral doses up to 5000mg/kg body weight. Behavioral changes like Loss of appetite, Hypo-activity, pilo-erection, lethargic, dizziness and a single episode of convulsion were observed with the dose of 2200mg/kg and above, with an increased severity as the dose increased. The symptoms mentioned above, however, disappeared after the first week of observation as indicated in the Table 1.

Table 1: Acute effects of *A. afra* aqueous leaf extract in rats as compared to the controls.

Dose of *A. afra* extract (mg/ kg)	**Death/experimented**	**Symptoms of toxicity**	
		During 1st wk of study	**During 2nd wk of study**
200	0/4	None	None
700	0/4	None	None
1200	0/4	None	None
2200	0/4	Loss of appetite,	None
3200	0/4	Loss of appetite, Hypo-activity, pilo-erection	None
4200	0/4	Loss of appetite, pilo-erection, hypoactivity	None
5000	0/4	Hypo-activity, lethargic, convulsion, loss of appetite, dizziness	None
Control	0/4	None	None

Both the experimental and control groups of rats had gained body weight during the two weeks observation (Table 2).

Table 2: Mean body weight of rats administered with extract as compared to the controls during two weeks observation (expressed as mean ± SDE, n = 4)

Group	Dose(mg/kg)	Initial mean body weight (gm)	Mean Body weight at the end of week 1 (gm)	Mean Body weight on day 14 (gm)
I	200	182.7± 17.9(0.56)	186.34 ± 18.9(0.56)	198.9±19.07(0.66)
II	700	157.8± 6.34(0.54)	160.4 ± 6.24(0.46)	169.07±6.98(0.52)
III	1200	182.42±5.64(0.9)	187 ± 5.8(0.99)	204.8 ± 8.95(0.90)
IV	2200	173.9±4.34(0.27)	177.32 ± 4.65(0.11)	188.55±5.22(0.07)
V	3200	194 ± 4.6(0.39)	198.5 ± 3.83(0.90)	208.55±4.62(0.34)
VI	4200	172 ± 8.55(0.25)	175.1 ± 9.17(0.26)	183.67±9.54(0.23)
VII	5000	187 ± 3.18(0.49)	190 ± 3.22(0.26)	199.22±4.94(0.18)
VIII	Control(vehicle)	169.5 ± 7.17	177.85 ± 7.41	188.5 ± 7.84

The figures under brackets indicate p-values, n –number of rats per group

4.1.2. Effects of acute administration of the extracts on gross pathology

Observation on the gross appearance of internal organs including liver and kidney of experimental rats did not show any abnormal changes in texture, shape, size or color in comparison to that of the control. No lesion was noted in these organs in all groups.

4.2. Subchronic toxicity study

4.2.1. Effects of subchronic administration of the extracts on behavior, gross pathology and body weight

During the period of 90 days of subchronic toxicity evaluation, rats that were orally administered with the repeated doses of the extract at 600mg/kg body weight showed no change in their general behavior as compared to the control group. Only rats in the group received the higher dose (1800 mg/kg) manifested minor signs of toxicity, comprising intermittent diarrhea, salivation and partial hypoactivity. Such signs started by the third day of treatment and stayed only for three days. There was no abnormal gross finding on skin and eyes as well as in gross observation of the liver and kidneys in any of the experimental and control groups. Moreover, there was no toxicity related death throughout the period of study.

As shown in the Table 3 there was a progressive body weight gain in nearly all groups of male rats with time over the whole period of the experiment. No significant change was

observed in the pattern of body weight gain among the different groups of rats in both experimental groups as well as the controls (Fig. 7).

Table 3: Mean body weights (in gram) of male rats administered with 600 and 1800mg/kg extract during the consecutive twelve weeks observation as compared to the controls.

Effect of *A. afra* extract on body weight of various groups of male rats (expressed as mean ± SDE, n = 4)			
Week	**Control**	**600mg/kg**	**1800mg/kg**
Initial mean body weight	211.5±1.6	226.7±3.7(0.56)	212.6±1.6(0.95)
1	216.7±1.3	231.1± 3.6(0.39)	218.3±1.4(0.79)
2	224.2±1.7	237.7±3.8(0.16)	228.3±1.1(0.56)
3	233±1.3	245.6± 3.2(0.35)	237.1±1.5(0.74)
4	242±2	252.2±2.8(0.27)	243.3±2.2(0.59)
5	252±2.7	258.8±3.4(0.58)	253±3.7(0.4)
6	263.5±2.7	265.5±3.5(0.31)	262±4.2(0.21)
7	274.3±3.2	275.6±3.6(0.49)	268.6±4(0.35)
8	286.5±2.6	286.2±3.9 (0.3)	275.2±3.1(0.2)
9	298.7±2	292.3± 4.1 (0.06)	283.3±2.4(0.69)
10	309±2.8	301.1± 4.7 (0.29)	292±2(0.97)
11	320.2±2.84	308.2±3.1(0.6)	295.7±3.3(0.48)
12	332±2.77	317.8±3.9(0.09)	302.8±4.3(0.35)

The figures under brackets indicate p-values, n –number of rats per group

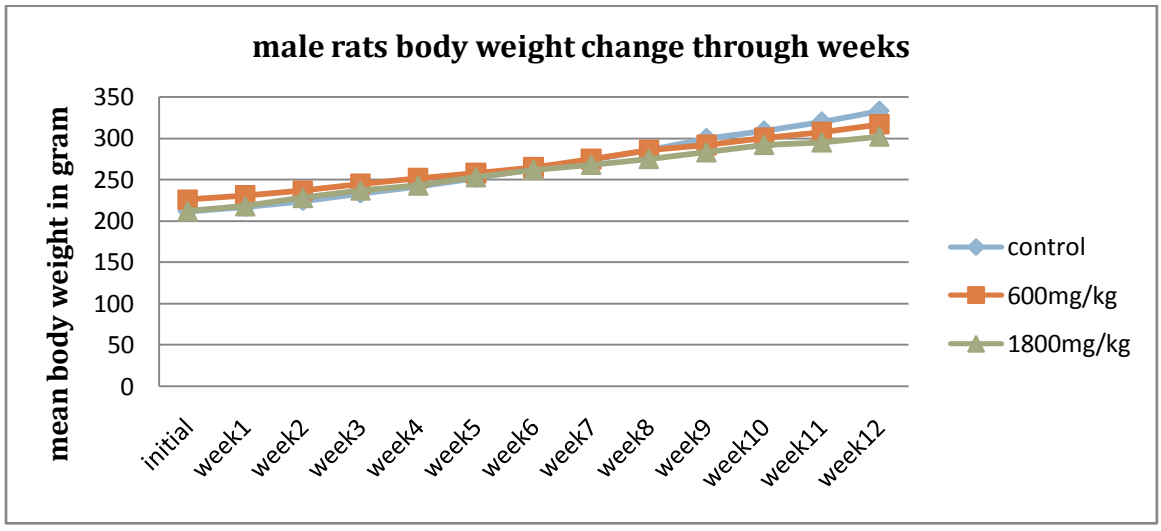


Figure 7: Body weight change in male rats administered with 600mg/kg and 1800mg/kg extract as compared to the controls.

The changes in the mean values of the body weights of female rats administered with 600mg/kg and 1800mg/kg of aqueous extract of *A. afra* as compared to the controls during the 12 weeks of study period is displayed in Table 4 and Figure 8. The change in body weight was not significantly different in both cases as compared to that of the control.

Table 4: Mean body weights (in gram) of female rats administered with 600 and 1800mg/kg extract in consecutive twelve weeks observation as compared to the control.

Effect of *A. afra* extract on body weight of various groups of female rats (expressed as mean ± SDE, n = 5)			
Week	**Control**	**600mg/kg**	**1800mg/kg**
Initial mean body weight	200.2±5.3	207.5±4(0.4)	205±2.8 (0.46)
1	208.5±6.2	214.7 ± 4.2 (0.52)	212.3 ± 2.5(0.54)
2	217.2±6.5	224.4±4.7(0.6)	219.8 ± 2.5(0.5)
3	226.9±6.6	231.8± 4.6 (0.9)	228.3± 1.7 (0.8)
4	231.9±6.9	238.8±4.1(0.87)	235.9±1.7(0.06)
5	237±7.3	243.4 ± 4.9(0.4)	243.5 ± 3.3 (0.2)
6	244.3±6.2	249.3± 4.9(0.57)	249.98 ± 3.8 (0.07)
7	251.4±5.5	255.5± 4.6 (0.95)	256.4± 3.6(0.2)
8	259.2±5.3	262.5±4.5 (0.98)	264±2.98 (0.17)
9	268±4.7	270.2 ± 5 (0.85)	272.5± 3.5(0.18)
10	276.8±4.5	276.4 ± 4.8 (0.87)	281.8 ± 2.7(0.97)
11	284.4±4.4	284.4±4.4(0.85)	288.6 ± 3.4(0.99)
12	288.7±4	289±3.9(0.47)	297 ± 3.4(0.19)

The figures under brackets indicate p-value, n – number of rats per group

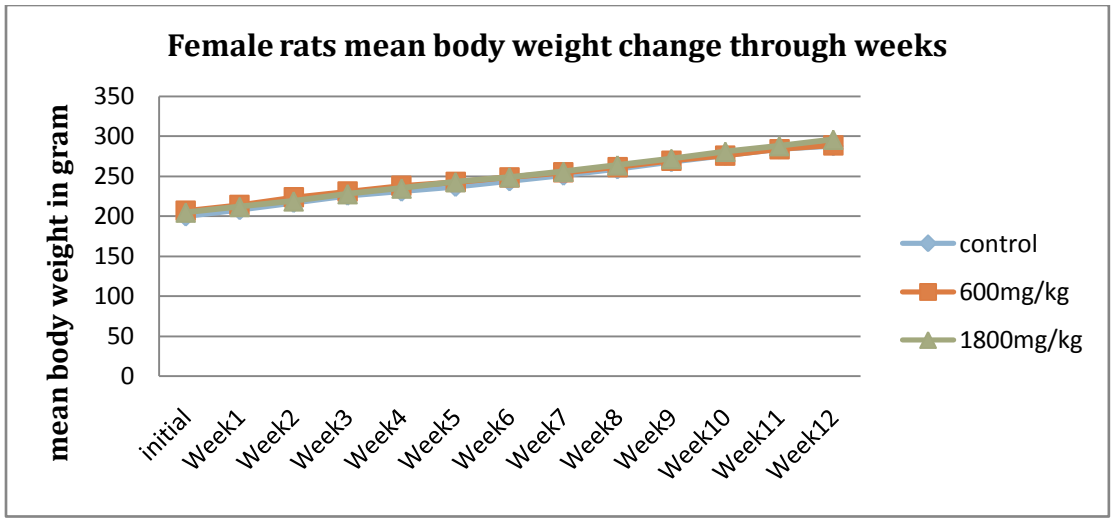


Figure 8: Mean Body weight of female rats administered with 600mg/kg and 1800mg/kg extracts as compared to the control.

4.2.2. Effect of subchronic oral administration of *Artemisia afra* on the liver and kidney of rats

At the end of the study-period, the liver and kidney of both control and experimental rats (male and female) were isolated, weighed and visually inspected for any histopathological changes in the tissue architecture and colour. Visual gross examination of the liver and kidney of both control and experimental rats showed normal architecture, no colour changes and no morphological disturbances. The results obtained are shown in (Figure 9&10)

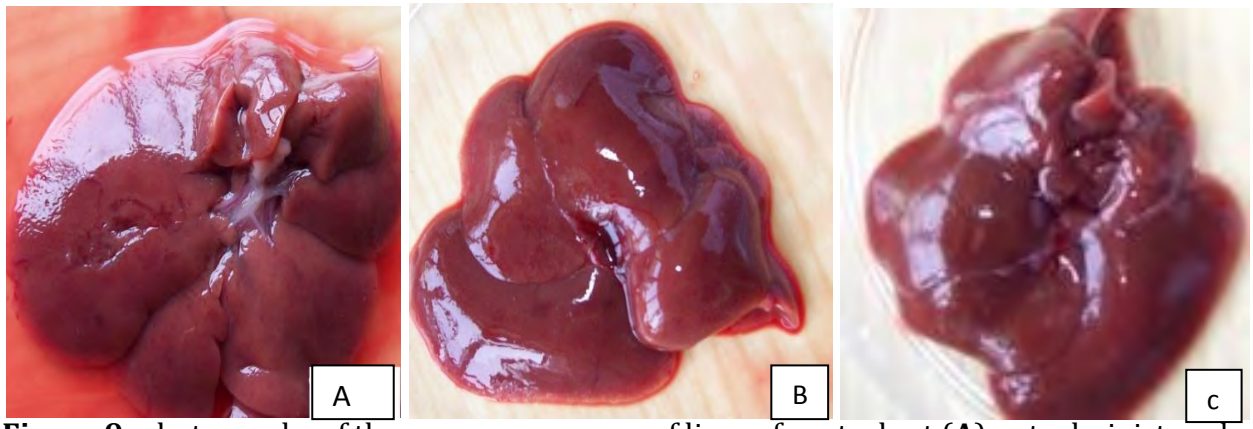


Figure 9: photographs of the gross appearance of liver of control rat (**A**), rat administered with 600mg/kg (**B**), and rat administered with 1800mg/kg (**C**).

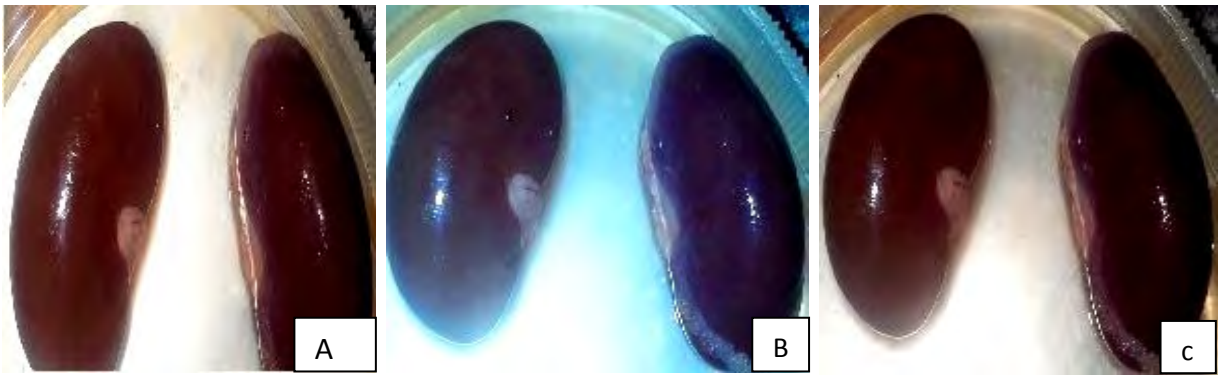


Figure 10: Photographs of the gross appearance of kidneys, control rat (**A**), rat administered with 600mg/kg (**B**), and rat administered with 1800mg/kg (**C**).

As indicated in the Table 5 (for male rats) and Table 6 (for female rats) treatment with the extract of *A.afra* did not produce any significant effect on weight of liver and kidneys at both dose of 600mg/kg and 1800mg/kg as compared to rats administered with disteld water.

Table 5: mean liver and kidney weight of male rats (in gram; mean ± SDE) chronically dosed with *A. afra* as compared to the controls.

Dose	Mean weight (in gram; mean ± SDE; n=4)	
	Liver	Kidney(single)
Control	15.775±0.69	1.58±0.046
600mg/kg	15.025±0.557(0.617)	1.5625±0.042(0.129)
1800mg/kg	13.92±0.97 (0.962)	1.56±0.06(0.939)

The figures under brackets indicate p-values, n – number of rats per group

Table 6: Mean liver and kidney weight of female rats (in gram; mean ± SDE) chronically dosed with *A. afra* as compared to the controls.

Dose	Mean weight (in gram; mean ± SDE; n=5)	
	Liver	Kidney(single)
Control	14.46±0.289	1.5±0.041
600mg/kg	15.04±0.435(0.643)	1.48±0.045(0.575)
1800mg/kg	15.18±0.449(0.944)	1.494±0.042(0.561)

The figures under brackets indicate p-values, n – number of rats per group.

4.2.3. Effects of aqueous leaf extract of *Artemisia afra* on Hematological and Biochemical parameters.

The aqueous leaf extract of *A. afra* did not produce significant change on any of the hematological parameters tested after its administration for 12 weeks in male rats as compared to the controls (Table 7).

Table 7: Effects of 600mg/kg &1800mg/kg aqueous leaf extract of *A. afra* on hematological parameters in male rats as compared to the controls. (Expressed as mean ± SDE, n = 4)

Hematological Parameters	600mg/kg	1800mg/kg	Control(disteld water)
WBC ($x10^3/\mu L$)	6.8±0.35(0.6)	6.9±0.67(0.4)	6.2±1.5
RBC ($x10^6/\mu L$)	8±0.25(0.16)	8.2±0.23(1)	7.8±0.3
HGB (g/dL)	15.4±0.35(0.8)	16.6±0.37(0.9)	14.5±0.9
HCT (%)	48.9±1.4(0.7)	50.9±2.5(0.64)	50.4±1.7
MCV (fL)	65.2±0.85(0.74)	63.8±1.7(0.98)	64.4±1
MCH (pg)	18.3±1.19(0.14)	18.9±1.6(0.35)	18.3±0.49
MCHC (g/dL)	26.9±2.6(0.25)	30.8±0.96(0.29)	28.5±1.2
PLT ($x10^3/\mu L$)	904.3±9.69(0.12)	881.4±51.6(0.4)	874.5±61.23
NEUT ($x10^3/\mu L$)	2.3±0.36(0.46)	2.4±0.4(0.9)	2.4±0.27
Lympho($x10^3/\mu L$)	3.8±0.12(0.46)	3.8±0.19(0.3)	3.6±0.15
Mono ($x10^3/\mu L$)	0.23±0.047(0.24)	0.19±0.04 (0.78)	0.23±0.04
EO ($x10^3/\mu L$)	0.053±0.0045(0.12)	0.054±0.004(0.27)	0.06±0.007
Baso ($x10^3/\mu L$)	0.02±0.0019(0.06)	0.017±0.003(0.36)	0.02±0.0012

The figures in brackets indicate the calculated p values of the treatment groups as compared to the control and n= no of rats per group.

Same results were obtained on hematological parameters with female rats, i.e., no significant changes were observed in the hematological parameters as compared to those of the control group as shown in Table 8.

Table 8: Effects of 600mg/kg & 1800mg/kg aqueous leaf extract of *A. afra* on hematological parameters in female rats as compared to the controls (expressed as mean ± SDE, n = 4)

Hematological Parameters	600mg/kg	1800mg/kg	Control(disteld water)
WBC ($x10^3/\mu L$)	5.97±0.98(0.34)	6.9±1.9(0.35)	5.4±0.44
RBC ($x10^6/\mu L$)	6.9±0.35(1)	7±0.3(0.84)	7.1±0.42
HGB (g/dL)	14.8±0.8(0.77)	14.9±0.9(0.86)	15.3±1.0066
HCT (%)	48.7±0.8(0.34)	46±2.3(0.74)	46.9±3.6
MCV (fL)	66.27±1.3(0.59)	65.4±0.78(0.96)	66.3±1.23
MCH (pg)	21.57±0.09(0.64)	21±0.44(0.25)	21.6±0.29
MCHC (g/dL)	32.1±0.12(1)	32.4±0.4(0.84)	32.6±0.4
PLT ($x10^3/\mu L$)	778±45.08(0.77)	806±9.24(1)	832±39.2
NEUT ($x10^3/\mu L$)	2.23±0.24(0.46)	2.04±0.089(0.64)	2.3±0.4
Lympho ($x10^3/\mu L$)	3.3±0.35(0.16)	3.77±0.17(0.64)	3.3±0.24
Mono ($x10^3/\mu L$)	0.18±0.018(0.64)	0.17±0.04(1)	0.15±0.035
EO ($x10^3/\mu L$)	0.046±0.0065(0.25)	0.042±0.006(0.83)	0.054±0.0044
Baso ($x10^3/\mu L$)	0.02±0.005(0.22)	0.019±0.0032(0.9)	0.021±0.0054

The figures in brackets indicate the calculated p values of the treatment groups as compared to the control and n= no of rats per group.

Similarly there was no significant change in (ALT) Alanine Amino Transferase, bilirubin, creatinine and urea) levels in both male and female groups that received *A. afra* at doses of 600mg/kg and 1800mg/kg when compared with the control group as shown in Table.9 (for the male) and Table 10 (for the female). However, while the values of AST (Aspartate Amino Transferase) were found not changed in the male groups at both doses and the female rats at 600mg/kg dose, there was 26% decrement in the female rats at dose of 1800mg/kg as compared to the controls. This was statistically significant.

Table 9: Effect of 600 & 1800mg/kg aqueous leaf extract of *A. afra* on biochemical parameters in male rats as compared to the control group (expressed in mean ± SDE, n = 4)

Parameters	Control	600mg/kg	1800mg/kg
AST(IU/L)	199.75±30.8	198±1.45(0.78)	194± 13.7 (0.16)
ALT (IU/L	175.25±26.1	177.3±2.07(0.84)	181 ± 34.9 (0.17)
Total Bilirubin	0.5525±0.09	0.53±0.08(0.747)	0.8 ± 0.13 (0.321)
Urea (mg/dL)	47.75±5.4	48±7(0.843)	53± 3.2(0.70)
Creatinine (mg/dL)	0.9825±0.08	1.04±0.02(0.46)	1.01± 0.13 (0.55)

The figures under brackets indicate p-values, n – number of rats per group

Table 10: Effect of 600 & 1800mg/kg aqueous leaf extract of *A. afra* on the biochemical parameters in female rats as compared to the control group (expressed in mean ± SDE, n = 5).

Parameters	Control	600mg/kg	1800mg/kg
AST(IU/L)	178±37.75	171.6±29.3(0.495)	131.3±16.169(0.034) **
ALT (IU/L	135.3±19.9	134±6.5(0.835)	119.6±24.3(0.385)
Total Bilirubin	0.8±0.13	0.76±0.109(0.051)	0.68±0.06(0.948)
Urea (mg/dL)	51±5.196	54.3±10.7(0.851)	36±2.517(0.22)
Creatinine (mg/dL)	1.0167±0.105	1.14±0.17(0.613)	0.76±0.026(0.878)

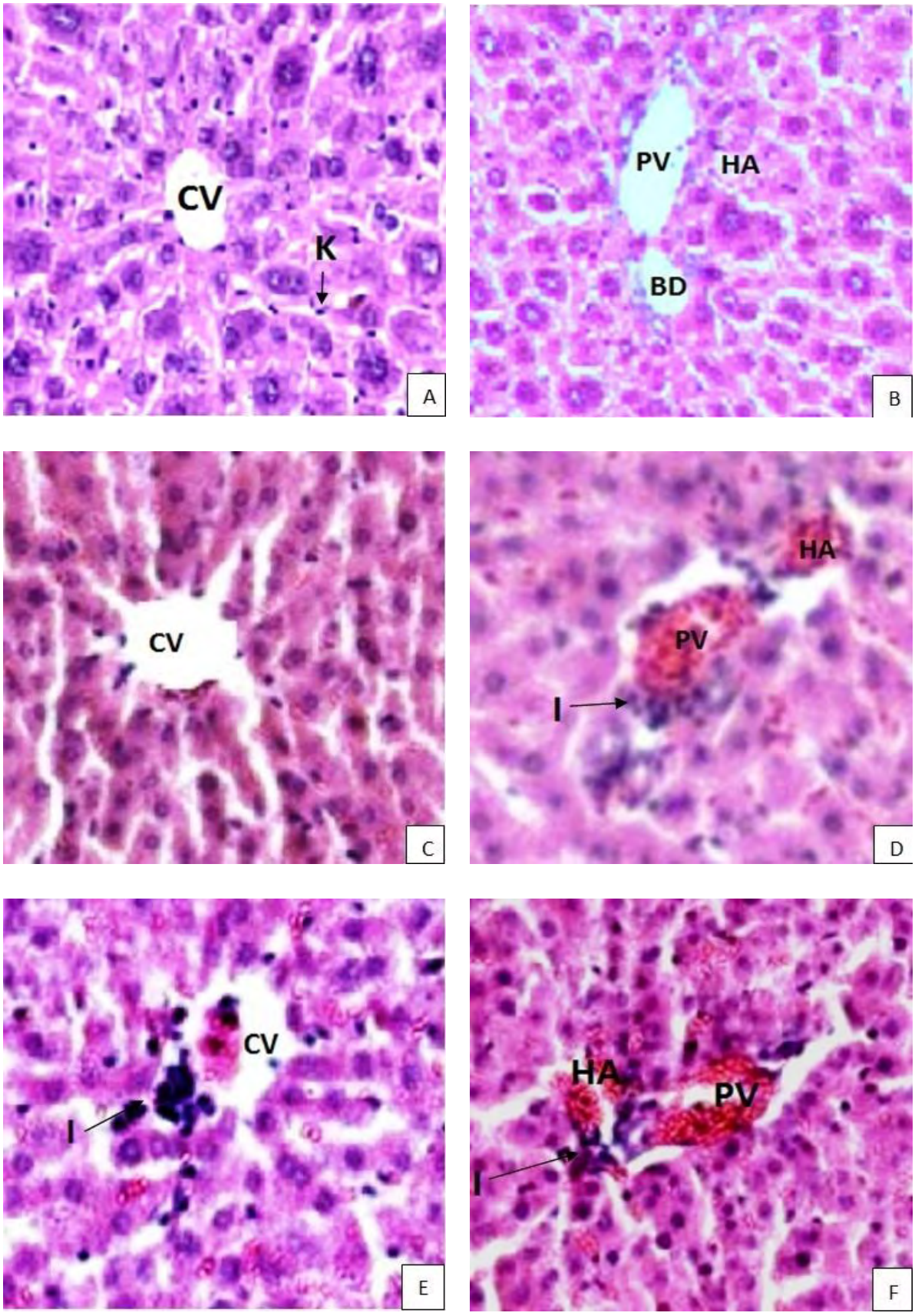
*The figures under brackets indicate p-values, **: significant, n – number of rats per group*

4.2.4. Effects of subchronic administration of the extract on histology of the liver

Microscopic examination of liver sections of the control rats (Figure 11A & B) showed the normal architecture of structural units of the liver, the hepatic lobules, formed by cords of hepatocytes separated by hepatic sinusoids. Additionally, the central vein and portal area containing branches of hepatic artery, billary duct and portal vein revealed normal appearance. In comparison to the control, the general microscopic architecture of the liver tissue sections of both male and female rats administered with 600mg/kg body weight dose (Figure 11C & D) of the extracts obtained from *A. afra* appeared to be not significantly affected after 90 days administration.

However, liver sections of some male and female rats administered with 600mg/kg body weight dose showed minor periportal mononuclear leukocytic infiltration (Figure 11D). In addition in the sections of male and female rats administered with 1800mg/kg body weight dose (Figure 11E & F), the liver appeared normal. However, minor mononuclear leukocytic cell infiltration near the central veins and portal area occurred. The histology of liver of male and female rat is similar and there was no difference in the liver sections of male and female experimental rats for each of the two doses as well as the controls.

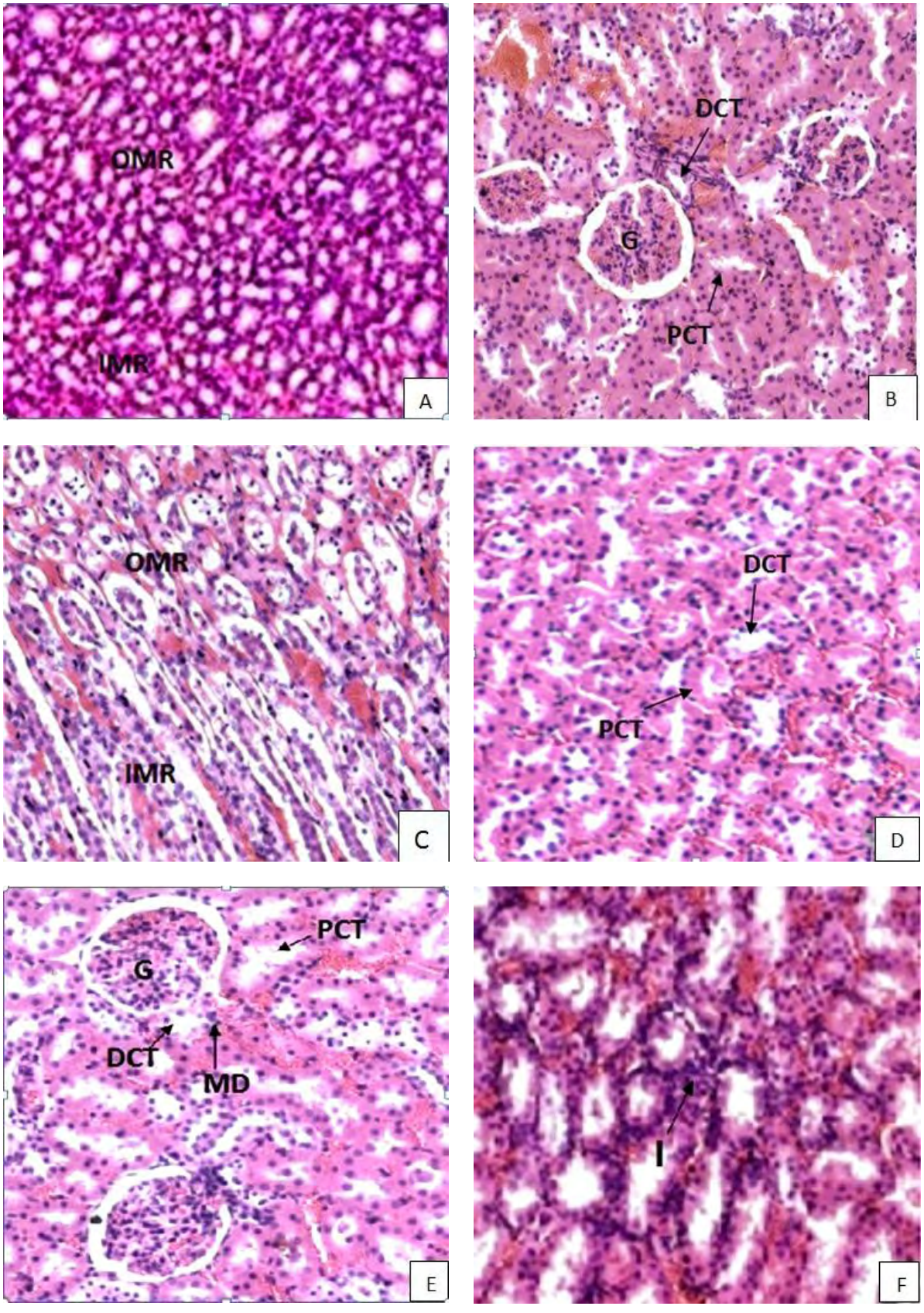
Figure 11: Photomicrographs of liver sections of control rats (A&B), rats administered with 600mg/kg (C&D), and rats administered with 1800mg/kg (E&F) sections are from female rats. **CV**=Central vein, **E** = Endothelial cells, **PV** = Portal vein, **BD** = Bile duct, **HA** = Hepatic artery, **K**=Kupffer cells, **I**= leukocytic Infiltration. (Sections were stained with H&E, X400). Note: leukocytic Infiltration (I) the liver sections of rats administered with the extract at dose 600mg/kg (D) and 1800mg/kg (E) and (F)


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

4.2.5. Effects of subchronic administration of the extract on histology of the kidneys

Examination of kidney sections of both male and female rats administered with the extract of *A. afra* at both 600mg/kg (Figure 12C & D) and 1800mg/kg doses (Figure 12E & F) indicated no structural disturbance as compared to the control rats (Figure 12A & B). The microscopic architecture of the kidney in experimental male and female rats had a similar appearance to that of the controls in which renal corpuscles maintaining their normal size of urinary space and normal tubular structures were observed. However, minor tubulointerstitial leukocytic infiltration was observed in small areas of kidney sections of both male and female rats administered with 1800mg/kg body weight dose (Figure 12F).

Figure 12: Photomicrographs of kidney sections of control rats (A & B), rats administered with 600mg/kg (C & D), and rats administered with 1800mg/kg (E& F) sections are from female rats. G = Glomerulus, DCT = Distal convoluted tubule, PCT = proximal convoluted tubule, I = leukocytic Infiltration, MD = Macula densa, OMR = outer medullary region, IMR = inner medullary region. (Sections were stained with H&E, X300). Note: Tubulointerstitial leukocytic infiltration with the extract at dose of 1800mg/kg (F)


OMR
IMR
A
DCT
G
PCT
B
OMR
IMR
C
DCT
PCT
D
PCT
G
DCT
MD
E
I
F

5. Discussions

Mixtures of many plants (often more than 10 together) are commonly employed by traditional healers, as combinations of medicinal plants are thought to have synergic effect. Products often do not contain any reference to the chemical constituents which may have toxic effects. Toxicity studies or tests are undertaken to characterize any toxicity of a substance there by to ensure safety (Curtis, 2007). In all the cases, the toxic effects are usually manifested either in an acute or a chronic manner, and occur mostly as a result of an acute or chronic exposure to toxic compound by oral ingestion, inhalation or absorption following skin contact (Timbrell, 2002). They are essentially performed on either mice or rats because of their availability, low cost and their manageable laboratory size than other higher laboratory animals such as rabbits in which further investigation is usually done. Toxicity screening models provide important preliminary data to help select natural remedies with potential health beneficial properties for future work (Pour *et al*., 2011). In this study, the focus was on acute and subchronic toxicity tests. The primary concern was to determine how toxic the aqueous extracts of *A. afra* might be after acute and subchronic administration to rats.

An acute toxicity test is a test that is conducted in a suitable animal species with a single dose and may be done for essentially all chemicals that are of any biologic interest. Its purpose is to determine the symptomatology consequent to administration of the compound and to determine the order of lethality of the compound. The test consists of administering the compound to the animals on one occasion (Pascoe, 1983; Loomis and Hayes, 1996). Accordingly, in the present study, the aqueous extract of *A. afra* did not show any mortality with single oral dose up to 5000mg/kg body weight. Dosing of the animals above 5000mg/kg was not done with the recognition of the need to protect animal welfare. Testing of animals at and above (5000 mg/kg) ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health, and no further testing should be conducted at higher dose levels (OECD, 2008). Besides, behavioral changes such as loss of appetite, hypo-activity, pilo-erection, lethargic, dizziness and convulsion started to appear at dose 2200mg/kg. The symptoms, however, disappeared after the 1st week of observation. The present result, therefore, suggests that the oral LD_{50} of the extract is

greater than 5000mg/kg. Hence, the plant extract may be considered to be safe when administered orally. In line with this a previous study did not also produce lethality up to 8460mg/kg following oral administration of *Artemisia afra* and the plant is therefore considered as relatively non-toxic (James, 2005).

In the 14 days acute toxicity study, the weight gain of rats administered with doses up to 5000mg/kg was not significantly different ($p>0.05$) from that of the control group. Furthermore, no gross pathological changes such as in color, organ swelling, texture and atrophy or hypertrophy were observed after single administration of the extract as compared with the control group. Therefore, the overall weight gain in both experimental and control rats and the absence of gross pathological changes indicate the good health status of the animals. In all groups these results of the acute toxicity study go in-line with other previous study by Mukinda and Syce (2007) in which the body weights and gross anatomy of some of the internal organs including liver and kidney of rat and mice studied with acute and chronic oral administration of *A. afra* also not significantly changed as compared to the control.

There was no noticeable deviation in the behavior of the rats administered with the low dose (600 mg/kg) as compared to that of the control group, and essentially all the experimental rats remained healthy during the 3-month period of chronic oral administration of *A. afra*, Only rats in the group which received the higher dose (1800 mg/kg) manifested minor signs of toxicity, comprising intermittent diarrhea, salivation and partial hypoactivity. Such signs started by the third day of treatment and stayed only for three days. Moreover, no deaths were occurred with both of the doses. Significant Changes in body-weight have been used as an indicator of adverse effects of drugs and chemicals (Hilaly *et al*., 2004). With respect to this study, there was no significant change ($p>0.05$) in weight of both male and female rats throughout the study period, but there was a progressive non significant weight increment in both experimental and the control groups. Increment in body weight determines the positive health status of the animals (Heywood, 1983). Therefore, the overall weight gain in both experimental and control rats indicate a good health status of the experimental animals. Suggesting for the lack of toxicity in all groups, these results of the subchronic toxicity study go in-line with other previous study by James (2005) in which the body weights of mice studied with acute and chronic oral and

intraperitonial administration of *A. afra* were not significantly changed as compared to the control.

According to Lu (1996), remarkable change in relative organ weights between experimental and control animals is an indicator of toxicity as organ weight is affected by the suppression of body weight. However, in the present study there was no significant change in organ (liver and kidney) weights and visual gross examination of the organs of both experimental rats and the control. These showed normal architecture, no colour changes and no morphological disturbances, indicating that the subchronic oral doses of *A. afra* extract administered had no effect on the organs of the rats and was well tolerated over the 3-month study period.

Hematological parameters were evaluated to obtain further toxicity related information, not detected by direct examination of organs and body weight analysis. Studies on haematological parameters can easily reveal abnormalities in body metabolic processes. The blood profile usually provides important information on the response of the body to injury or lesion, deprivation and stress (Raza *et al*., 2002). While the hematopoietic system is one of the most sensitive targets for toxic compounds (Harper, 1993), there were no significant ($P > 0.05$) differences in the red blood cell, white blood cell with its differential, haematocrits, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet counts of the experimental versus the control rats. These results, therefore, indicated that 3-month administration of *A. afra* had no effect on the circulating cells nor interfered with their production. The haemoglolobin and the RBC levels were not affected suggesting that haemolytic anemia and polycythemia, (that are characterized by decreases and increases in RBC count) respectively (Mdhluli, 2003), were not likely to be induced by *A. afra* in this species. The platelet levels were also not adversely affected indicating that the plant extract also did not affect the production of platelets nor induced thrombocytopenia, the latter normally being the first evidence of drug-induced toxic effects on haematopoiesis (haematogenesis) (Mdhluli, 2003). The levels of white blood cells, (which serve as scavengers that destroy microorganisms at infection sites, remove foreign substances and debris that results from dead or injured cells (Miller and Harley, 1996; Guilhermino *et al*., 1998; Mdhluli, 2003), were also not changed significantly suggesting that the aqueous extract of *A. afra* did not have toxic effect on the immune

system. Collectively, all the results suggest that the subchronic ingestion of the aqueous extract of *A. afra* did not alter the haematological parameters of the rats.

Liver and renal function tests have a significant importance to evaluate changes produced by a toxicant. This is because of their response to clinical signs and systemic symptoms. To assess the possible toxic effect of a drug, evaluation of hepatic and renal function is primarily preferred as these organs are functionally predisposed. Elevated serum levels of enzymes produced by the liver or nitrogenous wastes to be excreted by the kidney might be an indication to their spillage into the blood stream as a result of necrosis of the tissues (Rahman *et al*., 2001).

Many compounds are metabolized in the liver, but if too many demands are made on this organ's capacity, the continued function of its cells is no longer ensured (Mdhluli, 2003). It is known that the liver and kidneys play significant roles in various metabolic processes. Liver plays an important role in xenobiotic function; and the kidneys are the main organs involved in drugs elimination, and, therefore, particularly exposed to the toxic effects of exogenous compounds (Bidhe and Ghosh, 2004). It was thus important to investigate the effect of *A. afra* on the function of these organs.

Because of its wide range of functions, any abnormal change in liver will definitely affect complete metabolism (Paliwal *et al.*, 2009). Liver chemistry tests include several serum chemistries that reflect liver function. The most commonly used serum liver chemistry tests include serum transaminases (alanine aminotransferase ALT, aspartate aminotransferase AST), serum alkaline phosphatase ALP, Gamma-glutamyl transpeptidase GGT, bilirubin and albumin. The major intracellular enzymes of the liver which are alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful enzymes as biomarkers predicting possible toxicity (Rahman *et al*., 2001). Any damage to the parenchymal liver cells will result in elevations in both of these transaminases (Wolf *et al*., 1992). However, AST is not specific for liver, because it is also present in other tissues like kidneys, heart, pancreas, skeletal muscle, brain and red blood cells. It is also important to consider extrahepatic tissue damage as a possible source of serum AST when evaluating the enzyme in relation to the liver. On the other hand, AST found in the serum is of both mitochondrial and cytoplasmic origin and if it is raised that can be taken as a first sign of

cell damage that lead to the outflow of the enzyme into the serum (Mdhluli, 2003). The level of AST obtained in this study; showed a dose dependent significant ($p < 0.05$) decrease in the female administered group at dose of 1800mg/kg. In line with this, a previous study by James (2005) had shown significant decrement of the AST levels at higher dose treatment group at day 92 suggesting that at higher doses, *A. afra* does not adversely affect the cell mitochondria; in fact may even stabilized these organelles (e.g. brought level back to the day 0 levels). Also oral administration of aqueous extract of *A. afra* attenuated the elevated activities of all investigated liver enzymes including AST in diabetic rats (Taofik and Anthony, 2013). This may be an indication of nontoxic nature and protective action of the extract in reversing liver damage due to diabetes. Unlike AST, ALT is purely cytosolic and is more specific for hepatocytes. Serum transaminases, especially ALT, are the most important markers of hepatic injury (Amacher, 1998). In the present study there was no significant change of ALT in both male and female experimental groups at doses of 600mg/kg and 1800mg/kg. These suggest that *A. afra* did not adversely affect the hepatocytes; in fact it may even stabilize the organelles. This result goes in line with Komperlla (2005) in which his formulation and evaluation of rapid release Tablets manufactured from *A. afra* plant material, at higher dose have liver protective effect.

Liver is also the site of bilirubin synthesis. It is a catabolic end product from the breakdown of hem. The normal level is less than 1 mg/dL (18 mmol/L). Elevated serum bilirubin levels generally reflect an imbalance between production and conjugation followed by excretion. Total bilirubin can be segmented further into a water-soluble form referred to as ‘‘direct/conjugated bilirubin’’ or a lipid-soluble form, namely, ‘‘indirect/ unconjugated bilirubin.’’ An elevation in direct bilirubin is highly specific for billary tract obstruction. However, impaired billary excretion, which is an energy-dependent process, is thought to be the reason for increased levels observed in sepsis, total parenteral nutrition, and following surgery (Zimmerman, 1999). Due to its small molecular size and water-soluble properties, direct bilirubin appears in urine (Thapa and Walia, 2007). In this study there was no significant change of bilirubin on both experimental groups of male and female rats. This indicated that the plant *Artemisia afra* did not have a role in derangement of liver function.

It is known that a major function of the kidneys is to remove waste products and excess fluid from the body. These waste products and excess fluid are removed through the urine. The production of urine involves highly complex steps of excretion and re-absorption. This process is necessary to maintain a stable balance of body chemicals. The critical regulation of the body's salt, potassium and acid content is performed by the kidneys. The kidneys also produce hormones that affect the function of other organs. For example, a hormone produced by the kidneys stimulates red blood cell production. Other hormones produced by the kidneys help regulate blood pressure and control calcium metabolism. Blood and urine tests show how well the kidneys are doing their job.

Urea & creatinine are the parameters to diagnose functioning of the kidney. Changes in serum creatinine concentration more reliably reflect changes in Glomerular filtration rate (GFR) than do changes in serum urea concentrations. Creatinine is formed spontaneously at a constant rate, and blood concentrations depend almost solely upon GFR. Urea formation is influenced by a number of factors such as liver function, protein intake and rate of protein catabolism (Griffin *et al*., 2008). The rise in serum level of these chemicals indicates a decline or failure in renal function to filter waste products from the blood and excrete them in the urine (Paula and Mark, 2011).

In the present study the subchronic oral administration of the extract did not show significant alteration in the serum levels of urea and creatinine concentrations in rats administered with both doses as compared to the control. Thus, absence of change in the levels of the above renal function markers suggests that the extract does not cause deterioration in the renal function. This is in accordance with Mukinda and Syce (2007) who investigated the safety of *A. afra* aqueous extract (mimicking the traditional decoction dosage form) by determining its pharmaco-toxicological effects after acute and chronic administration to rats and mice, respectively.

Histopathological examinations provide information to strengthen the findings on biochemical and hematological parameters. Cell death due to toxicity (necrosis) or immunologically mediated occurs via apoptosis**,** in which isolated hepatocytes become shrunken, pyknotic, and intensely eosinophilic (Kumar *et al*., 2002). Pyknosis causes the formation of dark staining masses against the nuclear membrane (Young, 2006). In

addition damage from toxic or immunologic insult may cause hepatocytes to take on a swollen, edematous appearance (ballooning degeneration) with irregularly clumped cytoplasm and large, clear spaces (Kumar *et al*., 2002). Such hepatocytes damage were not occurred in the present study as there was no focal necrosis, pyknosis, enlarged or fragmented nucleus within cytoplasm of hepatocytes at both 600 and 1800mg/kg doses.

On the other hand, there were minor periportal leukocytic infiltration at dose 600mg/kg and mild mononuclear leukocytic cell infiltration near central vein and portal area at dose 1800mg/kg. However, such minor inflammatory changes obtained in this study were not accompanied by significant change in any of the hematological and biochemical markers of liver injury measured. The reason of occurrence of the leukocytic infiltration is not clear, but it might be a response to parenchymal cell death with causes ranging from infectious agents, exposure to toxicants, generation of toxic metabolites, and tissue anoxia.

In line with the present findings a study conducted by Mukinda and Syce (2007) had shown no significant change in haematological and biochemical parameters, except for transient decrease in AST level. Moreover, there were no effect on the levels of AST and ALT, which are considered to be sensitive indicators of hepatocellular damage and within limits can provide a quantitative evaluation of the degree of damage to the liver (Thierry *et al*., 2011*)*. It is reasonable to deduce, therefore, that *A. afra* does not induce a significant damage to the liver at both doses of the present study in rats.

In line with the biochemical findings (the amount of creatinine and urea), the general histological architecture were not affected in any of the treatment groups as compared to the control, although minor tubulointerstitial lymphocytic infiltration were observed in some kidney sections of rats administered with the extraction only at 1800mg/kg body weight dose. Related study conducted previously by Mukinda and Syce (2007), had also concluded that acute and chronic oral administration of aqueous extract of *Artemisia afra* was not toxic to the kidney in rat and mice, respectively.

6. Conclusion

In Wister rats administered with both 600mg/kg and 1800mg/kg doses of the aqueous leaf extracts of *A. afra*, there was no significant change in the evaluated hematological and biochemical parameters as well the gross structures of liver and kidneys. In addition, the general behavior, organ and body weights of experimental rats were not significantly affected as compared to the control. This indicates that the aqueous leaf extracts of the plant does not significantly affect these organs and hence, does not compromise the hepato-renal function.

Findings in the acute toxicity test suggest that *A. afra* is practically non-toxic to liver and kidney when administered orally. Subchronic toxicity study indicated that *A. afra* is relatively safe when administered orally for an extended period at therapeutic (600mg/kg) and triple of therapeutic doses (1800mg/kg). Therefore, it can be concluded from the findings of the present study that the aqueous extract of *A. afra* is relatively safe.

7. Recommendations

In addition, to the above conclusions the observed results also raised the following concerns.

First, although the chronic oral doses of *A. afra* extract used in this study did not produce any significant adverse effects in rats, further studies using other organs like GIT, parts of brain and CVS, and other animals are needed to be investigated.

Second, the effect of various factors in the state of the plant material, such as the growth stage and maturity of the plant, the specific parts of the plant (leaves, roots, bark, flowers, seeds, etc), seasonal variation, storage conditions, may have effect of the chemical composition, and the toxicity studied may be extended addressing these issues.

Third, studies to determine the effects of *A. afra* on the fetus, in a pregnant animal, on the reproductive capacity of the animals, on the genetic system and to determine the ability of this plant to produce tumors (tumorigenicity and carcinogenicity tests) may also be investigated.

8. References

Abebe, 1986. Traditional Medicine in Ethiopia, The attempts being made to promote it for effective and better utilization. *Ethnobiol and Ethnomedic J.* **9**:61-69.

Abebe, 1996. In Proceedings of the Workshop on Development Utilization of Herbal Remedies in Ethiopia. Addis Ababa: University Academic Press:25-34

Ahmedulla M, Nayer MP. 1999. Red data book of Indian plants. Calcutta: Botanical survey of India : 4**-**7.

Akhigbe A, Amaechina F, Ataman J, Idu M, Odia E, Omogbai E. 2006. Effect of Stachytarpheta jamaicensis L. (Vahl) on wistar rats: serum biochemistry and ultrasonography. *Med Scien J.* **6(4)**:646-649.

Aller M, Arias JA, Duran H, Lorente L, Rodriguez G. 1999. hepatectomy in the rat using a microsurgical technique. **84**:135-138.

Aller M, Lorente L, Rodriguez J. 1995. Surgical anatomy of the liver in Wistar rats. Surg Res Commun **17**:113-121.

Alli P, Anyika E, Igbokwe N, Mbaka G, Nwakakwa N, Ogbonnia S. 2010. Antimicrobial evaluation, acute and subchronic toxicity studies of Leone Bitters, a Nigerian polyherbal formulation, in rodents. *Agric and Biol J.* N America **1(3)**:366-376.

Amacher D. 1998. Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. *Regulatory Toxico and Pharmaco J*. **27**:119-130.

Andrews R, Berndt M. 2003. Platelet physiology: In cold blood dispatch.**13**:282-284.

Asres K, Bucar F, Burits M, . 2001. The Antioxidant Activity of the Essential Oils of *Artemisia afra, Artemisia abyssinica* and *Juniperus procera*. *Phytother Res J.* **15**:103-108.

Asres K, Flatie T, Gebre Mariam T, Gedif T. 2009. Ethnomedical survey of Berta ethnic group Assosa zone, Benishangul-Gumuz regional state, mid-west Ethiopia. *Ethnobiol and Ethnomedic J.* **5:**14-15.

Baracho N, Vicente B, Arruda G, Sanches B, Brito J. 2009. Study of acute hepatotoxicity of Equisetum arvense L. in rats. Acta Cirurg Brasil **24(6)**:21-26.

Barrett K, Barman S, Boitano S, Brooks H. 2010. Ganong's Review of Medical Physiology, 23rd ed. The McGraw-Hill Companies: 165-234.

Basuony M. 1997. Ecological variability and kidney structure of eight rodents. *Egypt J. Histol* **20**:417-434.

Begg EJ, Barclay ML. 1995. Aminoglycisides-50 years on. *British J. Clin Pharmacology* **39:** 597-603.

Bello I, Oduola T, Adeosun G, Ademosun A, Raheem G, Avwioro G. 2010. Hepatotoxicity and nephrotoxicity evaluation in wister rats exposed to Morinda lucida leaf extract. *American J. Medic Scien* **2(5)**:230-233.

Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. 1997. Uses and properties of citrus flavonoids. *Agricultural and Food Chemistry J.* **45**:4505-4515.

Bernstein S. 1993. Problems and potentialities of hematopoietic tissue transplants. New England Surg Socie **44**: 76-83.

Bidhe RM, Ghosh S. 2004. Acute and subchronic (28-Day) oral toxicity study in rats fed with novel surfactants. AAPS *Pharm Sci J.***14**:1-10.

Bremer K. 1994. In Asteraceae: Cladistics and Classification. Timber Press Oregon: 752-784

Charmi AM, Bahmani MM, Sajjadi RK. 2009. Morpho-histological study of kidney in farmed juvenile beluga. *Biol Sci J.* **12**:11-18.

China Great vista chemicals c. 2002. Herb Extracts: Luteolin. Am. www.greatvistachemicals.com /herb extracts /luteolin.htm J Respir Crit Care Med.

Clark M, Wallis M. 2003. Blood flow and muscle metabolism: A focus on insulin action. *American J. Physiol- Endocr and Metab* **284 (2)**:241-258.

Colson C, Broe M. 2005. Kidney injury from alternative medicines. Advances in Chro. Kidn. Disease. **12 (3)**:261-275.

Cook M. 2008. The anatomy of the laboratory mouse. Academic press, London:149-186

Čukuranović R, Vlajković S. 2005. Age related anatomical and functional characteristics of human kidney. *Medic and Biol J.* **12(2)**:61- 69.

Cunningham A, Hutchings A, Lewis G, Scott AH. 1996. Zulu Medicinal Plants: An inventory. South Africa, University of Natal press. Scottsville:327-421

Curtis D. 2007. Casarett and Doull's Toxicology: The basic science of poisons 7th ed. The McGraw-Hill Companies:21-65.

Debella A. 2002. Manual for phytochemical screening of medicinal plants. Department of Drug Research, EHNRI, Addis Ababa, Ethiopia:1-55.

Desta B. 1988. Ethiopian traditional herbal drugs potentiality and appropriate utilization. Proceedings on the 8th International Conference of Ethiopian Studies IES, AAU. Ethiopia:763-765.

Diggs, Bell , Sturm. 1975. The morphology of human blood cells. In Wright Stained Smears of Peripheral Blood and Bone Marrow, 3rd ed. North Chicago, Abbott Laboratories,.

Dyson A. 1998. Discovering indigenous healing plants of the herb and fragrance gardens at Kirstenbosch National Botanical Garden.Cape Town. National Botanical Institute, the Printing Press:9-10.

Ebo D, Bridts C, Hagendorens M, Aerts N, De Clerck L, Stevens W. 2008. Basophil activation test by flow cytometry: Present and future applications in allergology. Cytometry Part B (clinical cytometry) **74**:201-210.

El-Beltagy A. 2002. Studies on functional comparative anatomy of the kidney in some small mammals. *Mansora Fac of Sci J.*:345-421

El-Salkh A, Zaki T, Mohammad I, Khidr H. 2008. Anatomical, Histological and Histochemical studies on some organs of true desert rodents in the Egyptian Habitats. *Hosp Med J.* **33**:578- 806.

Elias H, Gershbein L. 1994. Observations on the anatomy of the rat liver. Anat Rec **120**:85-98.

Erlund I. 2002. Chemical analysis and pharmacokinetics of the flavonoids quercetin, hesperetin and naringenin in humans. Academic dissertation. Department of Applied chemistry and Microbiology, University of Helsinki. Helsinki:1-2

Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, O.M G, VanStaden J. 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and Toxicology. *Ethnopharmacology J.* **94(2-3)**:205-217.

Furugori M, Goda T, Hara Y, Okada H, Shimoi K, Suzuki M, Takase S, Yamamoto H, Kinae N. 1998. Intestinal absorption of luteolin and luteolin 7-O-ßglucoside in rats and humans. FEBS Letters, **438**:220-224.

Garciai F. 1997. Changes in blood,plasma and red cell volume in the rat, as a function of age. *Physiol J.* **190**:19-24.

Gartner L, Hiatt J. 2000. Color atlas of Histology, 3rd ed, Lippincott Williams and Wilkins. USA : 89-93.

Gericke N, Van OB, Van Wyk B-E. 2000. Medicinal plants of South Africa. 2nd ed. Tien Wah Press, Singapore: 44-75

Gericke N, van Oudtshoorn B, Van Wyk B. 1997. Medicinal Plants of South Africa. 1st ed. Briza, South Africa, Briza Publications, Pretoria, South Africa. 9:37-63.

Giboney. 2005. Mildly elevated liver transaminase levels in asymptomatic patient. Am Fam Physician.**71(76)**:1105-1110.

Gidey. 2011. Survey of medicinal plants used to treat human ailments in Hawzen district, Northern Ethiopia: 2-41.

Gordon S, Taylor P. 2005. Monocyte and macrophage heterogeneity Nature Reviews: Immun. **5**:953-964.

Graaf K. 2001. Human anatomy, 6th ed, The McGraw-Hill Companies, Inc :638- 640.

Griffin K, Kramer H, Bidani A. 2008. Adverse renal consequences of obesity. *American J. of Physiology - Renal Physiology* **294**:685-696.

Gude, Cosgrove, Hirsch. 1982 Histological Atlas of the Laboratory Mouse. Plenum Press, New York 54-65

Guilhermino L, Soares AMVM, Carvalho AP, Lopes MC. 1998. Acute effects of 3, 4-Dichloroaniline on blood of male wistar rats. *Chemosphere J.* **37**:619-632.

Guyton A, Hall J. 2006. Text book of Medical Physiology, 11th ed, Elsevier Saunders. 307-309, 859-862.

Harborne JB, Williams CA. 2000. Advances in flavonoid research since 1992. *Phytochemistry J.* **55**:481-504.

Harper HA. 1993. Review of physiological chemistry, 14th ed. California, Lange medical publications:185-402.

Harris L. 2002. An evaluation of the bronchodilator properties of Mentha Longifolia and *Artemisia afra*, traditional medicinal plants used in the Western Cape M. Thesis, Discipline of pharmacology. School of pharmacy, University of the Western Cape. Bellville:45-56

Health, Safety. 2004. Glossary of health & safety terminology www.delta.edu/slime/glossary.html.

Heywood. 1983. Long term toxicity. In: Balls M, Riddell RJ, and Worden AN, editors, *Animals and alternatives in toxicity testing,* London: Academic Press:79-89.

Hilaly JE, Israili ZH, Lyoussi B. 2004. Acute and chronic toxicological studies of Ajuga Ivain experimental animals. *Ethno-pharmacology J*. **91**:43-50.

Irshaid F, Mansi K. 2009. The effect of methanol extract derived from Utrica pilulifera leaves on some hematological and biochemical parameters of diabetic rats. *Res J. Bio Scien* **4(6)**:675-681.

Iwu M M. 1993. Handbook of African Medicinal plants. USA, Florida, CRC Press:121-122.

James T. 2005. Acute and chronic toxicity of the flavonoid-containing plant, *Artemisia afra* in rodents: 66-74.

Junqueira L, Carneiro J. 2005. Basic Histology, 11th ed. The McGraw-Hill Companies Inc:223-237 and 323-324.

Junqueria LC. 1998. Basic histology. 9th ed. Rio de Janeiro:178-182

Komperlla M. 2005. The formulation and evaluation of rapid release Tablets manufactured from *Artemisia afra* plant material:78-82

Kongure K, Kuwano H, Ishizaki M, Makuuchi MA, Nemoto M. 1999. comparative study of the anatomy of the rat and human livers. Hepatobiliary Pancreat Surg **6**:171-175.

Kroll D, Winslow L. 1998. Herbs as medicines. Archives of Internal Medic **158**:2192-2199.

Kumar V, Cotran R, Robbins S. 2002. Robbins Basic Pathology 7th ed. Elsevier Saunders, Philadelphia: 592-593.

Lewington. 1993. Medicinal plants and plant extracts: A Review of their Importation into Europe. Cambridge, UK:92-96

Liang XM, Jin Y, Wang YP, Jin GW, Fu Q, Xiao YS. 2009. Qualitative and quantitative analysis in quality control of traditional Chinese medicines. Chromatogr **1216**:2033-2044.

Liang Y-Z, Xie PS, Chan K. 2004. Quality control of herbal medicines. Chromatogr **812**:53-70.

Liu N, van der Kooy F, Verpoorte R. 2009. *Artemisia afra*: A potential flagship for African medicinal plants. *South Afr J. Bot* **75**:185-195.

Loomis TA, Hayes AW. 1996. Loomis's essentials of toxicology. 4th ed. California, Academic press:208- 245.

Lu F. 1996. Basic Toxicology: fundamentals, target organs and risk assessment, 3rd ed, Taylor and Francis, Washington:17-86.

Mander M. 1998. Marketing of Indigenous Medicinal Plants in South Africa: A Case Study in KwaZulu-Natal. Food and Agricultural Organization of the United Nations, Rome: 23-25

Mdhluli M. 2003. Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (Chlorocebus aethiops). PhD Thesis, Discipline of physiological sciences, University of the Western Cape. Bellville: 67-74.

Mercan U, Ahmet C, Hatice Ö, Nureddin C, Remzi E, Fevzi Ö, Hanefi Ö. 2008. Investigation of Acute Liver Toxicity and Anti-Inflammatory Effects of *Artemisia austriaca*.**1**:131-138.

Miller SA, Harley JP. 1996. Zoology, 3rd ed. Wm.C. Brown Publishers, USA:114-123.

Miruts G. 2007. Medicinal plants of Bench, Meint and Sheko cultural groups in Ethiopia with emphasis on use diversity, information consensus, abundance and habitat. Ph.D thesis submitted to Addis Ababa University:37-42

Miruts G, Zemede A, Thomas E, Zerhun W. 2003. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *Ethnopharmacol J.* **85**:43-52.

Mirutse. 2001. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia:78-89.

Miura K, MacGlashan D. 1998. Expression of protein kinase C isozymes in human basophils: Regulation by physiological and nonphysiological stimuli. Blood. **92(4)**:1206-1218.

Moges K, Robert M, Azeb R, Getachew A. 1998. In Vitro Test of Five Ethiopian Medicenal Plants for Antimalarial activity againest plasmodium Falciparum.*Ethiop. J. Sci* **21(1)**:81-89.

Monteiro E, Zanchet D. 2002. Pig liver sectorization and segmentation and virtual reality depiction. *Acta Cir Bras J.* **17**:381-386.

Moore K, Dalley A. 2006. Clinically Oriented Anatomy, 5th ed. Lippincott Williams and Wilkins:289-311.

MRC, Healthinfo S. 2004. Traditional medicines database: www.mrc.ac.za/Tramed3/Tramed3PlantPharmacologyDetails.

Mucciarelli M, Maffei M. 2002. Introduction to the genus Artemisia. In Wright CW (ed) Medicinal and Aromatic Plants - Industrial Profiles, Taylor & Francis, London:1-50.

Mukinda J, Syce J. 2007. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Ethnopharmacol J.* **112**:138-144.

Nwanjo H, Oze G, Onyeze G. 2007. Nephrotoxicity caused by the extract of Alstonia boonei (De Wild) stem bark in guinea pigs. *The Internet J. of Nutrition and Wellness* **3(2)**:1-3.

OECD. 2001. The Organization of Economic Co-operation and Development guideline for testing of chemical: 420 Acute Oral Toxicity. France:1-14.

OECD. 2008. Guidelines for testing of chemicals acute oral toxicicty - Fixed Dose Procedure: 1-12.

Oyewole I, Magaji Z, Awoyinka O. 2007. Biochemical and toxicological studies of aqueous extract of Tithonia diversifolia (Hemsl.) leaves in wistar rats. *Medic Plants Res J.* **1(2)**:30-33.

Paliwal A, Gurjar R, Sharma H. 2009. Analysis of liver enzymes in wistar rat under stress of λ-cyhalothrin and nuvan toxicity. *Biol and MedicJ.* **1(2)**:70-73.

Pappas R, Sheppard- Hanger S. 2004. *Artemisia arborescens*-essential oil of the Pacific Northwest: a high-chamazulene, low-thujone essential oil with potential skin- care applications. Atlantic institute, Reference manual www.atlanticinstitute.com/artemisia.pdf

Pascoe D. 1983. Toxicology. England, London, Edward Arnold limited:1-60.

Pass, Freeth. 1993. Biological Statistics of the Norway Rat:54-65

Paula A, Mark A. 2011. Kidney function test. Retrieved on 5 April 2013 from www.surgeryencyclopedia.com.

Piper M, Treuting D, Suzanne M. 2012. Comparative Anatomy and Histology:198-206

Popesko, Rajtovia, Horak. 1990. A Colour Atlas of Anatomy of Small Laboratory Animals,. Wolfe Publishing Ltd, London: 21-34

Pour B, Latha L, Sasidharan S. 2011. Cytotoxicity and oral acute toxicity studies of Lantana camara leaf extract.**16**:3663-3674.

Rahman MF, Siddiqui MK, Jamil K. 2001. Effects of Vepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. *Human and Experimental Toxicology J.* **20**:243-249.

Ramadori G, Moriconi F, Malik I, Dudas J. 2008. Physiology and Pathophysiology of liver inflammation, damage and repair. *Physiology and Pharmacol J.* **59**:107-117.

Rates S. 2001. Plants as source of drugs. Toxicon. **39**:603-613.

Raza M, Al-Shabanah O, El-Hadiyah T, Al-Majed A. 2002. Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. *Pharmace Scien J.* **70 (2)**:135-145.

Robbins S, Cotran R. 2005. Pathological Basis of Disease, 7th ed, Elsevier Saunders, Philadelphia: 879-882 and 960-970.

Roberts M. 1990. Indigenous healing plants. South Africa. Southern Book:226-228.

Saad B, Azaizeh H, Abu Hijleh G, Said O. 2006. Safety of traditional Arab herbal medicine. Evidence Based Complementary and Alternative Medic. **3(4)**:433-439.

Steenkamp V. 2003. Traditional herbal remedies used by South African Women for Gynaecological complaints. *Ethno-pharmacology J.* **86**:97-108.

Tabuti JRS, Lye KA, Dhillion SS. 2003 Traditional herbal drugs of Bulamogi, Uganda: plants use and administration. *Ethno-pharmacology J.* **88**:19-44.

Taofik O. Sunmonu and Anthony J. Afolayan. 2013. Evaluation of Antidiabetic Activity and Associated Toxicity of *Artemisia afra* Aqueous Extract in Wistar Rats. *African J. of Complementary and Alternative Medicine* **1**:8

Thapa B, Walia A. 2007. Liver function test and their interpretation. *Indian J. Pediatrics* **74**:67-75.

Thierry, Acha, Paulin, Aphrodite, Pierre, Tazoacha. 2011. Sub acute toxicity study of the aqueous extract from Acanthus montanus Djami Tchatchou. *Electronic J. Biol*.**7(1)**: 11-15.

Timbrell J. 2002. Introduction to toxicology. 3rd ed. London, Taylor & Francis:163-179.

Tortora G, Derrickson B. 2009. Principles of Anatomy and Physiology, 12th ed, John Wiley & Sons: 114-132 and 234-260.

Van der Walt L. 2004. *Artemisia afra*.page downloaded from the South African Biodiverisity Institute's Plant Information, accessed from plantzafrica.com.

Van Wyk B-E, Van OB, Gericke N. 2000. Medicinal plants of South Africa 2nd ed. Tien Wah Press, Singapore: 44-46

Verlander J. 1998. Normal ultrastructure of the kidney and lower urinary tract. *Toxicol Pathology J.* **26**:1-17.

Vipul G, Nilesh P, Venkat R, Nandakumar K, Gouda T, Shalam M, Kumar S. 2007. Hepathoprotective activity of alcoholic and aqueous extracts of leaves of Tylophora indica (Linn.) in rats. *Indian J. Pharmacol* **39**:43-47.

Waithaka J. 2004. The evaluation of markers for quality control studies of flavonoid-containing medicinal preparations. M. Thesis, Discipline of pharmacology. School of pharmacy, University of the Western Cape. Bellville :65-68.

Waynforth HB. 1980. Experimental and surgical technique in the rat. London, Academic press:17-68.

WHO. 2002. traditional medicine strategy. Geneva. **2**:2002-2005.

WHO. 2003. SARS: clinical trials on treatment using a combination of traditional chinese medicine and western medicine. Geneva : 53-61.

WHO. 2008. Traditional medicine fact sheet. Geneva:1-7.

Willcox M, Bodeker G. 2004. Traditional herbal medicines for malaria. **329**:1156-1159.

William JB, Linda MB. 2000. Color atlas of veterinary histology 2nd ed. Lippincott Williams & Wilkins:654-678.

Wolf PL, Williams D, Tsudaka T, Acosta L. 1992. Methods and Techniques in clinical chemistry. USA, John Wiley & Sons:132-196 and 375-383.

Yineger H, Yewhalaw D. 2007. Traditional medicinal plant knowledge and use by local healers in Sekoru District, Jimma Zone, Southwestern Ethiopia. *Ethnobiol and Ethnomedicine J.* 3:24.

Young A. 1999. The physiology of lymphocyte migration through the single lymph node in vivo. Seminars in Immunology:11-13.

Young A. 2006. The physiology of lymphocyte migration through the single lymph node in vivo. Seminars in Immunol **11**: 73-83.

Zimmerman H. 1999. Intrahepatic cholestasis. *Arch Intern Med J.* **139**:1038-1045.

9. Appendices

9.1. Appendix I: Preparation of working solutions

10% Neutral Buffered Formalin

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

Harris's Hematoxylin (H)

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

1% Alcoholic Eosin (E)

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

1% Acidic alcohol

70% alcohol	500 ml
Hydochloric acid, concentrated	5 ml

Bluing solution

Sodium bicarbonate	2.5 gm
Distilled water	1000 ml

9.2. 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	$1^1/_2$hrs
Absolute alcohol II	$1^1/_2$hrs
Absolute alcohol III	$1^1/_2$hrs
Absolute alcohol IV	overnight
Clearing	
Xylene I	$1^1/_2$hrs
Xylene II	$2^1/_2$hrs
Infiltration	
Paraffin wax I	$2^1/_2$hrs
Paraffin wax II	overnight

9.3. Appendix III: Hematoxylin and Eosin (H & E) Staining Protocol

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