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THE EFFECTS OF CHRONIC TREATMENT WITH *DODONAEA ANGUSTIFOLIA* SEED EXTRACTS ON SOME HAEMATOLOGICAL AND BIOCHEMICAL COMPOSITION OF BLOOD AND HISTOPATHOLOGY OF LIVER AND KIDNEY IN MICE

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THESIS

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfilment of the requirements for the Degree of Master of Science in Anatomy

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AAU	Addis Ababa University
ALP	Alkaline phosphatase,
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransfrase
BW	Body weight
DPX	Dibutyl Phthalet in Xyline
EDTA	Ethylenediaminetetra acetic acid
EHNRI	Ethiopian Health and Nutrition Research Institute
FOM	Faculty of Medicine
H & E	Haematoxylin and Eosin
HGB	Haemoglobin
LD_{50}	Lethal dose
L. f.	Linnaeus f
LYMP	Lymphcyte
MCH	Mean Morpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
NEUTR	Neutrophils
PLT	Platelete
RBC	Red Blood Cell
SEM	Standard Error of the Mean
SPSS	Statistical Package for Social Sciences
WBC	White Blood Cells
WHO	World Health Organization

ABSTRACT

In Ethiopia many medicinal plants are used for the treatment of malaria including *Dodonaea angustifolia* seed with out considering their side effects. Therefore, the present study attempts to evaluate the chronic effect of crude extracts of *D. angustifolia* seeds on some hematological and biochemical parameters and histopathology of liver and kidney of mice. For the chronic treatment, animals were administered with the crude extracts of the plant through the oral route using oral gavage for six weeks at doses of 400 mg/kg/bw and 800 mg/kg/bw of the aqueous extract and 400 mg/kg/bw and 600 mg/kg of the methanolic extract. At the end of the experiment, all mice under treatment were sacrificed after blood collection for hematological and biochemical analysis and liver and kidney sections were randomly taken for tissue processing. The results of hematological and biochemical analysis as well as the microscopic examinations of the liver and kidney sections were compared with control groups. The hematological and biochemical results showed no statistically significant differences between the aqueous extract treated mice at both doses of 400 mg/kg/bw and 800 mg/kg/bw and the control group. In contrast, statistically significant elevation in ALT, AST, urea and creatinine were observed in methanolic extract treated mice at a dose of 600 mg/kg/bw as compared to the control group. Histopathological examinations of the liver and kidney sections of aqueous crude extract treated mice at both doses of 400 mg/kg/bw and 800 mg/kg/bw showed similar histological appearance as that of the control group except some focal inflammation were observed around central vein at dose of 800 mg/kg/bw of the aqueous extract of the plant. In contrast, significant histopathological changes were observed in liver and kidney sections of methanolic crude extract treated mice at dose of 600 mg/kg/bw with focal cellular infiltration around central vein of the liver sections at 400 mg/kg/bw showing the dose dependent effect of methanolic crude extract of the plant. In conclusion, the aqueous crude extract is safe to use at effective dose of 400 mg/kg/bw whereas the methanolic crude extract of *D. angustifolia* at the same effective dose (400 mg/kg/bw) as that of aqueous crude extract has slight toxic effect but adversely affects the biochemical parameter and histology of liver and kidney as the dose level increases to 600 mg/kg/bw. As the aqueous extract did not show adversity at the effective dose, it is recommended that medicinal plant users choose the aqueous extract of the plant. Further investigation is also recommended to isolate metabolites that may contribute the toxic effect of methanolic crude extract on liver and kidney.

Key words: *Dodonaea angustifolia,* chronic toxicity, liver, kidney, mice

1. INTRODUCTION

1.1. Traditional Medicine

"Traditional medicine is the sum total of the knowledge, skill and practice based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health, as well as in prevention, diagnosis, improvement or treatment of physical and mental illnesses" (WHO, 2000). It has been in practice for a long period of time.

Plant derived drugs remain important, especially in developing countries, to treat different diseases. Approximately 60 – 80% of the world's population still relies on traditional medicine for the treatment of common illnesses and WHO also indicated that about 75% of the world population relies on traditional remedies for primary health care. As a result, the medicinal use of herb is deep rooted in human history and folklore, and has been included into the traditional medicine of all human cultures. Moreover, strong religious beliefs have been related to the healing properties of many herbs (Alemayehu, 2001).

Traditional medicine has been the back bone of the health care system of developing countries where the majority of the population is either not able to afford modern medicine or has no adequate information concerning the use of modern medicine and this may be due to various reasons, including limitation of conventional or modern medicine and lack of formal health care facilities.

WHO officially launched an international program to promote traditional medicines, which included the promotion and development of basic and applied research in traditional medicine (WHO, 1978). Hence herbal medicine has become a topic of increasing global importance to play a pivotal role in the health care system of large proportion of world's population particularly in developing countries where traditional system of medicine has a long history and is part of indigenous culture (Aniagu *et al.,* 2005; Fekadu, 2002).

Ethiopia is a country rich in its flora and fauna. The flora of Ethiopia consists of some 6,000 to 7,000 species, about 10% of which are believed to be native to the country (Hareya, 2005), and most of them are used as an important traditional medicine of Ethiopia (Teferi and Heinz, 2003) and occur throughout the diverse highland areas as well as in Southwest and central lowlands of the country. It was also reported that medicinal plants occur throughout

the country as important sources of traditional medicine and indigenous health care system of the county-Ethiopia (Aberra, *et al*., 2005; Teferi and Heinz, 2003). As a result, most of the rural population of the country depends on enormous resource of plants that are used as medicine as in many African countries. This continued dependence on traditional medicine is partly due to economic circumstances and partly because pharmaceuticals are out of reach for the majority of the population (Hareya, 2005).

Generally, traditional medicine practitioners believe that the traditional medicines are non – toxic without adequate knowledge of their adverse effects. As this herb continues to receive attention and more of its medicinal values discovered day by day, there is a need to investigate the toxicity effect of its consumption on liver and kidney functions of animal models (Oyewole and Massaquoi, 2008) due to the fact that liver and kidneys are organs commonly affected by toxicity.

The safety of several commercially available herbs has recently come into question due to reports of adverse effects and potential interactions with prescribed drugs (Popatal *et al*., 2001). Nowadays, one of the most severe examples of the potential for harm associated with herbal medication is the development of renal failure and hepatic dysfunction. Moreover, there are numerous examples of potential adverse effects associated with the most commonly used herbal and other types of complimentary and alternative medicine (Kefale, 2005).

By and large, real drawback in traditional medicine stem mostly from lack of precision in dosage and the imprecise nature of diagnosis, especially of chronic and complicated conditions (Dawit and Ahadu, 1993). The concept of side effects is more probably elaborated in the traditional medicine (Gilani and Rahman, 2005). However, significant achievement can be made in alleviating drug shortages through standardization of dosage and quality control of the total or partially purified extracts of widely used remedies that are proven safe and effective on the basis of *in vivo* and/or *in vitro* laboratory investigations (Dawit and Ahadu, 1993).

Inappropriate use of these medicinal plants may result in chronic damage to blood composition and tissue of vital organs such as heart, liver and kidney. For example, exposure of the kidneys to circulating toxins leads to pathological changes which in turn lead to disruption of glomerular functions. Subsequently, the renal tubular function will also be affected. From these points of view, the toxicity of undetermined traditional medicinal plant

could result in tissue or organ damage (Dapar *et al*., 2007). Likewise, the prolonged administration of ethanolic extract of *Khaya senegalenis* resulted in significant reduction in the alkaline phosphate activities of the kidney, and its body weight ratio. Prolonged administration of the same extract resulted in significant increase in the serum sodium concentration (Adebayo *et al*., 2003). According to Khleifat *et al* (2002), chronic treatment with 50 mg/kg of *Teucrium polium*, induced marked cytoplasmic vacuolation of liver and kidney tubular cells.

1.2. *Dodonaea angustifolia* L. f.

Dodonaea(D), which belongs to the family *Sapindaceae*, comprises many species most of which are used as traditional medicine in different countries of the world and is known for its folk remedies. *D. angustifolia* is an evergreen shrub widely distributed almost throughout the world specially in Mexico, Malesia, Australia, Ethiopia, Kenya, New Zealand, Oman, South Africa and Tanzania (Anilreddy, 2009; Orwa *et al.,* 2009; Tesfaye, 2000). In Ethiopia, *D. angustilolia* is widely distributed in different regions of the country. It is commonly called Dhitcha in "Afaan Oromoo" and "Kitkita" in Amharic and is a variable shrub or tree usually 4m – 8m tall with dark green barks. The leaves are simple inflorescence axillary, pale green with rounded tip. The flowers are unisexual and clustered at the end of the twigs. Petal is absent. Stamens are brown. Fruits are calving green and winged. The seeds are black, smooth, compressed–ovoid, and about 3mm long (Tesfaye, 2000).

The seed coats of most of the seeds are impermeable to water and there also appears to be a micropylar plug present where the hard seed coat may play a role in delayed germination (Burrows, 1995). As a result, the seeds can be stored for up to one year with germination rates ranging between 30% - 70%. There are about 100,000 seeds/kg of *D. angustifolia* (Tesfaye, 2000).

D. angustifolia grows best at 800 – 2650m above sea level in areas with rainfall range of 500 – 1500 mm/year (Tesfaye, 2000). It is the most aggressive colonizer on disturbed ground, even in rocky soil. It was observed that *D. angustifolia* by itself is free from any pests due to the presence of secondary metabolites (Subashini *et al.,* 2004). Due to the presence of insecticidal property and its wide distribution, it could be used as a botanical pesticide after exploring its toxicity and field trials (Subashini *et al.,* 2004).

Fig.1. Kitkita (*D. angustifolia*) plant growing in Harmania around Debresina, North Shewa, Amhara Region

D. angustifolia is reported to contain secondary metabolites like flavonoid, catechol, tannis, terpenoid, saponins, alkaloids, resins, diterpenoids, phenols, coumarins, and essential oils (Berhan, 2008; Subashini *et al.,* 2004; Amabeoku *et al.,* 2001). The major flavonoids detected in *D. angustifolia* include quercetine, isorhamnetin, narcissin and rutin (Subashini *et al*., 2004).

D. angustifolia is reported to possess several medicinal values. According to Subashini *et al*. (2004), *D. angustifolia* has a wide range of therapeutic applications since ancient times, and is used in India against pneumonia and other pulmonary diseases including tuberculosis. Moreover, these authors indicated that a decoction of the plant is used as tonic. In Mexico, it is also used for treatment of rheumatism, skin infection, diarrhea, pain of hepatic or spleenic origin, uterine, colic and other disorders involving smooth muscle (Rojas *et al*., 1996; Rojas *et al.,* 1995). The root infusion is used as remedy for common cold in East and South Africa. The leaves have anaesthetic properties and are chewed for their stimulating effect. Other

medicinal uses are for fever, sore throats, chest complaints, influenza, stomach disorders, dermatitis, cancer and haemorrhoid in Tanzania (Chhara *et al.*, 1991; Hedberg *et al.*, 1983) and for treatment of skin lesion and treatment of malaria in Ethiopia (Berhan, 2008; Desta, 1993). *D. angustifolia* is also used in South Arabia as household remedy for boils, as a mild purgative and in the treatment of inflammation of chest cold and for the treatment of hypertension (Mekkawi and Mossa, 1981).

The presence of quercetine and isorhanetin in large concentration in *D. angustifolia* are effective in the treatment of skin or mucous membrane inflammation (Getie *et al.,* 2002). Moreover, Australian aborigines use the leaves and roots as pain killer to soothe toothaches and head aches (Mekkawi and Mossa, 1981).

The crude extract of *D. angustifolia* along with other two medicinal plants, *Rumex nervosus* and *Rumex abssinicus*, possesses antibacterial activities but none of them exhibited antifungal activity (Getie *et al.,* 2003). The methanolic extract of the *D. angustifolia* has antimicrobial effect (Getie *et al*., 2003; Rojas *et al.,* 1992; Wagner *et al*., 1987). Ethanolic extract of the dried leaf of the Ethiopian provenance, however, showed no *in vitro* antimicrobial activity against mycobacterium tuberculosis (Asres, *et al*., 2001). *D. angustifolia* along with *Salvia africana-lulea* significantly reduced fever induced by lipopolysaccharide (Thring *et al.,* 2007; Amabeoku, 2001). Moreover, extract from the entire plant, (leaves, stem and roots), along with other medicinal plant is used as antiviral activity against HIV type one (HIV-1) and type two (HIV-2) having 50% cytotoxic concentration of 0.5mg/ml (Asres *et al.,* 2001).

The leaves of *D. angustifolia* are reported to contain flavonoid. Flavonoids are natural components of human and animal diet and have been shown to exert different biological effects, such as antiviral, anti-inflammatory, antimutagenic and anticarcinogenic function (Kefale, 2005). Some of its activities were reported to result partly from their antioxidant and antiradical properties of the flavonoids (Zielinska *et al.,* 2001). It was indicated that the isolated diterpenoid and flavonoid derivatives from *D. angustifolia* were responsible for the remarkable antioxidant and antimicrobial effect (Rojas *et al.,* 1992). The root juice is taken against ascaris while that of the leaf is taken against tape worm (Dawit *et al.,* 2003).

Kefale (2005) reported that quercetine serve as the backbone for flavonoid and is the most active flavonoid. Many medicinal plants have significant quercetine contents. Quercetine blocks mast cell and basophilic histamine deregulation, inhibits xanthine oxidase (the enzyme

producing uric acid) and aldose reductase (the enzyme converting glucose into sorbitol), and decrease neutrophil lysosomal enzyme secretion (Kefale ,2005).

Different literature surveys reported that some biological activity studies conducted on this plant species of different countries demonstrated variable results. For instance, methanolic extract of *D. angustifolia* leaf showed activity against *Escherichia coli*, *Pseudomonas aeroginosa*, and *Staphylococcus aureces* in Mexican species (Rojas *et al.,* 1992). Antibacterial activity was observed by ethanolic extract of the leaves of the Indian species of *D. angustifolia* and the Ethiopian species *D. angustifolia* has also reported to have antibacterial activity and strong antiviral activity against influenza (Getie, 2000).

The *in vitro* antimalaria activity of *D. angustifolia* was previously reported by Clarkson *et al* (2004). They reported that the extract of *D. angustifolia* seeds showed 50% in vitro concentration (IC_{50}) value of 15.5µg/ml. In the rural area of Ethiopia, crude aqueous extract of *D. angustifolia* seed is used for treatment of malaria (Berhan, 2008) and this is supported by the *in vivo* antiplasmodial activity against malaria. The effective dose for both extracts, aqueous and methanol, of *D. angustifolia* seeds was found to be 400 mg/kg/bw with 35.79% chemosuppressive effect of the earlier extract (Berhan, 2008). The adverse effects of *D. angustifolia* seeds on some haematological and biochemical parameters of blood and histopathology of liver and kidney of mice need to be evaluated.

1.3. The Blood and Its Composition

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

The formed elements of the blood are Red Blood Cells (RBC), White Blood Cells (WBC) and Platelets. RBCs are the most abundant formed elements and have a simple structure, with no nucleus or additional cytoplasmic organelles such as mitochondria at maturity. RBCs are packed with haemoglobin, the oxygen carrying protein pigment which gives red coloration to the blood and has red cell indices containing mean cell volume and mean cell haemoglobin concentration. These packed cell volume and the haemoglobin concentration may be reduced in number due to effect of administered nature cure bitters indicating that these herbs may produce some anaemic effect (Aniagu *et al.,* 2005).

WBC is larger than RBC, contains nucleus and other cytoplamic organelles. A normal WBC count is about 5000 – 10000/mm^3 in blood of average adult human individuals. White blood cells are capable of crawling movement known as amoeboid movement which allows them to squeeze through pores in capillary wall to reach the tissue and site of infection and perform their function outside the circulatory system using the blood stream as mode of transportation to reach their destination and play an important role in body's defence mechanisms against introduced foreign substances such as biologically active ingredients of administered herbal medicine (Taylor *et al.*, 1997). As a result, dose dependent increase in number of WBC count indicates that the administered herbal medicine contains biologically active components that stimulate the immune system through increasing the number of defensive WBC.

There are five types of WBC which constitute different concentrations. These include the neutrophils, eosinophils, basophils, monocyte and lymphocyte constituting about 40-75%, 1-6%, <1%, 2-10% and 20-40%, respectively in humans (Zhong Shi, 2004; Taylor *et al.*, 1997).

Neutrophils are abundant WBCs and are commonly squeezed between the cells of the capillary wall and wonder through the intercellular space where they actively phagocyte, engulf, digest and eliminate disease causing microorganisms. They play an important role in host-defence mechanisms against all classes of infectious agents and they are also involved in the pathology of various inflammatory conditions through accumulations and liberation of active proteolytic enzymes (Ramprasath *et al.*, 2006; Zhu, 2002).

Lymphocytes are component of WBCs and respond to presences of foreign substances that are introduced in to the body. It was indicated that the number of lymphocytes may be reduced in blood by some medicinal plants indicting that the medicine may contain biologically active ingredients that has anti-lymphocytic activities (Aniagu *et al.,* 2005).

Platelets are non-nucleated, disc like cell fragments, derived as fragmentation of a giant plyploid megakaryocyte of the bone marrow and are used in homeostasis and blood clotting mechanisms and they accounts about 150,000-400,000/cmm in humans (Taylor *et al.*, 1997).

Plasma is a pale straw-colored liquid consisting of 90% water and a variety of substances of low or high molecular weight that make up 10% of the plasma within solution and suspension with essentially an aqueous solution of inorganic salts which are constantly exchanged with the extracellular fluid of body tissue. Plasma consists of plasma proteins, such as albumin,

globulin and fibrinogen and other components including biochemical substances (Taylor *et al.*, 1997).

1.4. Structure, Function and Histopathology of Liver

Liver is the largest gland and the second largest organ in the body next to the skin. It weighs approximately 1500gm and account for approximately 2% of human adult body weight (Moore and Dalley, 1999) while in mice the percentage is variable according to their body weight (Kongure *et al.,* 1999). According to Martins *et al* (2007), the mouse liver consists of four distinct lobes of different size (caudate lobe, left lateral lobe, median lobe and right lobe). The caudate lobe consists of two parts: superior and inferior caudate lobes together comprise about 10% of the total liver weight. The superior caudate lobe is connected to the left lateral lobe via a thin interlobular ligament and is fully covered by the lesser omentum. The inferior portion of the caudate lobe is located behind the stomach where it is closely related to the pancreas and spleen and is covered by a fibrous capsule, attached to the dorsal wall of minor omentum. The left lateral lobe comprises about 34% of the total liver weight and is located in the left lateral position of dorsocaudal to the median lobe and cranio-ventral to the caudate lobes and the stomach where it is connected to the superior caudate lobe by interlobular ligaments (Madrahimov, *et al.,* 2006).

The median lobe represents about 26% of the total liver weight and is located under the diaphragm where it is fixed with the falciform ligament, which spans from the xiphoid and diaphragm to the liver forming the interlobular fissure. Besides, the median lobe consists of two parts, the right and the left part, that are separated by a deep fissure and the left part is smaller so that it represents about one third of the median lobe. The right liver lobe is located on the right side of the inferior vena cava and consists of two distinct portions, the right superior and the right inferior lobe. The right superior lobe comprises about 16% of the total liver weight with a wide base extending to the paracaval part of the liver and the pyramidal-shaped right inferior lobe with the tip pointing to the inferior vena cava comprises 14% of the total liver weight and is dorsally attached to the diaphragm (Martins *et al.,* 2007; Madrahimov, *et al.,* 2006).

The basic structural components of the liver are the liver cell, or hepatocyte which can be characterized by having darkly stained granules and few vacuoles in their cytoplasm (Ebaid *et*

al., 2007). These epithelial cells are grouped in interconnected plates and constitute two-third of the mass of the liver (Junqueira and Carneiro, 2005). The basic structural units of the liver, the lobule has a polygonal mass of tissue about 0.7 x 2 mm in size, with the portal space at the periphery and a central vein in the centre. It was around the central vein that inflammatory cellular infiltration was abundantly observed in response to drugs (Ebaid *et al.,* 2007). Portal spaces, region located in the periphery of the lobules, contain connective tissue, bile duct, blood vessels and lymphatics (Taylor *et al.*, 1997).

The hepatocytes in the liver lobules are radially disposed and are arranged like the bricks of a wall. These cellular plates are directed from the periphery of the lobules to its centre and anastomose freely, forming a complex and spongy like structure. The space between these plates contains capillaries called liver sinusoids. The sinusoidal space may be obliterated under toxic effect of herbal medicine (Ebaid *et al.,* 2007). A sub endothelial space known as the space of Disse separates the endothelial cells from the hepatocytes. The fenestrated and discontinuity of the endothelium allow the free flow of plasma but not of cellular elements into the space of Disse, permitting an easy exchange of molecules from the sinusoidal lumen to the hepatocytes and vice versa (Taylor *et al.*, 1997). This exchange is physiologically important not only because of the large number of macromolecules secreted into the blood by hepatocytes but also because the liver takes up and catebolizes many of the molecules including the drugs that are administered.

The sinusoid is surrounded and separated by a delicate sheath of reticular fibres. In addition to the endothelial cells, the sinusoids contain macrophages known as Kupffer cells. These cells are found on the luminal surface of the endothelial cells within sinusoid. The main functions of Kupffer cells are to metabolize aged RBC, digest haemoglobin, secrete proteins related to immunological processes, and destroy bacteria that enter the portal blood through the large intestine. Thus, Kupffer cells are the tissue macrophages in the liver and play a critical role in the defense mechanisms of the body (Ding *et al.,* 2003). And their nuclei appear to be pyknotic and their number increase indicating the toxicity of the drugs (Ebaid *et al.,* 2007). As a result most of them are located in the periportal region of the liver lobules, where they are very active in phagocytosis (Junqueira and Carneiro, 2005).

Liver is considered to be the most important organ for drugs or other metabolites (Effendy *et al.,* 2006). It performs many functions, such as detoxifying and accumulating metabolites, aiding food digestion, formation and secretion of bile, synthesis and release of plasma protein

and components such as urea, albumin, prothrombine fibrinogen and lipoprotein, fat metabolism, storage of vitamins, and cholesterol production (Taylor *et al.,* 1997). Moreover, it is involved in the removal of circulating cholesterol carried in lipoproteins manifesting the highest rate of sterol accumulation in both the human and mouse (Beltroy *et al.,* 2005). The cholesterol absorbed from the intestine is nearly completely cleared into the liver, using clathrin-coated pathway as indicated by the same authors. It is also used for storage of glycogen.

The most important function of the liver is to detoxify, eliminate toxins and chemical substances from the body or blood stream and change them into products that can be readily removed through the bile or urine. However, accumulation of these toxins in the body faster than the liver can process them results in damage to liver structure (Effendy *et al.,* 2006). Effendy *et al. (*2006) also indicated that some of the available chemotherepy medicinal plants are toxic and have the ability to cause damage to liver cells or hepatocytes.

1.5. Structure, Function and Histopathology of Kidney

Kidneys lie retroperitoneally on the posterior abdominal wall, one on each side of the vertebral column at the level of T_{12} through L_3 vertebrae in mice. Bonvalet *et al.* (1977) indicated that the male mice kidney were heavier than female mice kidney. However, no weight differences between left and right kidneys in both sexes.

Cross section of the kidney shows two distinctive regions, an outer cortex and an inner medulla. The cortex is covered by fibrous connective tissue forming a tough capsule and consists of Bowman's capsule, convoluting tubules and the beginning of collecting ducts, whereas the medulla consists of loop of Henle and collecting ducts as well as associated vasculatures which together forms a mass of pyramidal structure known as renal pyramids (Taylor *et al.,* 1997; Hill and Wyse, 1989). In rodents the medullary tissue of each kidney forms a single coherent structure. Moreover, the distribution of the various renal tubular segments within specific regions of the rat medulla is well-established and is divided macroscopically into an outer and inner position (Ernst and Schreiber, 1981). The functional unit of the kidney is the uriniferous tubule, consisting of the nephron and collecting tubules, each of which is derived from a different embryological primordia (Gartener and Hiatt, 2000;

Burkitt *et al.,* 1993). The laboratory rat has about 30, 000 nephrons in each kidney, the dog about 400,000 and humans about1.1 million (Hill and Wyse, 1989).

The kidney's nephron comprises the renal corpuscle, proximal convoluting tubule, Henle's loop, distal convoluting tubule (Carvalho and Junqueira, 1999). According to Carvalho and Junqueira (1999), renal corpuscle consists of a tuft of capillaries, the glomerulus, surrounded by a double walled epithelial capsule known as Bowman's capsule. The number of renal corpuscle of mice within the experimental age was around 12,675, each consists of vascular pole and urinary pole (Bonvalet *et al.,* 1977). At the urinary pole of renal corpuscle, the squamous epithelium of Bowman's capsule is continuous with the cuboidal or low columnar epithelium of the proximal convoluting tubules of the nephron and carries filters from renal corpuscle to loop of Henle. This tubule is longer than the distal convoluting tubule and is therefore, frequently seen near renal capsules in the renal cortex (Taylor *et al.,* 1997).

Distal convoluting tubule is a continuation of ascending limb of Henle's loop penetrating the cortex and is lined with simple cuboidal epithelium and has no brush border, no apical canaliculi and has small cells with more nuclei in it (Junqueira and Carneiro, 2005). Moreover, it is characterized by large, vertically disposed mitochondria assuming the aspect of ions transporting cells (Carvalho and Junqueira, 1999). Collecting tubules and ducts are lined with cuboidal epithelium but as they penetrate deeper into the medulla, their cells increase in height until they become columnar (Taylor *et al.,* 1997).

The kidney is the major excretory and osmoregulatory organ of mammals and has the following functions such as removal of metabolic waste products (e.g. body pigments), regulation of the water content of the body fluids, and regulation of chemical composition of the body by removal of substances which are in excess of immediate requirements (Taylor *et al.,*1997). Moreover, the kidney performs an important role in the body as regulatory organ that maintains the steady state of the extracellular fluid and the pH of the blood in normal functional range by excreting the metabolic wastes like blood urea nitrogen (BUN), creatinine and regulates the electrolyte balance (e.g. bicarbonates, phosphate, potassium, sodium and chloride). Therefore, nephrotoxic ability of xenobiotics is highly related to its ability to interfere with these normal structural and biochemical processes of the kidney. Consequently, it affects the functional units of the kidney due to damage to kidney structure (Kefale, 2005). As a result it is the second target organ for xenobiotics next to the liver for eternally absorbed toxic chemicals (Effendy *et al.,* 2006).

One of the main functions of the kidney is to maintain osmotic balance of fluid and this is done by selective reabsorption of ions most commonly Na^+ and K^+. The concentrations of these electrolytes are elevated in the blood (serum) in nephronic syndrome, and abnormalities kidney tubular structure especially in proximal convoluting tubules where the tubular cells appear enlarged with pink cytoplasmic granules and their nuclei appear to be slightly enlarged (Oyewole amd Massaquoi, 2008; Ebaid *et al*., 2007). It was reported that different segments of loop of Henle, thick descending limb, thin descending limb, the loop and thick ascending limb, were less affected by herbal medicine indicating that the main target of toxicity is convoluting and collecting tubules. Hence, the cells of convoluting tubules may be highly enlarged; as a result their lumens become obliterated containing some glomerular filtrate (Ebaid *et al*., 2007).

Pathological changes on kidney tubular structure may lead to leakage and rupture of the cell membrane of tubular cells resulting in elevation of those enzymes which are commonly concentrated on liver and kidney and are found in serum in significant quantities. Urea and creatinine are waste products which are passed into the blood stream to be removed by the kidney. Increase in concentration of these waste products in the blood is an indication of renal function impairment as a result of pathological changes on kidney tubular structure (Oyewole and Massaquoi, 2008). Furthermore, cytoplasmic vacuolation of kidney tubular cells occurs due to toxicity of drugs that are administered (Khleifat *et al*., 2002).

According to Ebaid *et al.* (2007), many researchers have identified that marked change in the fine structure of the cellular component of the proximal convoluting tubules occurs as a result of the treatment with different toxic substances. Moreover, many traditional medicinal plants have been found to cause renal tubular damage showing extensive interstitial fibrosis and severe tubular loss most commonly in the outer cortex (Dapar *et al.,* 2007).

1.6. Significance of the Study

Despite intensive efforts to control malaria, the disease continues to be one of the greatest health problems facing Africa. It is estimated that there are at least 300 million clinical cases of malaria per annum, making it one of the top killers among communicable diseases (Clarkson, *et al.,* 2004). Increase in prevalence and distribution of malaria has been contributed to a number of factors, one of them being the emergence and spread of drug resistance parasite. Efforts are now being directed towards the discovery and development of new chemically diverse anti-malaria drugs derived from medicinal plants. In Ethiopia many medicinal plants are used for the treatment of malaria including *D. angustifolia* seeds with out considering their side effect.

So far, to my knowledge, no report has been published on the effect of *D. angustifolia* seed extracts on hematological and biochemical composition of the blood and histopathology of liver and kidney. Therefore, the present study evaluates the chronic effect of crude extracts of *D. angustifolia* seeds on some haematological and biochemical parameters and histopathology of liver and kidney of mice.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

- To investigate the effect of aqueous and methanol crude extract of *D. angustifolia* seeds on some blood parameters, and on histology of liver and kidney in mice.

2.2. Specific Objectives

- To assess the effects of the extract on histopathology of liver and kidney.
- To assess the effects of the extract on the weights of liver and kidney in relation to the body weight in mice.
- To compare the haematological and biochemical parameters in the blood between the control and experimental groups.

3. MATERIALS AND METHODS

3.1. Study design: Laboratory-based experiment

3.2 Study setting: AAU, FOM, Histology, pathology, core laboratories and EHNRI, Department of Drug Research Laboratories

3.3. Plant Collection and Processing

Three kilograms of fresh, air dried seeds of *D. angustifolia* were collected from Debresina area, Northern Shewa, Amara Region, 190 km north of Addis Ababa during the month of July 2008. The seeds were further dried under shade at room temperature to ensure easy grinding of the seeds and protect the UV labile components of the seed. Any extraneous materials from the seeds were removed and crushed to fine particle powder using grinding mill. From 3kg of the dried seeds of *D. angustifolia*, 2.5 kg of fine powdered particles was obtained. The powder was stored in plastic bag till extracted with aqueous and methanol to obtain aqueous and methanolic crude extract respectively. The plants were identified by a botanist and deposited in the National Herbarium of Science Faculty, Addis Ababa University with voucher number of HM 02/2006.

3.3.1. Preparation of Aqueous Extract

Three hundred gm of fine powder of *D.angustifolia* seed was soaked/macerated with 1500ml of distilled water in 2 litter Erlenmeyer flask and allowed to rotate at a speed of 160 per minute for eight hours on orbital shaker to ensure complete mixing of the powdered particle with the solvent (water), and then allowed to settle for about 24 hours to separate the supernatant from residue. The supernatant was decanted from the residue, filtered with a clean cotton cloth on to petridish. The petridish containing filtrate was put into a refrigerator to deep freeze so that the filtrates become solidified. The solidified filtrate was allowed to freeze dry with lyophilizer and the crude extract was collected daily till the extract was completely obtained. The collected crude extract was weighed and stored in a desiccator at room temperature, where any moisture remained within the extract was completely removed, till used for the experiment.

3.3.2. Preparation of Methanol Extraction

For the methanolic extract, 300g of fine powdered particles of *D. angustifolia* seed was soaked with 1500ml of 80% methanol in 2 litter Erlymleyer flask and placed in orbital shaker at room temperature, and allowed to rotate at a speed of 160 per minute for eight hours to ensure complete mixing of the powdered particle with the solvent (methanol), and left for 72 hours to obtain supernatant. The macere was re-macerated twice, each using 500ml of methanol, for 48 hours each to get sufficient amount of the extract. The supernatant was decanted, filtered with Whatman No.1 filter paper. The filtrate was subjected to vacuum by rota vapour to remove the methanol by evaporation at a temperature of 40^{o}C The water left in the filtrate was removed by using water bath at a temperature of 40^{o}C , thereby, sticky crude extract was collected. The collected extract was weighed and the percentage yield was calculated. The crude extract was kept in refrigerator at -20^{o}C (Debella, 2002), till used for the experiment.

Table 1: Actual and percentage yield of aqueous and methanolic extracts of *D. angustifolia* seeds

Solvent for extraction	**Seed powder in gm**	**Actual yield in gm**	**Percentage yield**
Aqueous	300	31.5	10.5
Methanol	300	30	10

3.4. Experimental Animal Preparation

3.4.1. Acute Toxicity Study

3.4.1.1. Lethal Dose Determination of the Aqueous Extract

Thirty-six adult Swiss albino mice, 2 – 3 months old weighing 25 – 30 gm, were taken from animal breeding house of the Ethiopian Health and Nutrition Research Institute (EHNRI) and randomly assigned into six groups each containing equal number of males and females (n =6). The animals were acclimatized and fasted for eighteen hours. The weight of each animal within the group was recorded. Dose was calculated relative to their average body weight and administered orally to each mouse in a group. After oral administration of the desired dose as indicated in Table 2, each animal was returned to the cage in their respective groups. Animals were observed for toxicity manifestations and lethality for 24 hours. Deaths occurring in 24 hours were recorded and LD_{50} was calculated using SPSS version 15.0 computer software by probit analysis.

Table 2: Lethal dose determination of the aqueous crude extract of *D. angustifolia*

Group	**Dose (mg/kg/bw)**	**Number of mice used**
GI	Control	6
GII	4000	6
GIII	4500	6
GIV	5000	6
GV	5500	6
GVI	6000	6

3.4.1.2. Lethal Dose Determination of the Methanolic Extract

Forty-two adult Swiss albino mice, 2 – 3 months old weighing 25 – 30 gm, were taken from animal breeding house of the Ethiopian Health and Nutrition Research Institute and randomly assigned into seven groups each containing equal number of males and females (n = 6). The animals were acclimatized and fasted for eighteen hours, and the weight of each animal within the group was recorded. Dose was calculated in relative to their average body weight. After oral administration of the appropriate doses as indicated in Table 3, each animal was returned to the cage in their respective groups. Animals were observed for toxicity signs and

lethality for 24 hours. Deaths occurring in 24 hours were recorded and LD_{50} was calculated using SPSS version 15.0 computer software by probit analysis.

Table 3: Lethal dose determination of the methanolic crude extract of D. angustifolia

Group	**Dose (mg/kg/bw)**	**Number of mice used**
GI	Control	6
GII	2000	6
GIII	2500	6
GIV	3000	6
GV	3500	6
GVI	4000	6
GVII	4500	6

3.4.2. Chronic Treatment

Fifty adult Swiss albino mice weighing 25-30 gm at the age of eight weeks, equal numbers of male and female mice were used. The animals were taken from animal breeding house of the Ethiopian Health and Nutrition Research Institute. The animals were kept in metallic cage under standard laboratory condition, and they were exposed to 12 hours light and dark cycle at standard temperature (21 ± 2^0C). The animals were provided with standard commercial diet and drunk tap water *ad libitum* throughout the experimental periods.

Before the commencement of the experiment, all animals were weighed and randomly assigned into five groups of ten mice each with equal number of males and females, as per WHO guideline (WHO, 2003) and acclimatized to laboratory conditions for about seven days to experimental protocol to avoid any stress (Vipul *et al.,* 2007). On the day of experimentation, all animals were again weighed in their respective group and their average body weights were recorded to calculate the dose accordingly. (The weight recorded at the beginning of the experiment was the initial body weight that later compared with final body weight, the weight recorded at the end of the experiment).

There were one control group (common to aqueous and methanolic) and four experimental groups, two for aqueous (group II and III) and two for the methanolic extract (group IV and V). Each contained equal number of males and females but in separate cage for the treatment

with aqueous and methanolic extract. The control group was assigned as group I, and administered distilled water through oral route by oral gavage (WHO, 2000), throughout the duration of the experimental period. While the experimental groups were assigned as group II and III for the aqueous extract treatment at effective dose of 400 mg/kg/bw and 800 mg/kg/bw for about six weeks and group IV and V treated at the same effective dose of 400 mg/kg/bw and 600mg/kg/bw of the methanolic extract for six weeks whereby, each animal was given 0.5 ml/mouse of the extract in both cases in 24 hours interval. The administration rout throughout the duration of the experiment was through oral route using oral gavage as per WHO guideline (WHO, 2000).

3.5. Measurement of Body Weight

To investigate the effect of aqueous and methanolic extract of *D. angustifolia* seed on relative body weight and absolute weight of the organ, the body weights of animals were recorded at the beginning and end of the experiment. The liver and kidney were carefully removed from each mouse and weighed on electronic balance.

The relative organ weight was calculated using the weight of animal and weight of liver or kidney using the formula indicated by Aniagu, *et.al.,* 2005, so that comparison was made between the control and the experimental groups for both extracts.

$$ROW = AOW / FBW$$

ROW= relative organ weight

AOW = absolute organ weight

FBW = final body weight

3.6. Blood Collection for Haematological and Biochemical Analyses

At the end of the experimental period, animals belonging to each group were weighed on a digital electronic balance and were anesthetized under diethyl ether anaesthesia by placing each animal in an air tight dissector jar with cotton soaked in ether. Blood samples of 2-5ml were withdrawn quickly through cardiac puncture using a 5ml heparinized syringe and collected in a test tube with anti-coagulant EDTA and without anti-coagulant. Then, haematological parameters such as haemoglobin concentration (HC), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), lymphocyte, neutrophils and platelet count (PLC) were analyzed by using Auto-haematology analyzer CELL-DYN 3700cs system (ABOTT, USA) from the blood in a test tube with EDTA . The blood samples within a test tube containing no EDTA were allowed to clot, then centrifuged at 3000 revolution per minute for about 20 minutes to obtain the serum so that biochemical parameters, that are commonly used for liver and kidney function test such as, urea, creatinine, AST, ALP and ALT were analyzed by using BT-200 chemistry analyser (Bioteqnique, Italy) from the serum blood, thereby comparison were made between the control and experimental groups for both extracts (aqueous and methanol).

3.7. Animal Dissection and Tissue Collection

At the end of the experiments, animals belonging to each group were sacrificed after weighing on a digital electronic balance under diethyl ether anaesthesia. Immediately before the death of the animal the abdominal cavity was opened and the liver and kidney were carefully removed. The removed organs were cleared from any surrounding tissues and put on clean paper and weighed quickly on electronic balance and the organ weight was then recorded.

Randomly the coronal section of the right kidney and the transverse section of the lower parts of right lobe of liver were taken for histological processing.

3.8. Histological Processing

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

The resulting tissue blocks were sectioned in ribbons at a thickness of 5 µm with Leica microtome (Leica RM 2125RT Nussloch GmbH, Germany). The ribbons of the section were taken at every 5^{th} sections and put onto the surface of a warm water bath of temperature of $40^{o}C$. The floating ribbons over the surface of warm water were mounted onto precleaned slides spread with egg albumin. The slides containing paraffin wax were arranged within the slide holder and placed in an oven with temperature of 40 ^{o}C for about 20 minutes so as to remove the wax; fix the tissue to the slides and allowed to cool at room temperature for 30 minutes and stained regressively with routine Harris haematoxylin for 6 minutes and eosin for 17-20 seconds (H and E).

For routine H and E staining, two series of coupling jars were prepared. One for paraffin removal and hydration and the other was for dehydration and clearing. So sections were placed in xylene- I for 5 minutes and xylene II for 2 minutes again to remove the paraffin from tissue and hydrated with decreasing concentrations of absolute I, II and 95% of alcohol for two minutes each, 70% of alcohol for three minutes and 50% of alcohol for five minutes. The tissue sections were washed with tap water for five minutes and stained regressively with Harris haematoxylin for 6 minutes, then washed under running tap water for five minutes again. The slides were immersed in acidic alcohol for differentiation and controlling overstained haematoxylin for 1 second and then put in blueing solution (Sodium bicarbonate) until they became blue. After blueing, the slides were counterstained with eosin for 17-20 seconds and then washed in tap water for two minutes. The sections were dehydrated with

increasing alcohol concentration of 50%, 70%, 95%, absolute I and II for two minutes each. The dehydrated sections were cleared with xylene I and II for three minutes each and permanently mounted on microscopic slides using DPX and cover slips and then observed by light microscope for the investigations of any histological change, thereby the histology of the treated groups were compared with histology of the control groups.

3.9. Preparation for Microscope

The slides of liver and kidney were examined under light microscope for histological investigations fitted with different objective (10X, 25X, 40X and 100X). Photomicrographs of selected samples were taken using Fujicolor film at magnification of 40X using MC 80 DX Auto microscope camera (Zeiss,Germany) to observe changes on histology of liver and kidney. Based on observations made, comparisons were made between control and experimental groups.

3.10. Statistical Analysis

The result was analyzed statistically using one way analysis of variance (ANOVA) using the SPSS version 15.0 computer software to identify the possible difference between body weight , relative organ weight , haematological and biochemical values followed by student's t-test to compare the difference between control and treated groups. All data were expressed as mean $\pm$ standard error of the mean (SEM). Differences at $p<0.05$ were considered to be significant.

4. RESULTS

4.1. LD_{50} of *D. angustifolia* Seed

As it can be observed from the Figures 1 and 2, the mortality rate was found to increase with increasing dosage level. The dosages were plotted in X – axis and the mortality rate in probit units were plotted in Y- axis. These gave almost straight line indicating that increase in dosage level resulted in increase in mortality rate. Then LD_{50} was determined by drawing a vertical line on the X-axis from the point of the straight line where the probit units 0.5 (50% mortality) intercepted.

In the present study, the LD_{50} of the aqueous (Fig.1) and methanol (Fig.2) extract of *D. angustifolia* seeds was found to be 4672.02 mg/kg/bw and 2922.30mg/kg/bw, respectively.

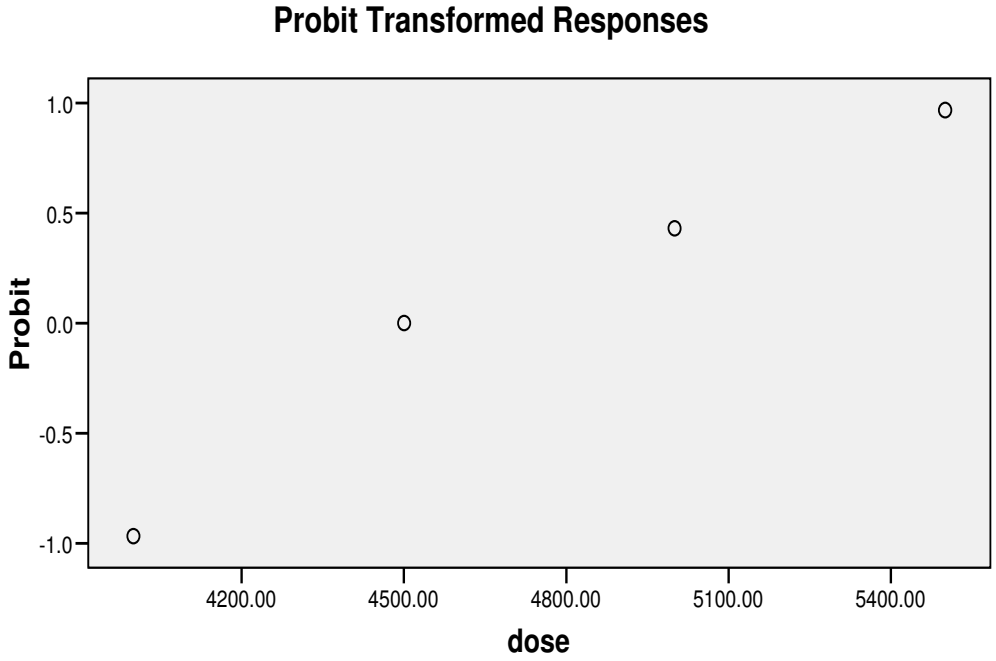

Fig 1: Probit transformed responses of mice treated with a single oral dose of aqueous extract of *D. angustifolia seeds*.

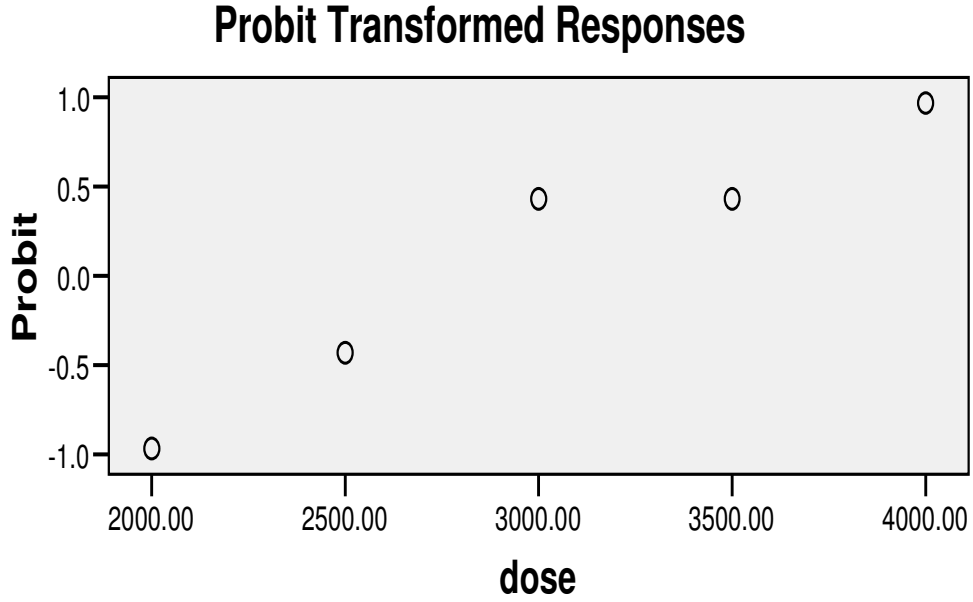

Fig 2: Probit transformed responses of mice treated with a single oral dose of methanolic extract of *D. angustifolia seeds*.

After administration of the aqueous and methanolic crude extracts of *D. angustifolia* seeds to the experimental mice and distilled water to the control mice, both the experimental and control groups were observed strictly for their behavioural changes for the 24 hours during investigations of lethal dose (LD_{50}). Accordingly, no toxicity manifestations or behaviour change were observed at doses lower than 2500 mg/kg/bw from the mice treated with aqueous extract of the plant. Depression, salivation and faster breathing were observed at higher doses up to 4000 mg/kg/bw of the aqueous extract.

Moreover, vomiting, diarrhea, distension of abdominal region, lacrimation and mortality were observed at doses between 5000 mg/kg/bw – 6000 mg/kg/bw. However, mortality began at dose of 4000 mg/kg/bw of aqueous extract of the plant. All treated mice died in 24 hours period following administration of the aqueous seed extract of the plant at dose of 6000 mg/kg/bw.

While in those mice treated with methanolic crude extract of *D. angustifolia* seeds, vomiting, diarrhea, distension of abdominal region and lacrimation were observed starting at the dose of 1000 mg/kg/bw. All treated animals died in 24 hours period after the administration of the methanolic crude extract of the plant at dose of 4500 mg/kg/bw.

4.2. Chronic Toxic Effect of Aqueous and Methanolic Extract of *D. angustifolia* Seed on the Behaviour of Mice

During the six weeks of treatment with the aqueous and methanolic extract of *D. angustifolia* seeds, animals were observed for behavioural change. No dose-dependent deaths were observed throughout the experimental period among those mice treated with aqueous extract of the *D. angustifolia* seeds at doses of 400 mg/kg/bw and 800 mg/kg/bw.

Mild signs of toxicity such as depression, loss of appetite, and less water intake were observed after a month of treatment among those mice treated with aqueous seed extract of *D. angustifolia* at doses of 400 mg/kg/bw and 800 mg/kg/bw as compared to the control group. However no dose dependent behavioural changes were observed.

Those mice treated with methanol crude extract of *D. angutifolia* seed at both doses of 400 mg/kg/bw and 600 mg/kg/bw were active and food intake was high at dose level of 400 mg/kg/bw. In contrast, depression, lacrimation, diarrhea and death were observed among mice treated with 600 mg/kg/bw methanolic extract of the plant after four weeks of treatment.

4.3. Effects of *D. angustifolia* Seeds Extract on Both Body Weight and Relative Organ Weight

As shown in Table 4, gain in body weight of control group and mice treated at both doses, (400 mg/kg/bw and 600 mg/kg/bw) of methanol extract were observed. However, the differences between the treated and control group were not statistically significant. In contrast, no weight gain was observed in those mice treated at doses 400mg/kg/bw and 800mg/kg/bw of aqueous extract showing slight weight loss as compared to the control group. No significant ($p > 0.05$) change in relative weight of liver and kidney in all treated mice in both extracts, aqueous and methanol were observed as compared to the relative weight of liver or kidney of the control group.

Table 4: Effects of aqueous and methanolic extract of D. angustifolia seeds on relative body weight and weight of liver and kidney of mice

Group	Dose in Mg/kg/bw	Initial body weight in g	Final body weight in g	Relative organ weight	
				Liver weight	Kidney weight
I	400(aqueous)	26.0000 ±.37	24.8922 ±.84	4.98±.06	0.60±.01
II	800(aqueous)	25.1000 ±.23	23.6963 ±.80	4.90±.05	0.55±.01
III	400(methanol)	24.7000 ±.45	29.9800 ±1.45	5.17±.07	0.62± .01
IV	600(methanol)	25.1000±.48	28.5083 ±1.34	5.79 ±.11	0.59±.01
V	Control	28.1000 ± 1.00	33.5150 ±1.56	4.83±.08	0.61 ±.02

Values are expressed as mean ± SEM.

4.4. Effects of Aqueous Extract of *D. angustifolia* seeds on Some Biochemical Parameters

Biochemical composition of serum blood such as ALP, ALT, and AST used for liver function test and creatinine and urea, used for kidney function test, analysed in the present study are summarised in Table 5. Comparison of those biochemical parameters of aqueous treated group were made against the control group. The result showed no statistically significant differences in concentrations of those biochemical parameters as compared to the control group. Concentration of ALP in blood serum seemed to decrease at both doses of 400 mg/kg/bw and 800 mg/kg/bw of aqueous treated group. However, the differences were not statistically significant as compared to the control group. No significant differences in concentration of ALT, AST, creatinine and urea in blood serum at both doses of (400 mg/kg/bw and 800 mg/kg/bw) of the aqueous extract of the plant were observed as compared to the control group.

Table 5: Effects of aqueous extract of D. angustifolia seeds on biochemical parameters.

Bichemical parameters	**Control**	**Doses**	
		400 mg/kg/bw	**800 mg/kg/bw**
ALP(IU/L)	138.33±14.24	113.75±8.51	135.00±53.31
ALT(IU/L)	50.00±2.04	46.25±9.44	47.50±4.79
AST(IU/L)	53.00±11.21	35.00±8.10	40.50±3.40
Creatinine(mg/dL)	1.00±.00	1.05±.05	1.10±.06
Urea(mg/dL)	18.50±5.51	19.75 ±3.47	16.25±1.11

The values are expressed as mean ± SEM.

4.5. Effects of Methanol Extract of *D. angustifolia* seeds on Some Biochemical Parameters

Some biochemical parameters in blood serum of mice treated with methanol crude extract of the plant were analysed and compared to the control group. The result showed that concentration of ALP in blood serum level decreased at both doses of 400 mg/kg/bw and 600 mg/kg/bw, and the differences were significant ($p< 0.05$) at dose of 600 mg/kg/bw as compared to the control group (Table 6). However, concentrations of ALT and AST in blood serum were significantly elevated at dose level of 600 mg/kg/bw as compared to the control group. Similarly, concentrations of creatinine and urea were significantly ($p<0.05$) elevated at dose of 600 mg/kg/bw as compared to the control group.

Table 6: Effects of methanol extract of D. angustifolia seeds on biochemical parameters

Biochemical Parameters	**Control**	**Doses**	
		400 mg/kg/bw	**600 mg/kg/bw**
ALP(IU/L)	138.33±14.24	130.00±5.00	122.50±7.50
ALT(IU/L)	50.00±2.04	66.67±3.33	96.67±21.86*
AST(IU/L)	53.00±11.21	60.00±6.43	107.33±3.33*
Creatinine (mg/dL)	1.00±.00	1.07±.18	2.33±.24*
Urea (mg/dL)	18.50±5.51	24.33±7.45	36.67±4.81*

* P< 0.05

The values are expressed as mean ± SEM.

4.6. Effects of Aqueous Extract of *D. angustifolia* Seeds on Some Haematological Parameters

Table 7 shows, the effect of aqueous extract of *D. angustifolia* seeds on some haematological parameters. The haematological analyses indicate that there were no significant differences in all haematological parameters at both doses (400 mg/kg/bw and 800 mg/kg/bw) of the plant extract as compared to the control group. Although statistically not significant, slight reduction of MCV at doses of 400mg/kg/bw and 800mg/kg/bw were observed in the aqueous extract treated group as compared to control group. Significant reduction ($p < 0.05$) in concentration of lymphocytes at doses of 400 mg/kg/bw and 800 mg/kg/bw of aqueous extracts of *D. angustifolia* seed, was observed as compared to control group.

The concentration of WBC in blood seems to be increased at the dose of 400 mg/kg/bw but decreased at the dose of 800 mg/kg/bw as compared to the control group, however, the reduction was not statistically significant.

Table 7: Effects of the aqueous extract of D. angustifolia seeds on haematological parameters.

Haematological Parameters	Units	Doses		
		Control	400mg/kg/bw	800mg/kg/bw
RBC	10^3 /µl	6.50 ± .92	7.08 ± .20	6.64 ± 1.28
HGB	10^6 / µl	9.65 ±1.28	10.23 ± .45	9.77 ± 1.91
HCT	%	32.38 ±5.23	33.30 ± 1.51	31.23 ± 6.41
MCV	fL	49.30±.76	46.97 ± .82	46.73 ± 1.16
MCH	Pg	14.88±.19	14.43 ± .27	14.67 ± .22
MCHC	g/dl	30.23±.72	30.77 ± .22	31.13 ± .66
WBC	10^3 /µl	3.24±.38	5.15 ± 1.37	2.49 ± .46
NEUTR	10^3 /µl	20.75 ± 2.83	24.95 ± 3.05	26.83 ± 6.66
LYMP	10^3 /µl	73.45 ± 2.44	55.80 ± 1.20*	51.93 ± 2.76*
PLT	10^3 /µl	627.50 ± 175.75	475.33 ± 95.19	747.33 ± 257.64

* P< 0.05

The values are expressed as mean ± SEM.

4.7. Effects of Methanol Extract of *D. angustifolia* Seeds on Some Haematological Parameters

Table 8 shows, some haematological parameters in methanol extract treated groups as compared with the control group. The results indicate no statistically significant differences in RBC, WBC, HGB, HCT, MCV, MCH, MCHC, and PLT levels as compared to the control group. However, increase in number of RBC and concentration of neutrophil at dose of 600 mg/kg/bw were observed. In contrast, a slight decrease in concentration of lymphocyte at dose 600 mg/kg/bw was also observed.

Table 8: Effects of the methanol extract of D. angustifolia seeds on haematology of mice under investigation.

Haematological Parameters	Units	Doses		
		Control	400mg/kg/bw	600mg/kg/bw
RBC	10^3 /µl	6.50±.92	6.23±1.19	7.22±1.43
HGB	10^6 / µl	9.65±1.28	9.30±1.68	10.50±2.60
HCT	%	32.38±5.23	31.00±5.57	34.40±9.10
MCV	fL	49.30±.76	49.90±.99	47.00±3.30
MCH	Pg	14.88±.19	14.97±.19	14.35±.75
MCHC	g/dl	30.28±.72	30.03±.37	30.65±.55
WBC	10^3 /µl	3.24±.38	4.94±1.71	3.13±.83
NEUTR	10^3 /µl	20.75±2.83	21.90±.00	28.35±2.65
LYMP	10^3 /µl	73.45±2.44	72.40±2.30	64.45±.95
PLT	10^3 /µl	627.50±175.75	611.33±179.15	469.00±302.00

The values are expressed as mean ± SEM.

4.8. Effect of the Aqueous Extract of *D. angustifolia* Seed on Histopathology of Liver

Histological examination of liver sections obtained from mice treated with aqueous extract of *D. angustifolia* seed at a dose of 400 mg/kg/bw shows no histopathological changes as compared to the control group. No inflammatory cellular infiltration around central vein and portal triad were observed. Normal hepatocytes with centrally located nucleus were seen like that of the control group. Fig. 5 indicates that no treatment related inflammations and cellular damage at the effective dose of (400 mg/kg/bw) of aqueous extract of the plant.

In contrast, in mice treated at a dose of 800 mg/kg/bw of the *D. angustifolia* seed aqueous extract, localized and focal lobular inflammatory cellular infiltration around the central vein were observed as compared to the control group. However, the change observed was not significant at this dose as compared to the control group. There were no significant

inflammatory cellular infiltration and cellular damage observed at this dose level (800 mg/kg/bw). Fig 4 & 5 below shows treatment related cellular infiltration at dose 800 mg/kg/bw of the *D. angustifolia* seed aqueous extract. Histological examination of the liver sections showed dose-dependent effects of the aqueous seed extract of *D. angustifolia* seeds. Although these histopathological changes were not significant at dose of 800 mg/kg/bw, focal inflammation were observed around central vein starting at this dose of the aqueous crude extract.

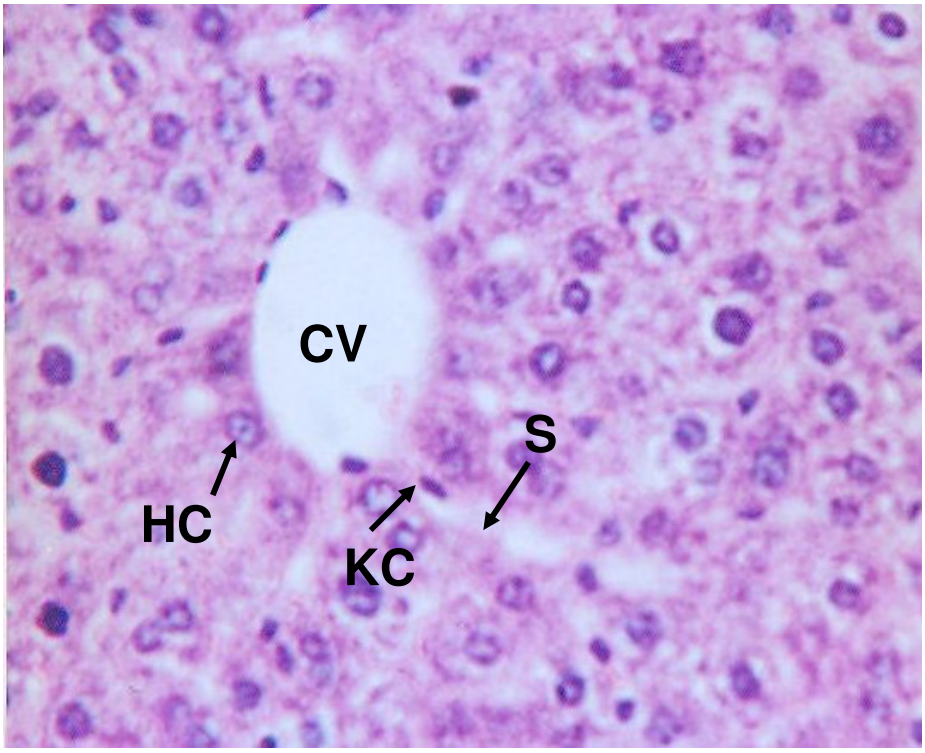

Fig.3: Photomicrograph of the H and E stained liver sections of control group showing the central vein (**CV**), and surrounding normal hepatocyte (**HC**), sinusoid (**S**), and Kupffer cells (**KC**) (H &E, x400)

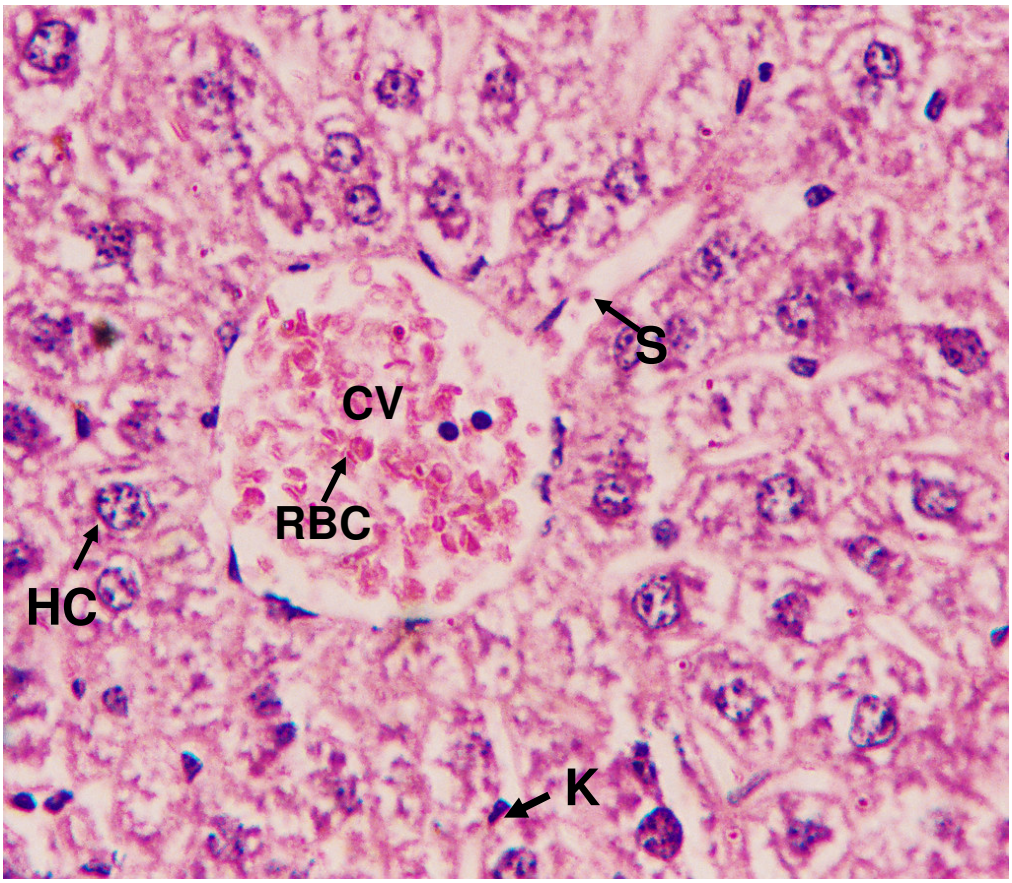

Fig.4: Photomicrograph of H and E stained liver section of mice treated at dose of 400mg/kg/bw of *D.angustifolia* seed aqueous extract showing normal hepatocyte (**HC**) around central vein (**CV**) filled with red blood cells (**RBC**), sinusoid (**S**), and Kupffer cell (**KC**), (H & E, x400).

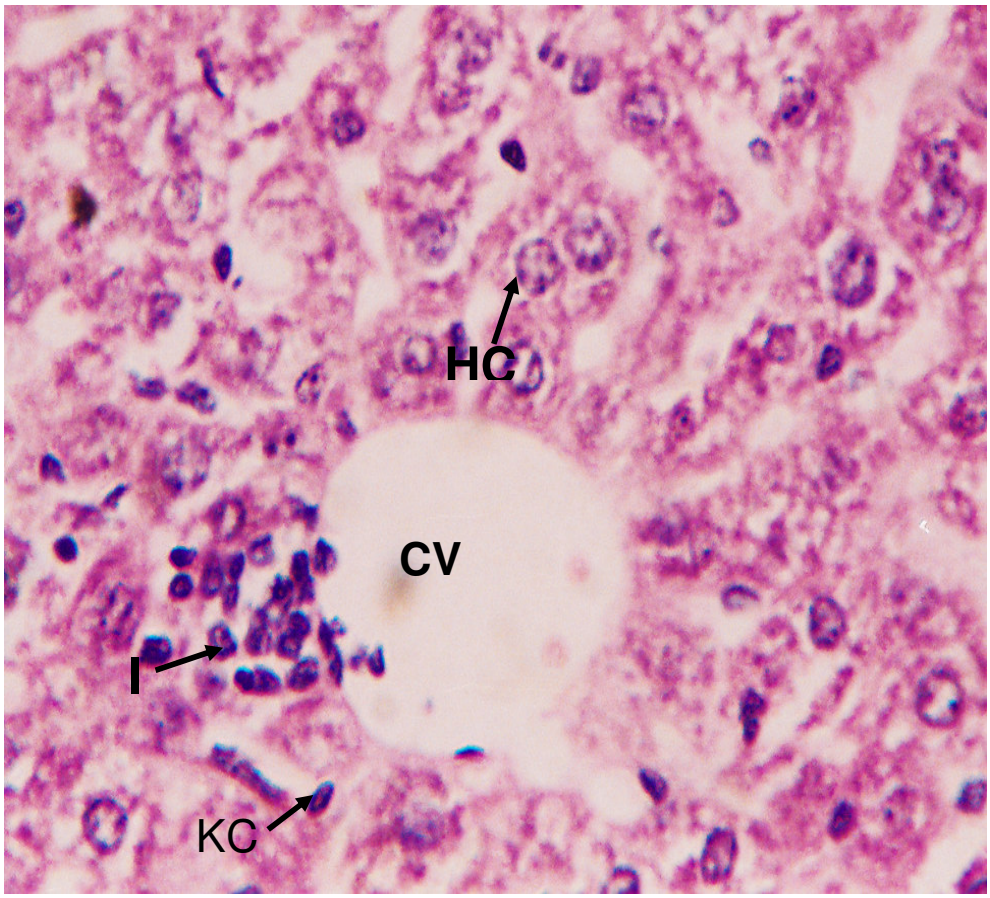

Fig.5: Photomicrograph of H and E stained liver section of mice treated at dose of 800 mg/kg/bw of *D. angustifolia* seed aqueous extract showing normal hepatocyte(**HC**), inflammatory cellular infiltrations (**I**) and activated Kupffer cells (**KC**) around central vein (**CV**) (H & E, x400)

4.9. Effect of the Aqueous Extract of *D. angustifolia* Seeds on Histopathology of Kidney

Microscopic observation of the kidney sections of mice treated at the dose of 400 mg/kg/bw of *D.angustifolia* aqueous seed extract revealed no marked histological change as compared to the control group. Evaluation of kidney sections obtained from mice treated with *D.angustifolia* seed aqueous extract at the dose of 800 mg/kg/bw showed similar histological findings as the control group. Histological sections of the kidney of the control group and experimental groups are shown in Figures 7 & 8 below.

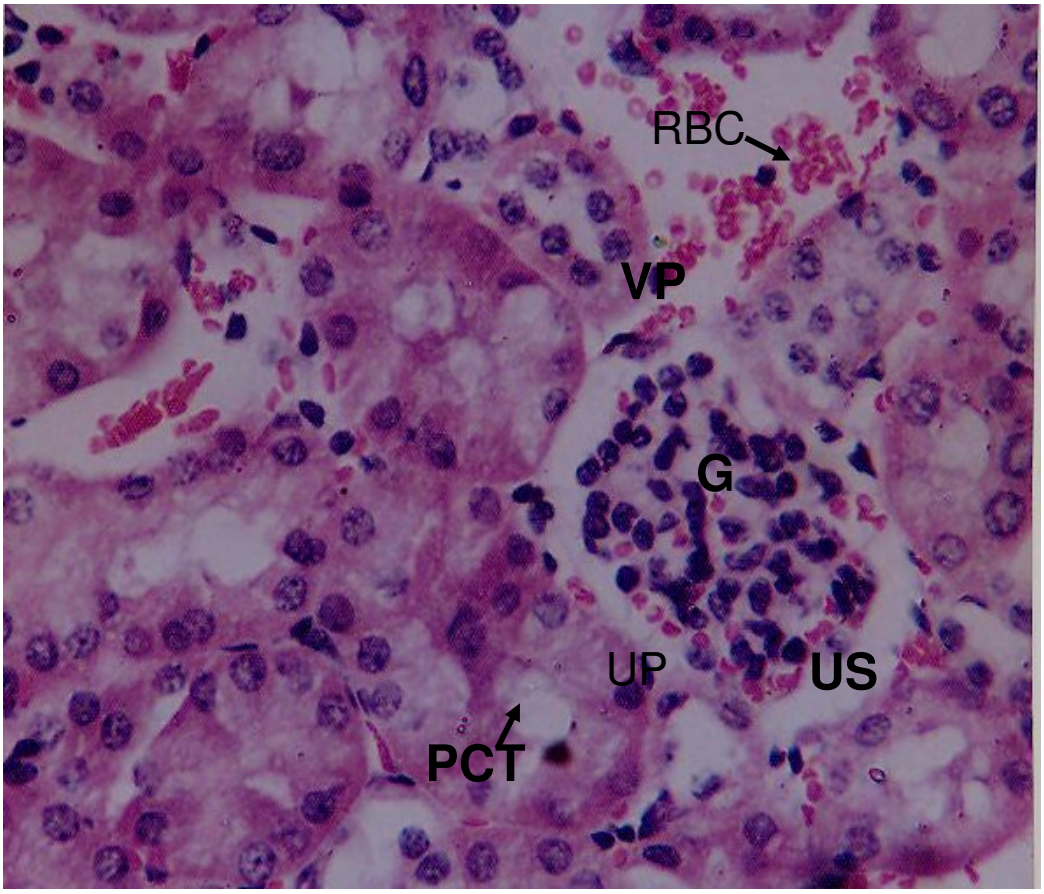

Fig.6: Photomicrograph of H and E stained kidney section of the control group showing normal histological feature (H and E, x400).

US = Urinary space, **G=** Glomerulus , **VP** = Vascular pole, **UP** = Urinary pole,

PCT = proximal convoluted tubule, **RBC** = Red blood cell

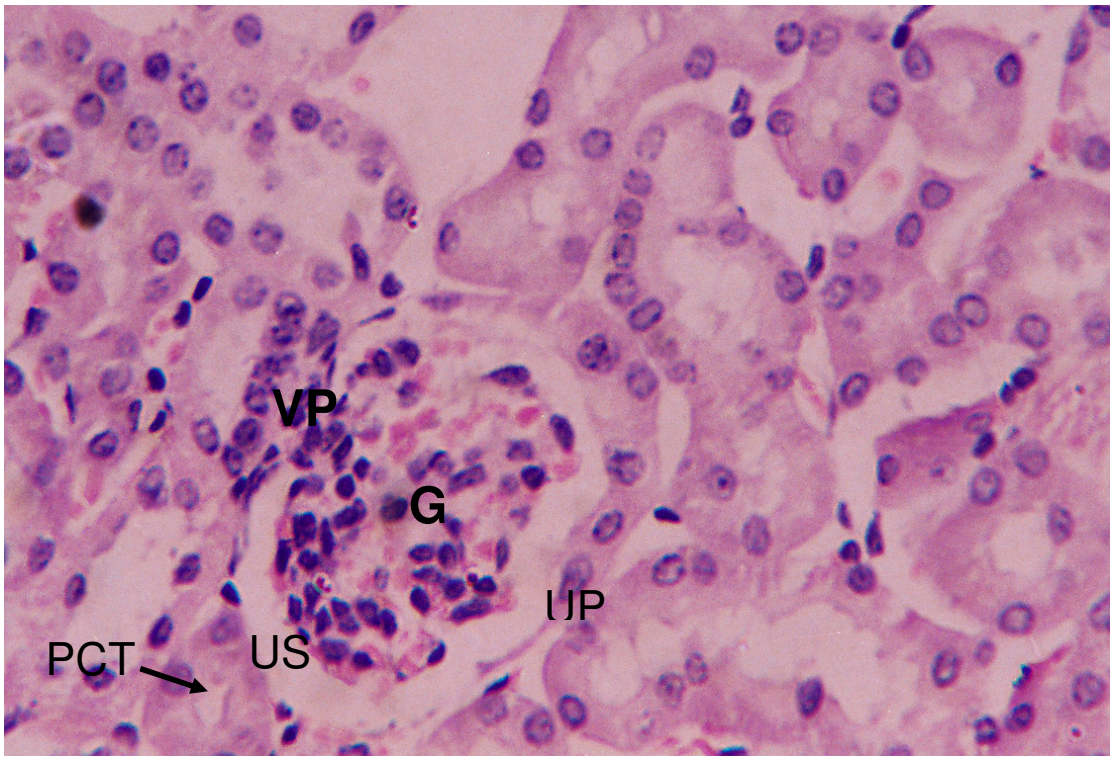

Fig.7: Photomicrograph of H and E stained kidney section of the mice treated at dose of 400mg/kg/bw of *D. angustifolia* seed aqueous extract. (H and E, x400). **US** = Urinary space, **G**= Glomerulus , **VP** = Vascular pole, **UP** = Urinary pole, **PCT** = proximal convoluted tubule

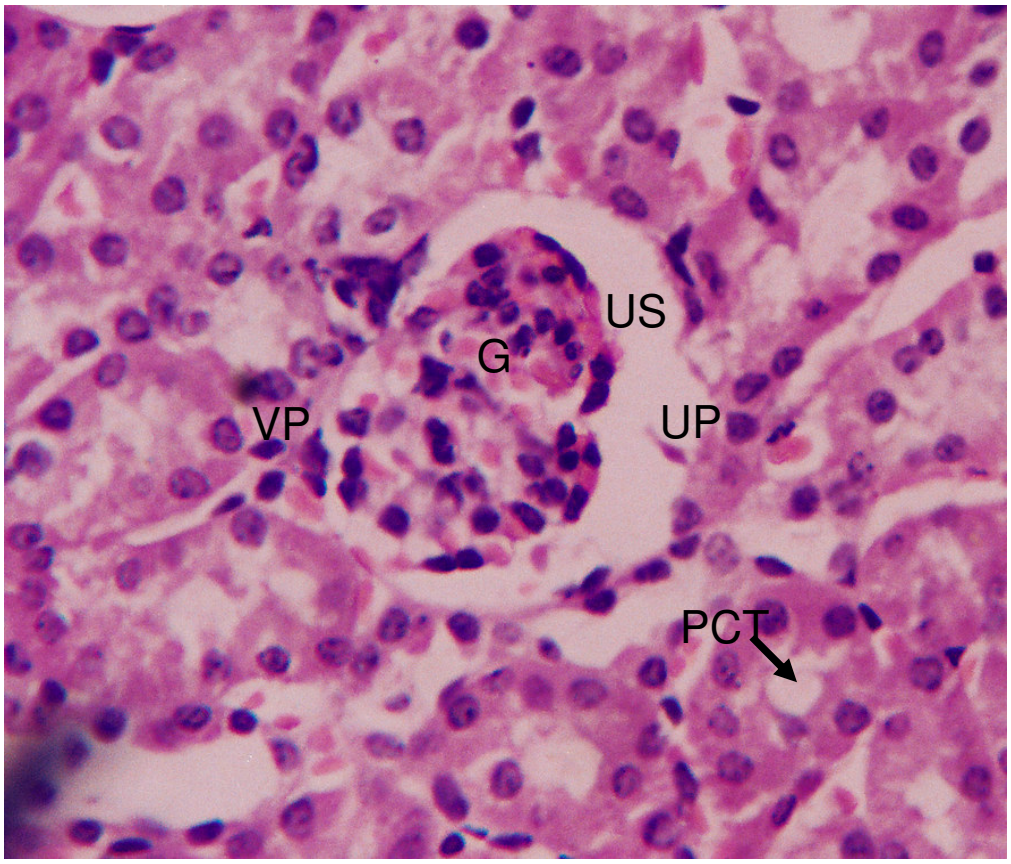

Fig.8: Photomicrograph of H and E stained kidney section of the mice treated at dose of 800mg/kg/bw of *D. angustifolia* seed aqueous extract. (H and E, x400). **US** = Urinary space, **G**= Glomerulus , **VP** = Vascular pole, **UP** = Urinary pole, **PCT** = proximal convoluted tubule

4.10. Effect of the Methanol Extract of *D. angustifolia* Seeds on Histopathology of Liver

Histological examinations of the liver sections of mice treated at a dose of 400 mg/kg/bw of the methanolic extract of *D. angustifolia* seeds indicated the presence of inflammatory cellular infiltration around central vein as compared to the histological examination of the liver of the control group. Although inflammatory cellular infiltration was observed around the central vein at dose of the 400 mg/kg/bw, the hepatocytes were normal and no vacuolation and no cellular necrosis was observed. Inflammatory cellular infiltrations were not only observed around central vein at this dose (400 mg/kg/bw) of the methanolic extract of plant but also observed in lobules of the liver sections.

The liver sections of mice treated at the dose of 600 mg/kg/bw of methanolic extract of the plant (Fig.11), showed significant inflammatory cellular infiltrations around central vein as compared to the control group. Moreover, cytoplasmic vacuolation, and pyknosis of nuclei as well as total damage of hepatocytes were observed as characterized by having darkly stained granules.

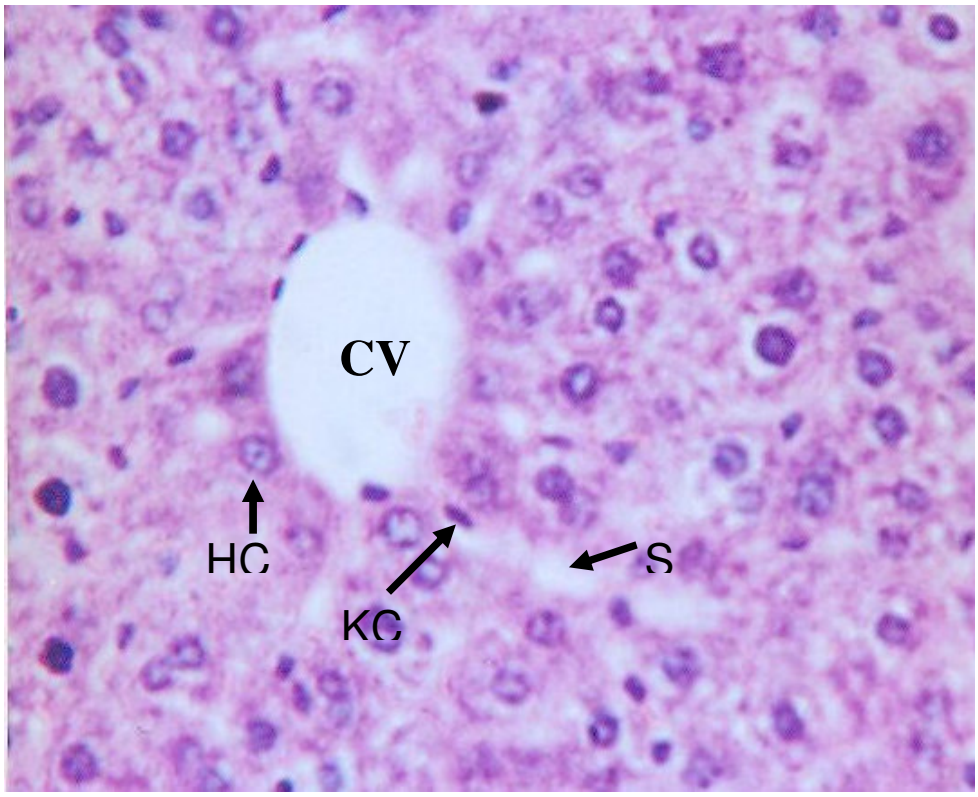

Fig.9: Photomicrograph of the H and E stained liver sections of control group showing the central vein (**CV**), and surrounding normal hepatocyte (**HC**), sinusoid (**S**), and Kupffer cells (**KC**). (H and E, x400)

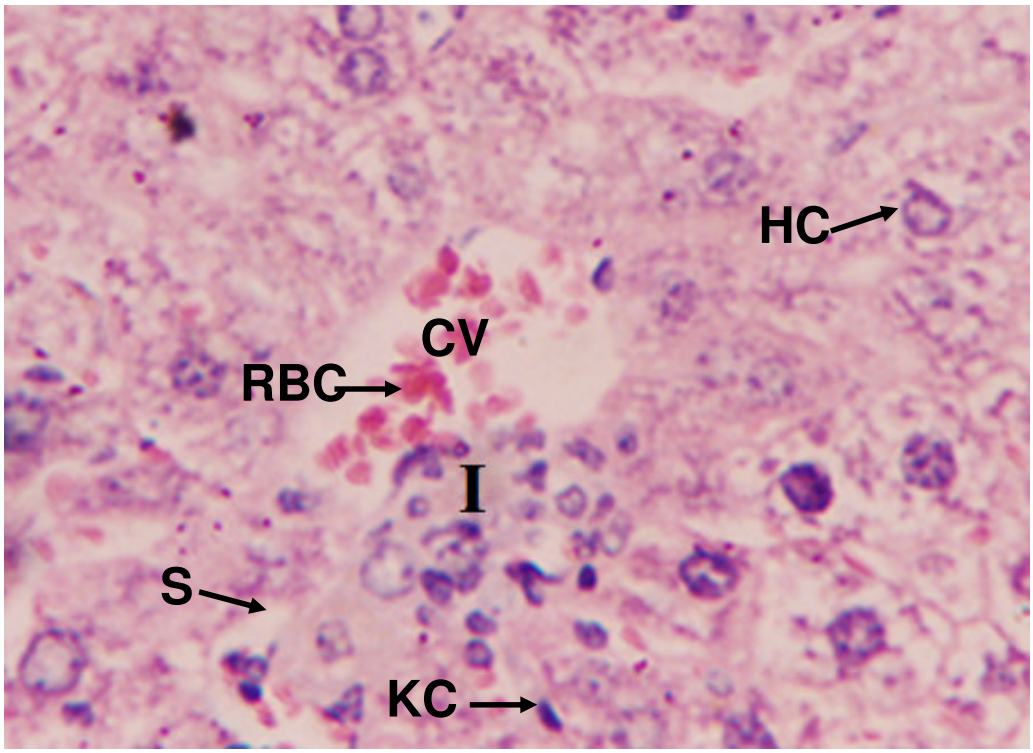

Fig.10: Photomicrograph of H and E stained liver section of mice treated at dose of 400 mg/kg/bw. *D. angustifolia* seed methanolic extract showing inflammatory cellular infiltrations (**I**) around central vein (**CV**)**,** and normal hepatocyte (**HC**), sinusoid (**S**), activated Kupffer cell (**KC**) and red blood cells (**RBC**) in **CV**. (H and E, x400)

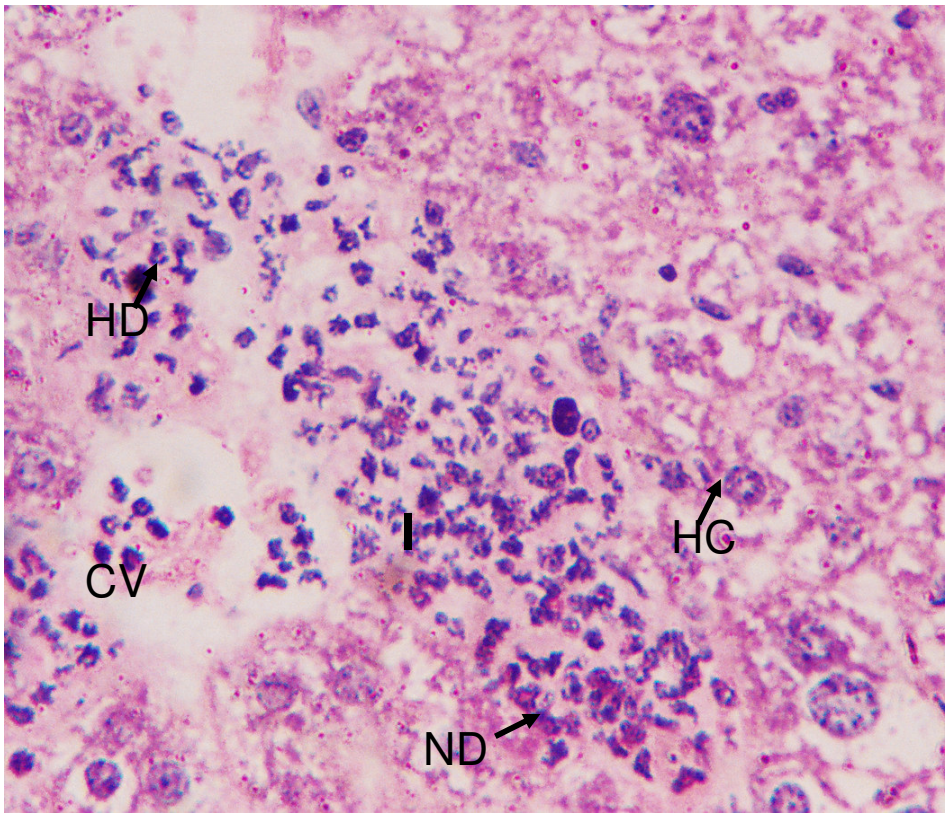

Fig.11: Photomicrograph of H and E stained liver sections of mice treated at 600 mg/kg/bw of *D. angstifolia* seed methanolic extract showing significant hepatocyte (**HC**) damage, inflammatory cellular infiltration **(I)** around the central vein (**CV**), and lobules, and nuclear death (**ND**) as well as hydropic degeneration (**HD**) (H & E x400).

4.11. Effect of the Methanol Extract of *D. angustifolia* Seed on Histopathology of Kidney

Histological examinations of the kidney sections of mice treated with *D. angustifolia* seed at dose of 400 mg/kg/bw methanolic extract of the plant showed normal histological appearance with no abnormal cellular alteration and tubular necrosis as compared to the histological examinations of control mice kidney sections (Fig.13).

Unlike the normal histological appearance of mice kidney sections treated at the dose of 400 mg/kg/bw, significant cellular damage, tubular necrosis, congested glomerulus and cellular infiltration around Bowman's capsule and complete obliteration of the urinary space with disturbed epithelial linings of the urinary space (Fig.14) were observed in mice treated with

methanol extract of *D. angustifolia* seed at dose of 600 mg/kg/bw, suggesting that the effect of methanolic extract of this plant is dose dependent.

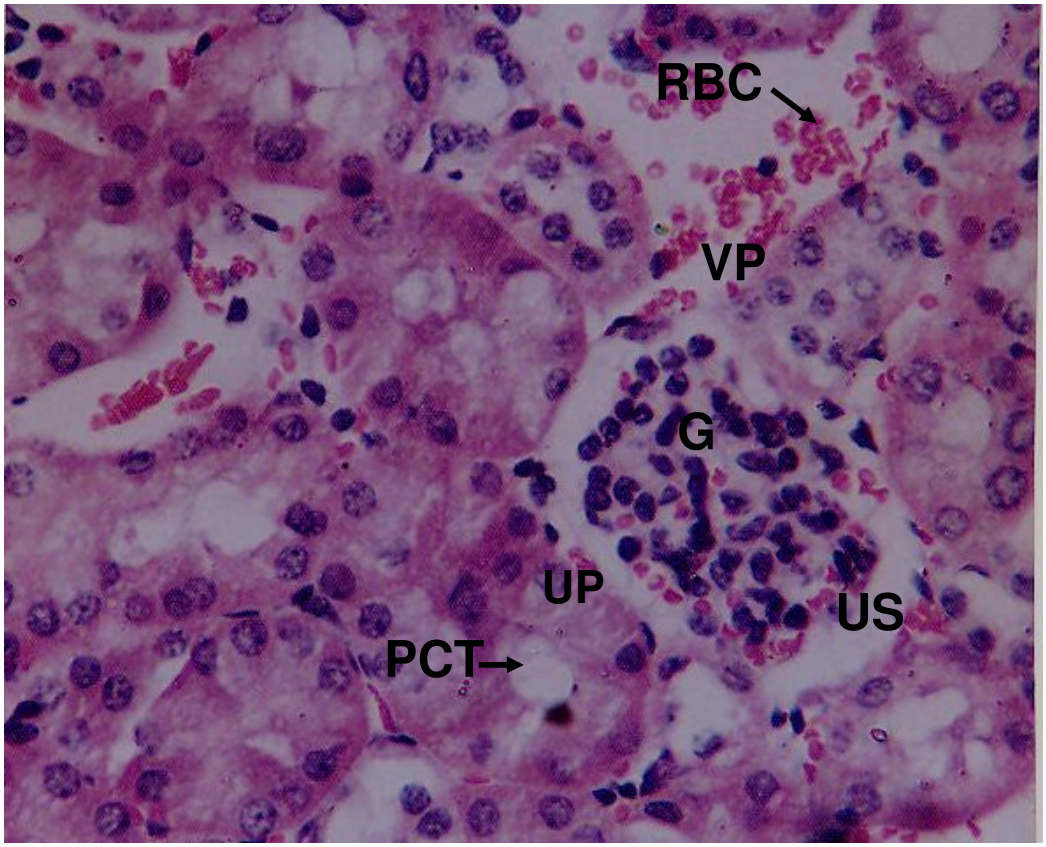

Fig.12: Photomicrograph of H and E stained kidney section of the control group showing normal histological appearance (H and E, x400).

US = Urinary space, **G**= Glomerulus , **VP** = Vascular pole, **UP** = Urinary pole, **PCT** = proximal convoluted tubule, **RBC** = Red blood cell

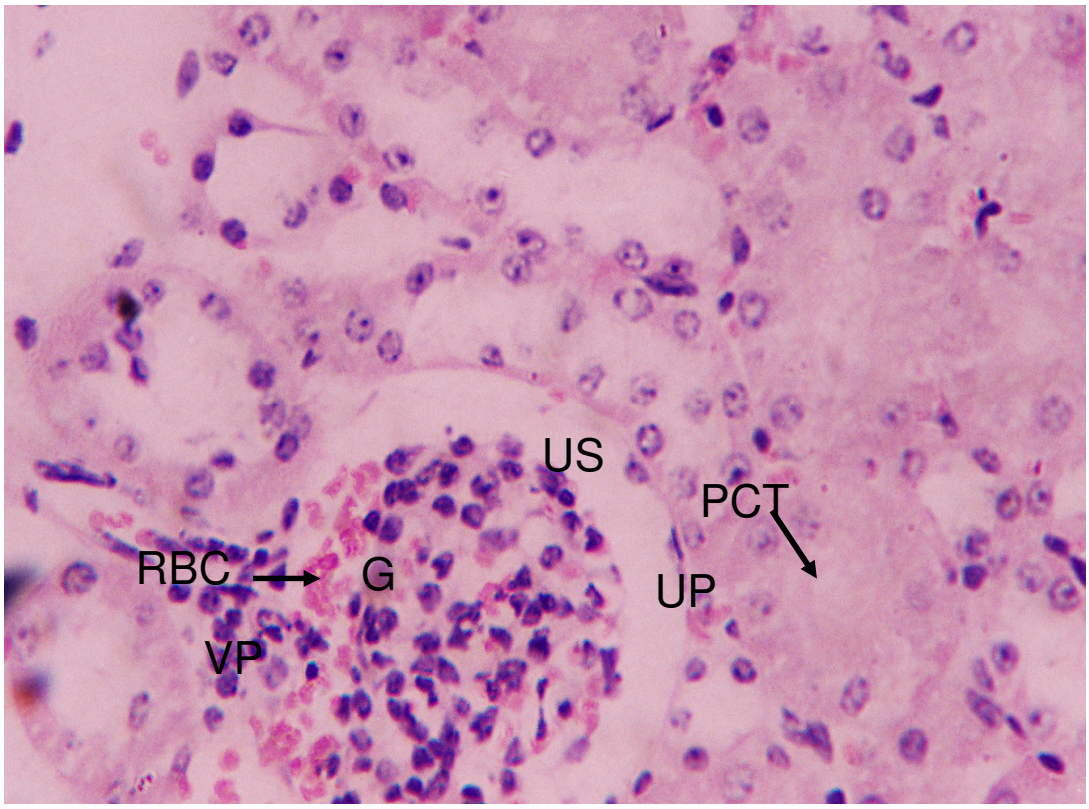

Fig.13: Photomicrograph of H and E stained kidney section of mice treated at dose of 400 mg/kg/bw of *D. angustifolia* seed methanolic extract. (H and E, x400). **US** = Urinary space, **G**= Glomerulus , **VP** = Vascular pole, **UP** = Urinary pole, **PCT** = proximal convoluted tubule, **RBC** = Red blood cell

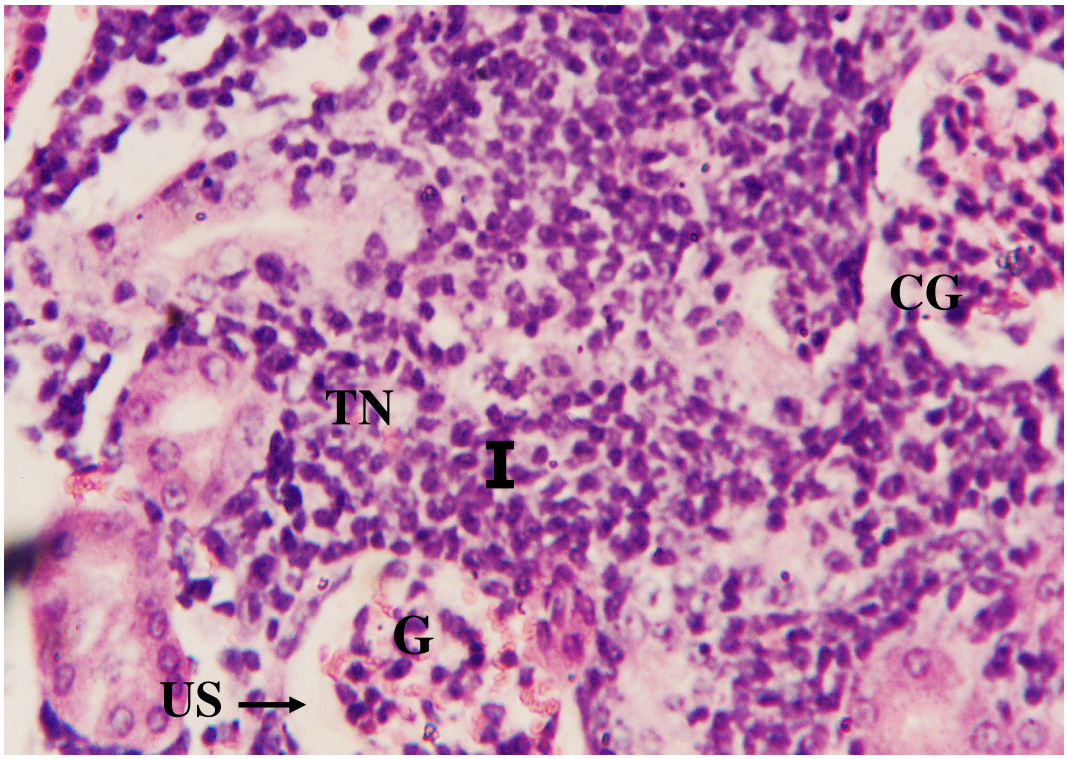

Fig.14: Photomicrograph of H and E stained kidney section of the mice treated at dose of 600 mg/kg/bw of *D.angustifolia* seed methanolic extract showing tubular necrosis (**TN**), congested glomerulus (**CG**) and significant inflammatory cellular infiltration (**I**) (H and E, x400). **US** = Urinary space, **G**= Glomerulus

5. DISCUSSION

Even though, the acute toxicity test has been widely criticized as a parameter for assessing toxicity there are still certain occasions when some useful information could be obtained from such studies. Apart from giving a clue on the range of doses that could be used in subsequent toxicity testing, it could equally reveal the possible clinical signs elicited by the substance under investigation (Anaigu *et al*., 2005).

Determination of LD_{50} is usually an initial screening step in the assessment and evaluation of the toxic characteristics of medicinal plants as it helps to identify the safety margin of the medicinal plant under investigation. In the present study, the $LD_{50,}$ a single oral dose at which 50 % of mortality occurs, of aqueous and methanolic crude extract of *D. angustifolia* seeds was found to be 4672.02 mg/kg/bw and 2922.30 mg/kg/bw respectively. The result of the present study is in agreement with that of the previous study (Berhan, 2008), which reported that the LD_{50} of aqueous crude extract of *D. angustifolia* seeds was greater than 4500 mg/kg/bw while that of methanolic extract was greater than 2000 mg/kg/bw. The difference in LD_{50} between aqueous and methanolic extract of *D. angustifolia* seed may be due to the presence of additional secondary metabolites in methanolic crude extract of *D. angustifolia*.

The behavioural manifestations such as salivation, lacrimation, vomiting, diarrhea and distension of abdominal region observed in the mice treated at higher doses of aqueous and methanolic extract of *D. angustifolia* seeds during determination of the LD_{50} were in agreement with the previous studies reported on toxic effects of the plants at higher dose by (Berhan, 2008).

Mild signs of toxicity such as depression, loss of appetite, and less water intake observed after four weeks administration of the aqueous crude extract at doses of 400 mg/kg/bw and 800 mg/kg/bw, during the chronic treatment period, may be due to the prolonged effect of the aqueous crude extract that may contribute to slight weight loss of the mice under treatment.

Depression, lacrimation, diarrhea and death observed among methanolic crude extract treated mice at higher dose of 600 mg/kg/bw may be due to the adverse effect of this extract on the organ – system of the treated mice.

At the end of the experiment, gain in body weight of the mice treated with methanolic crude extracts of the plant at both doses of 400 mg/kg/bw and 600 mg/kg/bw was observed. This

may be due to the presence of appetite stimulating secondary metabolite in the methanolic crude extract hence food intake increases resulting in increase in body weight of the mice. This showed that methanolic crude extract didn't adversely interfere with the nutritional benefits such as stability of appetite in treated mice (Aniagu *et el*., 2005). In contrast, slight body weight loss was observed in those mice treated with the aqueous crude extract of the *D. angustifolia* seed at doses of 400 mg/kg/bw and 800 mg/kg/bw.

The loss of body weight in aqueous extract treated mice was possibly due to appetite suppressant effect of any of the metabolites present in aqueous crude extract of the plant. Berhan (2008) also reported that the aqueous crude extract of *D*. *angustifolia* seed caused body weight loss of the mice under treatment.

No significant difference in relative weight of liver and kidney were observed in all aqueous extract treated mice when compared with that of the control group. This may be due to the absence of cellular inflammation of the target organs (liver and kidney) in treated mice. However, the slight increase in relative weight of liver and kidney observed among all methanolic crude extract treated mice may be due to the increase in body weight.

The biochemical compositions of blood such as blood urea, uric acid, creatinine, cholesterol, blood glucose level, alkalin phosphatase (ALP) aspartate aminotransfrase (AST), alanine aminotransferase (ALT) and electrolytes are used to evaluate the toxic effect of medicinal plants. It was indicated that elevation of blood glucose level, blood urea nitrogen, cholesterol, AST, ALT and albumin in serum blood shows that the administered medicinal plant has a toxic effect on animal model (Khleifat, 2002; Oyewole and Massaquoi, 2008). On the other hand, reduced level of blood glucose level, blood urea nitrogen and uric acid shows that the medicinal plant has limited toxicity effect and does not interfere with renal tubular function. From these points of views, change in these biochemical compositions of blood as well as enzymatic activities in the serum blood used to indicate the earlier toxic effect of medicinal plants. However, these changes in biochemical compositions of blood are induced due to damage to hepatocyte and severe acute liver failure (Khleifat, 2002).

Liver cell damage is indicated by increase in plasma enzymes such as AST, ALT, and ALP (Khleifat, 2002; Oyewole and Massaquoi, 2008). In the present study, the decrease in concentrations of ALP in the blood serum level of aqueous crude extract treated mice shows that the aqueous crude extract of the plant has no cholestasis effect. The cholestasis of the

liver was characterized by increase in level of ALP concentration in blood serum level (Babayi *et al*., 2007). Babayi *et al*. (2007) reported that the significant reduction in ALP levels by medicinal plant shows that no possible cholestasis occurred at the dose levels tested since increase in ALP level is usually a characteristic finding in cholestatic liver disease.

Aniagu *et al*. (2005) indicated that AST concentrations were consistently higher than ALT levels which are expected since body cells contain more AST than ALT. Usually, about 80% of AST is found in the mitochondria whereas ALT is a purely cytosolic enzyme. Therefore, AST appears in higher concentrations in tissues of liver and kidneys and is released slowly in comparison to ALT. Moreover, AST is not specific for the liver but is also found in other organs like the heart, brain, kidney and skeletal muscle. Since ALT is primarily found in the cytoplasm of the hepatocytes, it is considered to be a more sensitive marker of hepatocellular damage than AST (Aniagu *et al*., 2005).

In the present study, concentrations of both ALT and AST in blood serum level were slightly reduced at doses of 400 mg/kg/bw and 800 mg/kg/bw of aqueous treated mice indicating that the aqueous crude extract of the plant has no adverse effect on the histology of the liver. However, the slight elevation in the concentrations of ALT and AST at 400 mg/kg/bw of methanolic crude extract and significant increase at 600 mg/kg/bw of the methanolic crude extract is an evidence of hepatotoxic effect of the extract and that the effect is dose dependent. This is in agreement with the findings of Wurochekke *et al*., 2008 who reported that the significant increase in ALT level at a given doses of the herbal medicine is an indication of hepatocellular damage induced by the crude extracts of the herbal medicine.

Increase in concentration of urea and creatinine in the serum blood level indicates inability of the kidney to excrete these products suggesting a decrease in glomerular filtration rate (Ovuru *et al*., 2004). On the other hand, the reduced levels of urea and creatinine probably indicate that the medicinal plant did not interfere with the renal capacity to excrete these metabolites (Aniagu *et al.*, 2005).

In the present study, the concentration of urea and creatinine in the serum blood level of the aqueous crude extract treated mice with 400 mg/kg/bw showed similar concentrations with that of the control group. The concentrations of urea and creatinine were slightly reduced at the dose of 800 mg/kg/bw of the aqueous crude extract of *D. angustifolia* seed indicating that the aqueous crude extract of the plant has no toxic effect on the histology of kidney at this dose. In contrast, significant increase in the concentration of urea and creatinine in the serum

blood level of methanolic crude extract of *D. angustifolia* seed at the dose of 600 mg/kg/bw indicates the methanolic crude extract of the plant has adverse effect on the histology of kidney. However, the elevation at the effective dose (400 mg/kg/bw) of the methanolic crude extract of the plant shows no significant effect on the function of kidney. Slight increment of these biochemical parameters at the dose of 400 mg/kg/bw and significant increment of these biochemical parameters at the dose of 600 mg/kg/bw of the methanolic crude extract indicates that the effect of methanolic extract has dose dependent toxic effect on histology of the kidney. This result is supported by previous studies of Ovuru *et al*., (2004) and Aniagu *et al*., (2005) which reported increase in concentration of urea and creatinine in blood serum level shows the toxic effect of the medicinal plant on histology of the kidney. On the other hand reduction in the concentrations of these biochemical parameters in the serum blood level indicates the plant has no toxic effect on histology of the kidney.

In the present study, the result of haematological analysis such as RBC, WBC, HGB, HCT, MCV, MCH, MCHC, and PLT in both aqueous and methanolic extract treated mice showed no statistically significant difference ($P > 0.05$) when compared to the control group. This is in agreement with toxicological investigation of chronic treatment with other medicinal plant of C. *myricoids* on blood, liver and kidney tissues of mice done by Hayelom, (2009). In contrary, increase in concentrations of WBC seen in the present study at the effective doses of both aqueous and methanolic crude extract treated mice is in agreement with the toxicity studies reported in rats fed nature cure bitters (NCB) of other medicinal plant done by Aniagu *et al. (*2005).

Increase in the total WBC counts with both extracts (aqueous and methanolic) in the present study may be due to the presence of biologically active ingredients that have the ability to stimulate the immune system through increasing the population of defensive white blood cells (Aniagu *et al.,* 2005). In contrast, the reduction in the concentrations of lymphocyte at all doses of aqueous and methanolic extract treated mice in the present study may be due to the presence of anti – lymphocyte activity which may be compensated by increase in concentration of neutrophils at all doses of both aqueous and methanolic crude extracts of the plant.

Slight reduction of MCV observed at the dose of 800 mg/kg/bw of aqueous extract and 600 mg/kg/bw of methanolic extract may be due to failure of osmoregulation in the blood

(Moussa, 2004). This is in agreement with toxicological investigation of chronic treatment with *C. myricoids* on blood, liver and kidney tissues of mice reported by Hayelom (2009).

Because of its functional roles in the body, liver has been the major organ of toxicity for many xenobiotics. It is the first organ that comes in contact with internally absorbed chemicals, so in excess these chemicals may cause liver injury. Injury to the liver may affect the integrity of hepatocyte leading to the release of enzymes and damage to the hepatobilliary system causing the release of essential enzymes and impair the biosynthetic and catabolic capacity of the liver (e.g. cholesterol, protein, albumin and glucose) (Kefale,2005). In addition to these biochemical markers a change in the liver structure can contribute to the diagnosis of liver injury (Kefale, 2005).

Early evidence of liver damage is usually indicated by the fatty change which is further indicated by the form of cytoplasmic vacuoles in the hepatocyte. Consequently, the vacuoles will displace the nucleus to one side. Moreover, the hepatocytes will enlarge and the nuclei become darkly stained (Ebaid *et al.,* 2007). Zhang and Wang (1984) also suggested that the cytoplasmic vacuolation is mainly a result of considerable lipid inclusion and fat metabolism occurring during pathological changes on the liver structure. When the metabolic interference is becoming more severe, hydropic degeneration will be observed and the cell will become enlarged (Effendy *et al.,* 2006). Eventually, the affected cells will undergo necrosis. Retention of water inside hepatocyte resulted in oedema, which may have occurred due to the reduction of energy necessary for the regulation of ion concentration in the cell.

As it was indicated by Effendy *et al.* (2006), ‘‘unless the restoration of the normal structure of the liver is in place, the liver function will be impaired’’. Therefore, this pathological change in liver structure that results in impaired liver function interferes with the secretion of plasma protein (Ebaid *et al.,* 2007).This results in subsequent change in drainage of tissue fluid due to decrease in blood osmotic pressure (Ebaid *et al.,* 2007).

The focal cellular infiltration observed around the central veins at the dose of 800 mg/kg/bw and the absence of infiltration at dose 400 mg/kg/bw of aqueous extract of the plant indicated the dose dependent effect of the aqueous extract. This is in agreement with chronic toxicity study of *H. suaveolens* by Attawish *et al*. (2005), which reported that the water extract of *H suaveolens* given orally did produce dose dependent sign of toxicity in rats.

Unlike normal histological observation of aqueous extract treated mice liver sections, at effective dose of 400 mg/kg/bw of aqueous extract, focal cellular infiltrations observed in the liver sections of mice treated at the same effective dose of 400 mg/kg/bw of methanolic crude extract, shows that the methanolic crude extract of the plant may contain additional metabolites that adversely affect the liver. This is evident as the dose level increased to 600 mg/kg/bw whereby significant cellular infiltration around central vein, cytoplasnic vacuolation, pyknosis of nucleus as well as total damages of liver cell were observed. This is in agreement with the effect of other medicinal plant *T. polium* at higher dose of 50 mg/kg/bw that induced marked cytoplamic vacuolation, and damage of the liver tissue reported by Khleifat *et al*. (2002).

Early evidence of liver damage may be indicated by fatty changes which result in cytoplasmic vacuoles in hepatocyte which may impair the normal function of liver which may be further indicated by hydropic degeneration (Ebaid *et al*., 2007; Effendy *et al*., 2006).

In this study, treatment with methanolic crude extract showed changes in the histology of kidney sections at the dose of 600 mg/kg/bw including tubular necrosis, congested glomerulus, significant cellular damage and complete obliteration of urinary space. This may be due to the fact that kidney is the second target organ next to the liver where circulating toxins will cause pathological changes and disruption of glomerular functions (Ebaid *et al*., 2007). This is highly suggestive of the presence of additional secondary metabolites in the methanolic crude extract of *D. angustifolia* seeds which may adversely affect the liver and kidney.

The aqueous crude extract *D. angustifolia* seed is safe at the effective dosage (400 mg/kg/bw) for treatment of malaria with only some focal inflammations around the central vein at two folds of the effective dose (800 mg/kg/bw). Many herbal preparations have been found to induce renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss most prominently, in outer cortex (Dapar *et al*., 2007). This is in agreement with the adverse effect of the methanolic crude extract of *D. angustifolia* seeds at dose of 600 mg/kg/bw on histology of kidney sections observed in the present study.

6. CONCLUSION

From the present study, the following conclusion may be made:

- The aqueous extract of *D. angustifolia* seed has less toxic effect on the histology of liver and kidney than the methanolic extract. Furthermore, the methanolic extract could damage the liver and the kidney at a dose which produces anti malaria effect.
- The aqueous crude extract of *D. angustifolia* seed is safe at effective dosage (400 mg/kg/bw) with only some focal inflammations around central vein of the liver at two folds of the effective dose (800 mg/kg/bw).
- While the methanolic crude extract of *D. angustifolia* seed at the same effective dose (400 mg/kg/bw) as that of aqueous extract has slight toxic effect and adversely affects the biochemical parameter and histology of liver and kidneys of the mice under treatments, as the dose level increased to 600 mg/kg/bw, it may be concluded that using the methanolic crude extract of the plant at effective dose may not be safe.

7. RECOMMENDATIONS

Based on the result of the present study, the following recommendations are made:

- Further investigation should be recommended to isolate metabolites that may contribute to the toxic effect of methanolic crude extract on liver and kidney.
- Further investigations should be performed to identify the effect of the crude extract of the plant on other organs such as central nervous system, gastrointestinal tract, ovary and uterus.
- As the aqueous extract did not show adversity at the effective dose for malaria, it is recommended that medicinal plant users choose the aqueous extract of the plant.

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9. APPENDICES

SOLUTION PREPARATIONS

9.1. Fixative

Neutral buffered formalin

Formalin (~ 40% aqueous solution of formaldehyde) – 100 ml

Sodium dihydrogen orthophosphate (monohydrate) – 4 g

Disodium hydrogen orthophosphate (anhydrous) – 6.5 g

Distilled water - 900ml

9.2. Tissue processing

70% Alcohol..... 2 hrs
90% Alcohol..... 2 hrs
Aboslute Alcohol.....1 ½hrs
Absolute Alcohol 2.....1½hrs
Absolute Alcohol 3.....1½hrs
Absolute Alcohol 4.....Over night
Xylene 1 .....1½hrs
Xylene 2 .....2½hrs
Wax 1 .....1½hrs
Wax 2 .....2½hrs
Wax 3.....Overnight
Embedding

9.3. Staining Solution

9.3.1. Harris haematoxylin

Haematoxylin ----------2.5 g

Absolute alcohol ----------25ml

Potassium alum ----------50 g

Distilled water ----------500ml

Mercuric oxide ----------1.25 g

Glacial acetic acid----------20 ml

9.3.2. Preparation of Alcoholic Eosin

EosinY(yellowish)----------0.5 g

Ethanol(95%) ----------100 ml

Glacial acetic acid ----------0.5 ml

9.4. Bluing solution (0.1%)

Sodium bicarbonate ($NaHCO_3$) ----------1 gm

Distilled water----------1000 ml

9.5. Preparation of acid alcohol

Absolute alcohol (ethanol) ----------70 ml

Distilled water ----------30 ml

Hydrochloric acid ----------0.5 ml

9.6. Preparation of albumin coated glass slides

Dissolve 50ml of egg albumin in 50ml of glycerin in 1:1 ratio.

Dip slides, arranged in a clean slide rack, into the solution for 2 minutes.

Leave the slides to air dry for 10 minutes.

DECLARATION

I, the under signed, declare that this M. Sc thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for this thesis have been duly acknowledged.

M.Sc candidate Soressa Abebe Geneti

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Ato Kelbessa Urga

Signature _____

Date _____