

**ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES,
SCHOOL OF MEDICINE, DEPARTMENT OF ANATOMY**



**EVALUATIONS ON THE SUB-CHRONIC TOXICITY OF 70%
ETHANOLIC SEED EXTRACTS OF *ALBIZIA GUMMIFERA* AND
MILLETTIA FERRUGINEA ON BLOOD PARAMETERS AND LIVER AND
KIDNEY TISSUES IN ALBINO WISTAR RATS**

BY: MEKONNEN DEBEBE

AAU, Ethiopia

October, 2014

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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF AAU IN
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Table of Contents	page
1. INTRODUCTION.....	1
1.1. Background	1
1.2. Traditional medicine	3
1.3. Plant medicines	4
1.3.1. <i>Albizia gummifera</i>	4
1.3.1.1. Ethnobotany	4
1.3.1.2. Ethnomedicinal values.....	5
1.3.1.3. Bioactive ingredients	5
1.3.2. <i>Millettia ferruginea</i>	6
1.3.2.1. Ethnobotany.....	6
1.3.2.2. Ethnomedicinal values.....	6
1.3.2.3. Bioactive ingredients	7
1.4. Blood: Composition and functions	7
1.5. Liver: Structure and functions.....	9
1.6. Kidneys: Structure and functions.....	12
1.7. Significance of the study.....	14
2. OBJECTIVES	15
2.1. General objective	15
2.2. Specific objectives	15
3. MATERIALS AND METHODS.....	16
3.1. Plant Material Preparation.....	16
3.1.1. Plant Collection and Processing.....	16
3.1.2. Plant Material Extraction	17
3.2. Experimental Animals.....	18
3.3. Ethical Considerations	18
3.4. Sub-chronic toxicity test of <i>Albizia gummifera</i> and <i>Millettia ferruginea</i> seeds extract in rats.....	19
4. RESULTS	23
4.1. Sub-chronic toxicity study	23

4.1.1. Effect of the extract of both plants on general health and body weight.....	23
4.1.2. Effect of <i>Albizia gummifera</i> and <i>Millettia ferruginea</i> hydro alcohol seed extract on hematological parameters.....	29
4.1.3. Effect of <i>Albizia gummifera</i> and <i>Millettia ferruginea</i> hydro alcohol seed extract on serum biochemical parameters	35
4.1.4. Macroscopic observations and organ weights	37
4.1.5. Microscopic observations	39
5. DISCUSSION	45
6. CONCLUSION	52
7. RECOMMENDATIONS	53
8. REFERENCES.....	54
9. APPENDICES	65
9.1. Appendix I: Preparations of working solutions.....	65
9.2. Appendix II: Tissue processing schedules to form paraffin blocks for manual technique	66
9.3. Appendix III: Routine heamatoxylin and eosin (H and E) staining schedule for tissue sections. ...	67

List of figures	page
<i>Figure 1.1: Major causes of death worldwide among children less than five years of age and neonates ...</i>	1
<i>Figure 1.2: Photograph of Albizia gummifera showing the leaves and its pods</i>	5
<i>Figure 1.3: Photograph of seeds of Millettia ferruginea</i>	6
<i>Figure 1.4: Photograph of different lobes of rat liver.....</i>	11
<i>Figure 3.1: Map showing different cities of Ethiopia.....</i>	16
<i>Figure 3.2: Photograph showing different steps of extraction through orbital shaker, filtration and crude extraction by Rota vapor.....</i>	17
<i>Figure 4.1: Time course and dose effect of seeds extract of Albizia gummifera on body growth pattern of male rats</i>	26
<i>Figure 4.2: Time course and dose effect of seeds extract of Albizia gummifera on body growth pattern of female rats.....</i>	26
<i>Figure 4.3: Time course and dose effect of seeds extract of Millettia ferruginea on body growth pattern of male rats</i>	28
<i>Figure 4.4: Time course and dose effect of seeds extract of Millettia ferruginea on body growth pattern of female rats.....</i>	28
<i>Figure 4.5. Photomicrograph of H and E stained liver sections from rats administered with hydroethanolic seeds extracts of Albizia gummifera</i>	40
<i>Figure 4.6. Photomicrograph of H and E stained liver sections from rats administered with hydroethanolic seeds extracts of Millettia ferruginea</i>	41
<i>Figure 4.7. Photomicrograph of H and E stained kidney sections from rats administered with hydroethanolic seeds extracts of Albizia gummifera</i>	43
<i>Figure 4.8. Photomicrograph of H and E stained kidney sections from rats administered with hydroethanolic seeds extracts of Millettia ferruginea</i>	44

List of table's	page
<i>Table 4.1: Effect of the subchronic administration of Albizia gummifera seeds extract on the body weight of male and female rats</i>	25
<i>Table 4.2: Effect of seed extract of Millettia ferruginea on the body weight of male and female during the 13th week</i>	27
<i>Table 4.3: Hematological parameters of male rats treated with 125 and 250mg/kg body weight of seed extract of Albizia gummifera for 13 weeks</i>	31
<i>Table 4.4: Hematological parameters of female rats treated with 125 and 250mg/kg body weight of seed extract of Albizia gummifera for 13 weeks</i>	32
<i>Table 4.5: Hematological parameters of male rats treated with 125 and 250mg/kg body weight of seed extract of Millettia ferruginea for 13 weeks</i>	33
<i>Table 4.6: Hematological parameters of female rats treated with 125 and 250mg/kg body weight of seed extract of Millettia ferruginea for 13 weeks</i>	34
<i>Table 4.7: serum biochemical parameters of male rats treated with 125 and 250mg/kg body weight of seed extract of Albizia gummifera for 13 weeks</i>	36
<i>Table 4.8: Serum biochemical parameters of female rats treated with 125 and 250mg/kg body weight of seed extract of Albizia gummifera for 13 weeks</i>	36
<i>Table 4.9: Serum biochemical parameters of male rats treated with 125 and 250mg/kg body weight of seed extract of Millettia ferruginea for 13 weeks</i>	36
<i>Table 4.10: Serum biochemical parameters of female rats treated with 125 and 250mg/kg body weight of seed extract of Millettia ferruginea for 13 weeks</i>	37
<i>Table 4.11: Organ weights of male rats treated with 125 & 250 mg/kg body weight doses of the seed extracts of Albizia gummifera</i>	38
<i>Table 4.12: Organ weights of female rats treated with 125 & 250 mg/kg body weight doses of the seed extracts of Albizia gummifera</i>	38
<i>Table 4.13: Organ weights of male rats treated with 125 & 250 mg/kg body weight doses of the seed extracts of Millettia ferruginea</i>	38
<i>Table 4.14: Organ weights of female rats treated with 125 & 250 mg/kg body weight doses of the seed extracts of Millettia ferruginea</i>	39

LIST OF ACRONYMS

AAU	-----	Addis Ababa University
ALP	-----	Alkaline phosphatase
ALT	-----	Alanine aminotransferase
AST	-----	Aspartate aminotransferase
BASO	-----	Basophil's
°C	-----	Degree Celcius
EPI	-----	Ethiopian public Health Institute
EO	-----	Eosinophil's
FC	-----	Female rats control group
HCT	-----	Hematocrit
HGB	-----	Hemoglobin
LYMPH	-----	Lymphocytes
LD ₅₀	-----	Lethal dose for 50 percent of the population
MC	-----	Male rats control group
MCH	-----	Mean corpuscular hemoglobin
MCHC	-----	Mean corpuscular hemoglobin concentration
MCV	-----	Mean cell volume
mg/kg	-----	Milligram per kilogram
MONO	-----	Monocytes
NEUT	-----	Neutrophils
PCV	-----	Packed cell volume
PLT	-----	Platelets
RBC	-----	Red blood cells
RDW-CV	-----	Red cell distribution width cell volume
SEM	-----	Standard error of the mean
SPSS	-----	Statistical package for social sciences
WBC	-----	White blood cells

ABSTRACT

Albizia gummifera and *Millettia ferruginia* are plants found in Ethiopia that have different medicinal values. The objective of the present study was to evaluate the toxicological effects of sub-chronic administered hydro-ethanolic (70%) seeds extract of *Albizia gummifera* and *Millettia ferruginea* in albino Wistar rats. The seeds of these plants were collected from different areas of Ethiopia. They were dried and crushed to powder and macerated with hydro-alcohol and placed in orbital shaker. The extract was then filtered through Whatman filter paper No.1 and the filtrate was evaporated to dryness by Rota vapor and further concentrated by water bath at 40 °C. The extract was packed in air tight brown glass bottles and kept in a refrigerator at 4 °C. The extract was then administered to rats at different doses to determine the LD₅₀ of the extract and at doses of 125mg/kg/day and 250mg/kg/day for the sub-chronic toxicity study. The LD₅₀ of *Albizzia gummifera* and *Millettia ferruginea* were found as 4000mg/kg and 3500mg/kg, respectively. Statistically significant difference in body weight was observed in female rats in the 10th week at 250mg/kg body weight of seeds extract of *Albizia gummifera* administered group and in the male rats at lowest dose during the 9th and 10th weeks of administration period for seeds extract of *Millettia ferruginea*. The seeds extract of *Albizia gummifera* statistically decreased ($p \leq 0.05$) MCH in the male rats at both doses; MCHC at both 125mg/kg and 250mg/kg in the female rats; and MCH in the female rats at higher dose and increased RDW-CV in the male rats at both doses. It increased NEUT at the highest dose in both females and males. The seeds extract of *Millettia ferruginea* decreased ($p \leq 0.05$) in the MCHC and MONO of female rats at the highest doses. ALP, ALT and urea were found significant in the female rats administered with 250mg/kg of *Millettia ferruginea* seeds extract. While, seeds extract of *Albizia gummifera* increased only urea in male rats at 250mg/kg. Some histopathological changes in liver and kidney were also observed for both plants extracts. There were inflammation, congestions and focal hepatocellular necrosis of the liver tissue. The extracts also produced atrophy of the glomeruli of the kidney. The observed changes in both of the plant seeds extract might have resulted because of the presence of some bioactive ingredients in the extract. Therefore, the active ingredients which might be responsible for toxic insult should be researched with their mechanisms of actions.

Key words: *Albizia gummifera*, *Millettia ferruginea*, seeds, hydroethanolic extract, toxicity, Wistar Albino Rats.

1. INTRODUCTION

1.1. Background

Human health is directly related to the quality and quantity of readily available water in nature. In turn, exposure to waterborne pathogens influences the occurrence of diarrheal illness. This is true for the developing world particularly of the African continent, where poverty, water quality, and sanitation deficiencies have strongly contributed to the declining human health levels and, in particular by diarrheal disease (Alexander & Blackburn, 2013). As indicated in the figure 1.1 below, diarrheal disease accounts for about 17% of death in children less than five years of age. In the presence of reasonably high amount of intestinal parasite and *Salmonella* and *Shigella* species that are drug resistant to the commonly prescribed drugs (ampicillin, amoxicillin, and cotrimoxazole) diarrheal disease is a threat to the children and community at large (Beyene & Tasew, 2014). Such increasing development of bacterial resistances against the known antibiotics necessitates the search for new antimicrobial agents (Sergeev *et al.*, 2014).

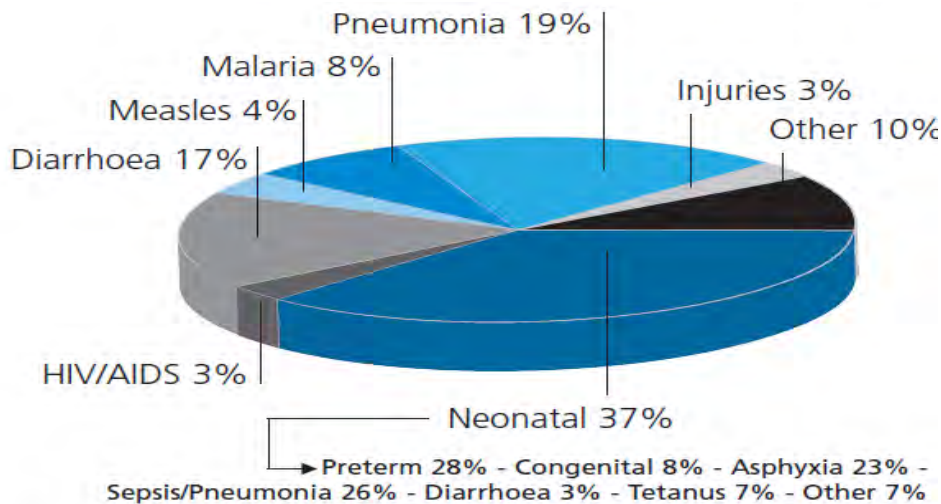


Figure 1.1: Major causes of death worldwide among children less than five years of age and neonates (Bryce *et al.*, 2005)

On the other hand schistosomiasis, leishmaniasis and malaria remain major problems to society. Schistosomiasis is also a water-based disease caused by trematodes of the genus *Schistosoma*. The parasitic larvae live in fresh water and can penetrate human skin, placing people at risk

through everyday activities. Inside the victim's body, adult female worms lay thousands of eggs and that cause significant damage to internal organs, such as the intestines, bladder, kidneys, liver, and lungs. The five schistosome species that are known to infect humans are *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum*, *S. mekongi*, and *S. japonicum*. It remains one of the most prevalent parasitic diseases in the tropics and subtropics. About 122 million peoples in eastern Africa are currently infected with either *S. mansoni*, or *S. haematobium*, or both species concurrently (Schur *et al.*, 2013).

Leishmaniasis is a protozoan parasitic disease caused by infection with different species of *Leishmania*. The life cycle of this parasite is maintained through the bite of phlebotomine sand flies. It is often referred to as a group of diseases because of the varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction. The most common forms of leishmaniasis in the Old World are cutaneous leishmaniasis, causing skin sores (Reithinger *et al.*, 2007), and visceral leishmaniasis (also known as kala-azar), affecting internal organs of the body such as spleen, liver, and bone marrow (Chappuis *et al.*, 2007). The rare clinical form of the disease is mucocutaneous leishmaniasis (also known as espundia) which causes destruction of mucosal areas of the oral cavity, nasal cavity and pharynx which occurs exclusively in Latin America (Myler & Fasel, 2008).

Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium*. Female Anopheles mosquitoes are responsible for maintaining the life cycle and transmitting of the disease. In the life cycles of these parasite gametes are taken up by mosquitoes and develop into sporozoite stage which will be injected into human (Cox, 2010).

A large proportion of the population of developing countries, use traditional medicine alone or in combination with Western drugs to treat a wide variety of ailments including the above mentioned parasites. With the renewed interest from Western countries in herbal remedies, and the increasingly urgent need to develop new effective drugs, traditionally used medicinal plants have recently received the attention of the pharmaceutical and scientific communities (Taylor *et al.*, 2001). Traditional and modern medicine research directorate of the Ethiopian public health research institute is currently developing drugs from plant remedies having antimicrobial, anti leishmaniasis, antimolluscidal (snails the intermediate host of schistosomiasis) and

antilarvacidal (to eradicate malaria at the larval stage) as treatments to the above diseases. Two of such screened plants, which are also the objectives of this study, are *Albizia gummifera* and *Millettia ferruginea*.

1.2. Traditional medicine

Global traditional medicine usage is widespread and growing in various parts of the developing world such as Africa, China, India, Japan and Latin America in which about 80%, 40%, 65%, 60-70% and 40%-71% respectively of their population use traditional medicine to meet their primary health care needs (WHO, 2002). Despite the pressure from the national health policy that promotes biomedicine, traditional medicine is still the major source of health care service for many patients in Ethiopia (Teshome, 2013). According to the national policy on traditional medicine and regulation of herbal medicine report of WHO global survey 2005, various traditional medicine practices have been developed in different regions but without a parallel development of international standards and appropriate methods for evaluating them. Traditional medicine is at the crossroads of two different clusters of competences: values and responsibilities (WHO, 2008).

Two factors could be identified which made traditional medicine persistent despite the absence of official technical and financial support to healers. First, traditional medicine remains the source of culturally appropriate therapy for the illness that biomedicine does not recognize their relevance such as illnesses of personalistic origin. Secondly, the dissatisfaction with treatment outcomes at anyone of the biomedical clinics or hospitals pushes patients to consult traditional healers and hence contributes to their persistence (WHO, 2008). Traditional medicines includes various forms of therapy out of which phytotherapy is the most common form.

1.3. Plant medicines

In Africa, more than 2,000 plants have been identified and used as remedies to treat several ailments, but very few of these plants have been screened for their safety (Fennell *et al.*, 2004). Ethiopia has a long history of using plants as traditional medicine, but knowledge about the extent and characteristics of traditional healing practices is constrained and it has even been ignored in the national health care system (Deribe *et al.*, 2006). Medicinal herbalism is the study of herbs and their medicinal uses (Ameh *et al.*, 2011). This definition can be extended to include the cultivating, collecting and dispensing of aromatic plants, particularly those considered to have medicinal properties. The folk knowledge and traditions of Ethiopia utilize the herbal resources available in nature. This knowledge is transferred from generation to generation orally as guarded secrets (Yadav, 2013). Among the many plants used for traditional medicine, *Albizia gummifera* and *Millettia ferruginea* are in Ethiopia.

1.3.1. *Albizia gummifera*

1.3.1.1. Ethnobotany

One of the plants used as traditional medicine in Ethiopia is *Albizia gummifera* (peacock flower- in English, Sessa- in Amharic, Ambabesamuka- in Oromifa). It belongs to the family Leguminosae, and a sub family Mimosoideae. *Albizia gummifera* is a large deciduous tree with flattened canopy, growing up to 35m high and trunk up to 75cm in diameter. It is found in east Africa, the Democratic Republic of Congo, Madagascar, and West Africa, ranging from dry or wet lowlands to up land forest edges, and in riverine forest, at an altitude of 2400m above sea level. It is indigenous in few countries namely; Angola, Cameroon, Democratic Republic of Congo, Ethiopia, Kenya, Madagascar, Nigeria, Tanzania, Uganda, and Zambia. It is, however, exotic in Brazil (Schmidt and Mwaura, 2010). The picture of the plant is shown in figure 1.2.



A

B

Figure 1.2: Photograph of *Albizia gummifera* showing:- A) the leaves and its pods containing seeds and B) indicate its stem

1.3.1.2. Ethnomedicinal values

Albizia gummifera seeds, has shown activities against different bacteria at different gradient of dilution (Geyid *et al.*, 2005). It has also shown promising molluscidal activities against *Biomphalaria pfeifferi*, *Bulinus sp.* and *physa acuta* (Woldemicheal *et al.*, 2006). According to Debella *et al.* (2007), the seed extract of *Albizia gummifera* shows larvicidal activities against *Aedes aegypti*, *Aedes africanus*, and *Culex quinquefasciatus*. Traditionally stem bark decoction of *Albizia gummifera* is used to treat malaria and an extract from fresh crushed pods is used to treat stomach pains. Its roots are used to cure skin diseases such as acne, itching and eczema (Kokwaro, 2009). Furthermore, crude hydro-alcoholic (20-80 %) extracts of *Albizia gummifera* was effective against reference strain of *N. gonorrhoeae* (Tefera *et al.*, 2010).

1.3.1.3. Bioactive ingredients

The therapeutic effects of plant species are determined by their constituents. Phytochemical studies have shown the presence of various bioactive ingredients in some traditionally used plant extracts, which are responsible for their medicinal uses. Aqueous seed extracts of *Albizia*

gummifera contain chemical constituents such as alkaloids, polyphenols, unsaturated sterol/or triterpens, saponins, glycosides and carbohydrates (Woldemichael *et al.* 2006).

1.3.2. *Millettia ferruginea*

1.3.2.1. Ethnobotany

Millettia ferruginea (birbira- in Amharic, sotallo- in Oromiffa, sari- in Oromiffa Arsi, Yego- in Oromifa Harar, Enghe diksho- in Sidama, Zaghia- in Wallayita), belongs to the family Leguminosae and a sub family Papilionoideae. It is an indigenous plant species found only in Ethiopia. There are two sub-species known to be found in this country. These are: *Millettia ferruginea* which is confined to the northern part of the country and *Millettia darasana* which is found in southern provinces, particularly Sidama region. This plant (*Millettia ferruginea*) is umbrella-shaped or flattened at top, and grows up to a height of 25-35m (Karunamoorthi *et al.*, 2009, Kothai and Befirdu, 2012). The picture of the plant seeds is shown in figure 1.3.



Figure 1.3: Photograph of *Millettia ferruginea*: -A) Stem and its leaves; B and C indicate its seeds

1.3.2.2. Ethnomedicinal values

The seed extract of *Millettia ferruginea* showed promising larvicidal activities against *Aedes aegypti*, *Aedes africanus* and *Culex quinquefasciatus* (Debella *et al.* 2007). Traditionally, bark and mature fruit and seeds of *Millettia ferruginea* are used as fishing poison (Banzouzi *et al.*, 2008).

The fruits, leaves, seeds and stem decoction of *Millettia ferruginea* are used respectively for the treatments of pain, earache and bacterial infection of nails, insecticidal properties and toothaches (Banzouzi *et al.*, 2008). Furthermore, *Millettia ferruginea* leaf has activities against wound causing bacteria (Taye *et al.*, 2011).

1.3.2.3. Bioactive ingredients

Pytochemically screened, aqueous seed extracts of *Millettia ferruginea* contain chemical constituents such as polyphenols, tannins, unsaturated sterol/or triterpens, glycosides and carbohydrates (Woldemichael *et al.* 2006).

1.4. Blood: Composition and functions

Like the other connective tissues, blood consists of cells and an extracellular component. Blood cells are erythrocytes, leukocytes and thrombocytes. Plasma is the liquid extracellular material that imparts fluid properties to blood. In humans the relative volume of cells and platelets, and plasma in whole blood is approximately 45% and 55%, respectively. Total blood volume in the average adult person is about 6 L or 7% to 8% of the total body weight (Ross and Pawlina, 2011). The mean blood volume for male rats was found to be 55.6 μ l/gm and the corresponding value for female rats was 53.1 μ l/gm (Everett *et al.*, 1956).

The RBCs are biconcave disks with no nucleus and under normal physiologic conditions they never leave the circulatory system. The major function of erythrocytes is to transport hemoglobin, which in turn carries oxygen and carbondioxide (Guyton and Hall, 2006; Tortora and Derrickson, 2009; Ross and Pawlina, 2011). An estimate of the volume of packed erythrocytes per unit volume of blood is referred to as hematocrit (Junqueira and Carneiro, 2005; Ross and Pawlina, 2011). In contrast to mammals, RBCs in birds, reptiles, and other lower vertebrates have nuclei (Zhang *et al.*, 2011). The anucleated erythrocyte, which is seen in mammals, is considered evolutionarily more advanced (Snyder and Sheafor, 1999).

The WBCs are the diffuse units of the body's defense system and most of them are specifically transported to areas of infection and inflammation. When they reach their destination, they leave the blood stream by migrating between the endothelial cells of the post capillary venules and

capillaries by a process known as diapedesis, and enter the connective tissue spaces to perform their function (Junqueira and Carneiro, 2005; Guyton and Hall, 2006; Ross and Pawlina, 2011).

Ottesen (1954) also asserted that it is possible to calculate the time cells have spent in the organism by incorporation of radioactive phosphorus into their deoxyribonucleic acid during their formation. This remains in the cells during their life time. In other words the age of the cells is from the time of their labeling until they are sampled from the blood stream. Accordingly, Ottesen (1954) found that the mean age of the granulocyte is 8.7 to 9.4 days. The majority of the granulocytes enter the blood stream at an age of about 6 days. Less than 5 % of the granulocytes in the blood stream are less than 5 days old, and a negligible percentage is older than 3 weeks. That of lymphocytes have a mean age of about 100 to 200 days.

Circulating blood monocytes undergo further maturation (which differ in terms of phenotype, morphology and function) up on leaving the vasculature and migrating into the various tissues and body cavities (Kreutz *et al.*, 1992). The successful completion of the differentiation pathway from the immature precursor blood monocytes to mature macrophages seems to be a central event in the ontogeny of the mononuclear phagocyte system (Kreutz *et al.*, 1992). Macrophages play a significant role in immune system of the body. They assume a defensive role exhibited by their ability to carry on phagocytosis of parasites and microbes. They regulate lymphocyte activation and proliferation and they are essential in the activation process of T- and B-lymphocytes by antigens and allogenic cells (Elhelu, 1983).

Blood platelets (thrombocytes) are non-nucleated, disk-like cell fragments that are formed in the bone marrow from megakaryocytes. Their transport towards the vessel wall is influenced by the hematocrit, red blood cell (RBC) size, and shape. They are the primary cells responsible for the control of bleeding and under normal circumstances their activation in response to bleeding triggers the clotting process (Aarts *et al.*, 1986 ; Junqueira and Carneiro, 2005; Guyton and Hall, 2006).

Plasma is liquid medium in which blood cells are suspended, and involved in the transport of nutrients from their site of absorption or synthesis and distributing them to various areas of the

organism. It also transports metabolic residues which are removed from the blood by excretory organs (Ross and Pawlina, 2011).

1.5. Liver: Structure and functions

The human liver is the largest internal organ in the body (Tortora and Derrickson, 2009). It is located in the right upper quadrant of the abdominal cavity beneath the diaphragm. It is well protected by the rib cage in the dome of the diaphragm and maintains its position through peritoneal reflections (attachment). It is divided into right larger lobe and a smaller left anatomic lobe by the attachment of the falciform ligament on the anterosuperior surface (Moore and Dalley, 2006; Tortora and Derrickson, 2009). The liver is a highly vascularized organ which has dual blood supply uniquely by the hepatic artery, which contributes 20-30% of the blood supply, and the portal vein, which is responsible for the supply of the remaining 70-80% (Junqueira and Carneiro, 2005; Ross and Pawlina, 2011).

As described by Kogure *et al.*, (1999) and Martins and Neuhaus, (2007), 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 measures from 3.8 to 4.2 cm, while the anterior–posterior diameter ranged from 2.2 to 2.5 cm.

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. The superior (parietal) surface comprises a part of the left lateral and medial lobes, and, as a whole, is convex, and fits under the vault of the diaphragm. It is completely covered by the peritoneum, except along the line of attachment of the falciform ligament. The inferior (visceral) surface is uneven, concave and is in relation to the stomach, duodenum, and right colic flexure, the superior part of the pancreas, the right kidney and suprarenal gland. The rat liver inferior surface does not have the fossae in the shape of the letter H as in humans. This surface is almost completely invested by the peritoneum. Through the porta hepatis (transverse fissure) the portal vein, the hepatic artery

and nerves, the hepatic duct and lymphatic's pass. The posterior surface is not covered by the peritoneum over some part of its extent, and is in direct contact with the diaphragm. It extends obliquely between the caudate lobe (CL) and the bare area of the liver. The inferior vena cava is completely intrahepatic (Kogure *et al.*, 1999; Martins and Neuhaus, 2007).

Although the rat liver is lobated, it has rather uniform surfaces as the lobes lie flat against each other. The only exception to this is the posterior CL, which is separated from the remainder of the liver by the stomach. The line of attachment of the falciform ligament divides the liver into two parts, termed the right and left lobes. Different from human liver, in which the right lobe is much larger than the left one, the rat left and right liver have approximately the same volume. The rat liver lobes, like that of the human liver, are named after the portal branches that supply them, as among mammals, the portal system is the most constant anatomical reference. 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, makes 2/3 of the volume of the medial lobe) and a smaller left medial lobe (LML, makes 1/3 of the volume of the medial lobe). The RML has both left and right hepatic vascular components (Kogure *et al.*, 1999; Martins and Neuhaus, 2007).

The right lobe (RL) is located on the right of the venacava 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 right lobe (SRL, also called the right posterior lobe) and inferior right lobe (IRL, also called the right anterior lobe). 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 fissures. The 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 and is divided into two portions: the paracaval portion (caudate process), which accounts for 2–3% of the liver mass, encircles the inferior vena cava and bridges the CLs and the right lateral

lobe, and the Spiegel lobe, which has an anterior (superior) and a posterior (inferior) portion in the form of discs, each representing 4% of the liver mass. The anterior part of the CL is located anterior to the esophagus and stomach and its pedicle lies superior, while the posterior is located behind these structures and its pedicle lies inferior. Both are covered by a very thin layer of peritoneum, the hepatoduodenal and hepatogastric ligaments (Kogure *et al.*, 1999; Martins and Neuhaus, 2007). The above mentioned different lobes of the rat liver is illustrated in the figure 1.4 below.

Liver impressions (colic, renal, duodenal and suprarenal) are not as evident as in the human liver. 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 one, the round ligament, is a fibrous cord, which is the obliterated umbilical vein. The liver is also attached to the lesser curvature of the stomach by the hepatogastric ligament and to the duodenum by the hepatoduodenal ligament (Kogure *et al.*, 1999; Martins and Neuhaus, 2007).

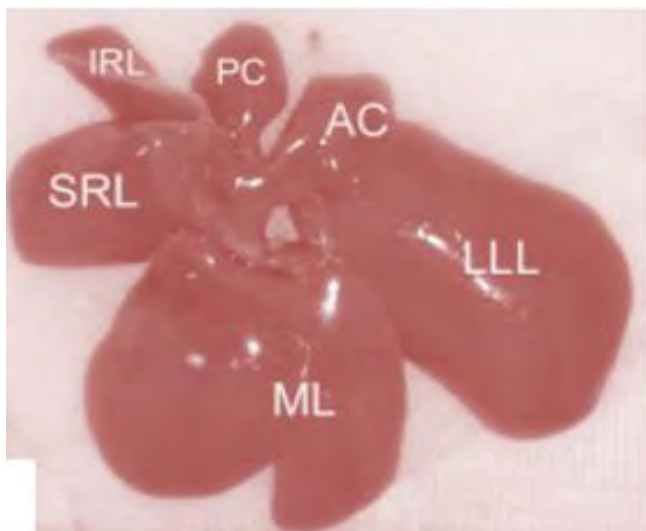


Figure 1.4: Photograph of the different lobe of rat liver. Note that ML=median lobe, LLL= left lateral lobe, AC= anterior caudate, PC= posterior caudate, IRL=inferior right lobe and SRL=superior right lobe (Kogure *et al.*, 1999)

The major part of the liver is composed of cords of parenchymal cells, the hepatocytes (Ross and Pawlina, 2011). 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; Ross and Pawlina, 2011). The space between these hepatic plates contains dilated capillaries known as hepatic sinusoids. In light microscope sections, structural units of the liver called liver lobules can be seen. The liver lobule is formed of a polygonal mass of tissue with portal spaces or areas at the periphery and a vein called the central vein in the center (Junqueira and Carneiro, 2005; Ross and Pawlina, 2011). The portal areas contain (house) slender branches of the hepatic artery, tributaries of the relatively large portal vein, interlobular bile ducts and lymph vessels.

1.6. Kidneys: Structure and functions

According to Onyeanusi *et al.*, (2009), both African giant and Wistar rats, each kidney has dorsal and ventral surfaces, medial and lateral borders, an upper and lower poles. The right kidney is situated more cranially than the left. The right kidney is related to the liver while; the left is related to the stomach, pancreas, descending colon, spleen and small intestine. The kidneys of both rats are bean shaped and smooth and covered with a thin fibro-muscular capsule but those of the African giant rats are being bigger in size. The nephrons are the structural and functional units of the kidney. Each kidney is composed of more than 1 million nephrons (Guyton and Hall, 2006). Nephron consists of two parts: a renal corpuscle, where blood plasma is filtered, and a renal tubule into which the filtered fluid passes. The two components of a renal corpuscle are the glomeruli and the glomerular (Bowman's) capsule (Tortora and Derrickson, 2009). The glomeruli are arterial capillary tufts, that are responsible for filtration of the blood (Ross and Pawlina, 2011).

The wall of the proximal convoluted tubule is made up of a single layer of cuboidal cells that interdigitate with one another and is united by apical tight junctions. The luminal edges of the cells (cell apices) have abundant microvilli about 1 μm in length, which form a striated brush border (Ross and Pawlina, 2011). Many substances are actively reabsorbed at a high rate in the proximal tubule, including sodium, potassium, calcium, phosphate, glucose, amino acids, and water (Guyton and Hall, 2006).

The distal convoluted tubule and the connecting tubule form the distal tubule of the kidney. The distal tubule extends from the macula densa region to the point of confluence with another

nephron to form the cortical collecting tubule (Reilly and Ellison, 2000). This tubule plays an important role in the fine-tuning of renal Na^+ and K^+ excretion. It reabsorbs 5–10% of the filtered sodium and chloride and participates importantly in net K^+ secretion. Furthermore, it has function in systemic calcium and magnesium homeostasis as it is the site of regulated trans cellular Ca^{+2} and Mg^{+2} transports in the kidney (Loffing *et al.*, 2004 and Guyton and Hall, 2006).

1.7. Significance of the study

Although traditional medicine is widely used to treat several ailments, and is often more available and affordable than western medicine, it is not without limitations. To this effect, there are research articles showing toxicological studies of different medicinal plant extract on different organs of administered animals so as to indicate caution not to use them as medicine unless they are proven to be safe by other findings (Afolayan and Yakubu, 2009; Alferah, 2012; Ajibade and Famurewa, 2012; Builders *et al.*, 2012 and Ogunka-Nnoka *et al.*, 2012). The safety of extract of different medicinal plant species which play important roles in managing various health problems showing no histopathological manifestations have also been reported (Jaijoy *et al.*, 2010; Oduola *et al.*, 2010 and Panunto *et al.*, 2011).

There are also a number of plants that are widely used as herbal medicine in Ethiopia and yet some of them are toxic to both humans and livestock (Abebe *et al.*, 2001). Despite the widespread use of *Albizia gummifera* and *Millettia ferruginea* in the treatment of various health problems (especially in non-orthodox medical care), the issues of appropriate dosage and harmful side effects of these plants have not been researched. So far, no literature is available on histopathologic implications in sub-chronic oral administration of the seeds extract of these two plants. This work therefore intends to investigate if there is any histopathologic effect, of the plant extract on the liver and kidney, and on blood parameters.

2. OBJECTIVES

2.1. General objective

➤ To evaluate the toxicological effect of sub-chronic administered hydroethanolic (70%) seeds extract of *Albizia gummifera* and *Millettia ferruginea* in albino wistar rats.

2.2. Specific objectives

- To determine the LD₅₀ of seeds extract of the two plants.
- To assess the sub-chronic effect of the extract on general body weight.
- To evaluate the effects of sub-chronic administration of the extract on hematological and biochemical parameters.
- To observe the sub-chronic effect of the extracts on weight of liver and kidneys.
- To investigate gross and microscopic histopathological changes of liver and kidneys on sub-chronic administration of the extract.

3. MATERIALS AND METHODS

3.1. Plant Material Preparation

3.1.1. Plant Collection and Processing

The seeds of *Albizia gummifera* were collected in Metu 400km west of Addis Ababa, whereas seeds of *Millettia ferruginea* were collected in Bodetti, Welayeta Soddo destrict 300km south of Addis Ababa in November 2013 in the wild at altitudinal range of 900 –3900 meter above sea level. They were identified and confirmed by a taxonomist using standard Flora, and voucher specimens of *Albizia gummifera* (Voucher No. AG-2006) and of *Millettia ferruginea* (Voucher No. MF-2049) which were pre-deposited in the Herbarium of the Traditional and Modern Medicine Research, Ethiopian Public Health Institute, Addis Ababa. The seeds of the plants were dried and crushed to powder at the Traditional and Modern Medicine Research Directorate of the Ethiopian Public Health Institute (EPHI).

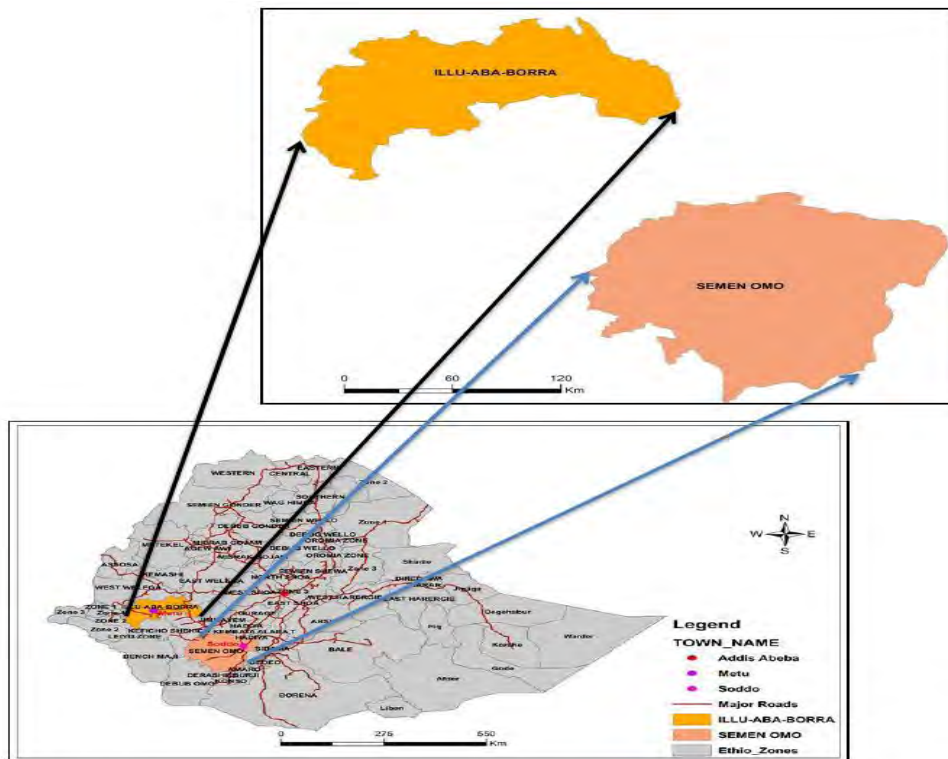


Figure 3.1: Map showing different cities of Ethiopia. Notes: the different arrows indicate Illu-Aba Borra where Metu is located and South Omo where Sodo is located, from which the plant materials collected.

3.1.2. Plant Material Extraction

1250gm of the powdered seeds of *Albizia gummifera* and 1230gm of the powdered seeds of *Millettia ferruginea* using wooden-made pestle and mortar were macerated separately with hydroalcohol (70% ethanolic) in 1:4 solute to solvent ratio and placed in orbital shaker at room temperature for 72hrs (Figure 3.1). This step was repeated three times to extract exhaustively until the extract gave faint or no coloration. The extract was then filtered through Whatman filter paper No.1 and the filtrate was evaporated to dryness under reduced pressure by Rotavapor (figure 3.1) and further concentrated by water bath at 40 °C. Then, the gummy residue extract was weighed that packed in air tight brown glass bottles with proper label and kept in a refrigerator at 4 °C until used for the preparation of stock solutions required in the subsequent experimental tests. For preparation of tested doses, appropriate amount of the crude extract was weighed and dissolved in 2-5ml distilled water immediately before administration. Some of the steps involved in the extraction phases are shown in figure 3.1.



Figure 3.2: Photograph showing different steps of extraction through orbital shaker, filtration and crude extraction by Rota vapor.

3.2. Experimental Animals

A total of 108 female rats were used for acute toxicity study and total of 50 rats (25 female and 25 male) were used for the sub-chronic toxicity study. All the experimental animals used in this study were bred at EPHI animal rearing unit. The male and female rats were kept in separate cages and were maintained on a 12hrs light/dark cycle, at room temperature and with free access to water and food. They were all acclimatized prior to drug administration. The leftover food and water were changed daily and the cages were cleaned with the husk changed every three days. All the animals seemed healthy.

3.3. Ethical Considerations

All the experiments had been conducted following the approval by the responsible bodies of the school of Medicine, AAU and EPHI in line with the highest standard for the humane and compassionate use of animals in biomedical research.

3.4. Acute oral toxicity test of *Albizia gummifera* and *Millettia ferruginea* seeds extract in rats

Twelve groups (Group I to XII) of female rats with four rats in each group were used for the acute toxicity studies of the two plants. The rats used in this study were starved overnight but allowed free access to drinking water prior to experimentation. Rats in group I- XII were given the extract at single oral doses of 50, 100, 150, 250, 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 mg/kg body weight, respectively for both plants separately in an attempt to see sign of toxicity and to determine the LD_{50} of the extracts of the plants. A control group of four rats was given only the vehicle. The animals were kept under observation after post-treatment in order to check for any behavioral and/or clinical manifestations such as CNS effect (excitement, ataxia, and sleep), altered feeding, vomiting and diarrhea. At the end of two weeks, one animal from each group was randomly sacrificed humanely after anesthesia (diethyl ether) by cervical dislocation and post-mortem gross observations were carried out on the internal organs (liver and kidneys).

3.5. Sub-chronic toxicity test of *Albizia gummifera* and *Millettia ferruginea*

seeds extract in rats

This study was conducted to investigate the effect of sub chronic treatment with seed extracts of the two plants on general body weight and weight of the organs (liver and kidneys); and on blood parameters as well as histopathology of liver and kidney tissues.

25 male and 25 female rats were used. They were randomly assigned to two groups of ten animals, five males and five females each for one of the plant extract. Similarly, the animals were randomly assigned to two groups of ten animals, five males and five females each for the other plant extract. One group containing ten animals (five male and five females) was assigned as control group. Throughout the experimental period, the female and male rats were housed in separate cages. Animals in one group received single daily dose of 125mg/kg body weight and the second group was administered with a single daily dose of 250mg/kg body weight for 90 days of the seeds extract of *Albizia gummifera*. Similarly, the seeds extract of *Millettia ferruginea* were given for one group a single daily dose of 125mg/kg body weight and for the other, 250mg/kg body weight for a period of 90 days. The control group received only vehicle daily throughout the period of study. The actual dose of the plant extract corresponding to each group was calculated on the basis of the body weight. The extract was dissolved in vehicle (distilled water) immediately before administration.

Throughout the study period, animals in all the study group were carefully monitored/ followed/ for any clinical signs of toxicity. The body weight of each rat in each group was measured before the beginning of drug administration and once a week thereafter throughout the study period. Finally, mean body weights were calculated and used for analysis of body weight progress.

3.5.1. Specimen Collection

At the end of dose administration, blood samples were collected through cardiac puncture using a gauge needle mounted on a 5ml syringe. Blood from each animal was drawn and placed in a sample tubes containing anti-coagulant, Ethylene Diamine Tetra-Acetic Acid (EDTA) and set for determination of hematological parameters. Another sample of blood from each animal was collected into tubes without anti-coagulant and allowed to clot for 3hrs. The coagulated blood was centrifuged and serum obtained was used for blood chemistry (biochemical assay).

Before specimen collection all animals were anesthetized with diethyl ether. After blood collection, each of the rat in the treated and control groups were sacrificed by cervical dislocation. After death, the animals were placed in the supine position on dissection board. The limbs were stretched and fixed to make the autopsy of the organs of interest easy. At autopsy liver and kidneys were visually examined for any signs of gross lesions. The organs removed from each rat were blotted on the filter paper. Then each of these organs was weighed on a semi-microbalance. After rinsing in normal saline, sections were taken from each of these organs. These specimens were placed in a pre-labeled sample bottles containing fixative (10% neutral buffered formalin) and used for histopathological studies.

3.5.2. Hematological and Biochemical Analyses

Hematological and serum biochemical parameters were conducted at core laboratories of EPHI, Addis Ababa. Hematological parameters including total counts of RBC, HCT, HGB, RDW, PLT count, total count of WBC, and differential count of each of the WBCs were measured in an automatic hematology analyzer, cell-DYN-3700 (Abbott Diagnostic Division, USA). In addition, red cell indices such as MCV, MCH and MCHC were also analyzed with the automatic analyzer. Similarly, serum biochemical parameters including ALP, ALT, AST, and urea were determined in clinical chemistry analyzer, Human star 80 (Human GmbH, Germany).

3.5.3. Histopathological studies

3.5.3.1. Tissue Processing

For histopathological studies under light microscopy, tissue samples taken at autopsy were processed in Histology laboratory, Department of Anatomy, College of Health Sciences, AAU. Each of the tissue samples harvested from liver and kidneys of all extract treated and non-treated animals were fixed separately in 10% neutral buffered formalin (Appendix I).

Following fixation, the tissues were rinsed in running water overnight to remove excess fixative. The wet fixed tissues were dehydrated in ascending grades of ethyl alcohol: in 70% alcohol, 80% alcohol, 95% alcohol, absolute alcohol I and absolute alcohol II (Appendix II). After dehydration the specimens were cleared in xylene I and xylene II (Appendix II). The tissues were transferred to liquid paraffin wax I and then to liquid paraffin wax II (Appendix II). The wax impregnated tissues were then embedded in paraffin blocks. All tissue blocks were labeled and allowed to dry at room temperature. These tissue blocks were sectioned with a Leica Rotary Microtome (Leica Rm2125RT, Model Rm2125, China) at 5-6 μ m thickness. The ribbons of these sections were collected and gently floated on a tissue flotation water bath at a temperature of 20 °C to stretch the paraffin wax impregnated tissue. The stretched floated ribbons were picked up on glass microscopic slides. The slides were placed in a warm oven overnight to help the slice adhere to the slides. The slides were allowed to cool at room temperature and kept ready for routine staining steps.

3.5.3.2. Tissue Staining

Before carrying out the staining of tissues, the sections were deparaffinized by xylene I and xylene II (Appendix III). Then the tissues were hydrated successively by running through a down series of alcohols: in absolute ethanol I, absolute ethanol II, 95% ethanol, 80% ethanol and 70% ethanol (Appendix III). The slides were then rinsed in distilled water followed by Harris' hematoxylin stain. These slides were washed in tap water and dipped into 1% acid alcohol (Appendix I) for differentiation and remove excess stain. The sections were rinsed briefly in running tap water to remove excess acid. The slides were then dipped in bluing solution followed by counter stain in eosin. The H and E stained sections were dehydrated by running through

increased grade of ethyl alcohols: 70% ethanol, 80% ethanol, 95% ethanol, absolute ethanol II and absolute ethanol I (Appendix III). Lastly, these slides were mounted using DPX mountant and glass cover slips.

3.5.3.3. Microscopy and Photomicrography

Microscopic slides of organs under study were examined carefully under compound light microscope at Histology Laboratory of Anatomy Department, College of Health Sciences, AAU. Slides from the extract treated groups were evaluated for any toxic insult to the organs compared to slides from their respective control groups. Finally, photomicrographs of selected slides were taken using (LEICA ICC50 HD, Germany) automated built-in digital photo camera.

3.6. Statistical Analysis

The numerical data obtained from the experiment were analyzed statistically on SPSS version 20, computer software package. The values of body weight changes were analyzed and the results were expressed as $M \pm SEM$. Differences between the treated and control groups were compared by 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.

4. RESULTS

4.1. Acute Toxicity Study

The acute toxicity study was a single dose toxicity test conducted to estimate lethal dose of the 70% ethanolic seeds extract of *Albizia gummifera* and *Millettia ferruginea* in rats' model. The administration of the extract orally at 50, 100, 150, 250, 500, 1000, 1500, 2000 and 2500 mg/kg body weight did not produce mortality of the administered group for the extracts of both plants with the exception of 250mg/kg for *Millettia ferruginea* where only one animal died. One and two rats died among the 3000 and 3500 mg/kg *Millettia ferruginea* seed extract administered groups, respectively. While two rats died among the 4000mg/kg *Albizia gummifera* seed extract treated group. The LD₅₀ of *Albizia gummifera* and *Millettia ferruginea* were therefore found as 4000mg/kg and 3500mg/kg, respectively. These animals have shown some behavioral changes such as altered feeding, low locomotion and pilo-erection before death. Also acute manifestation like diarrhea and loss of appetite were observed. No abnormal gross necropsy of liver and kidneys were observed.

4.2. Sub-chronic toxicity study

4.2.1. Effect of the extract of both plants on general health and body weight

During the 90 days of sub-chronic toxicity evaluation, all the male and female rats that were orally administered with the repeated doses of both 125 mg/kg and 250mg/kg body weight showed no extract related noticeable changes in their general behavior as compared to the control group for both of the plant extracts. One animal was found dead from *Millettia ferruginea* extract administered groups during early stage (3rd week) of administration period. No abnormal findings on gross observation of the liver and kidneys were observed in this rat. Otherwise, there were no toxicity related deaths throughout the period of study.

The effect of the seed extracts of *Albizia gummifera* and *Millettia ferruginea* on the body weight of male and female rats during the 13 weeks of sub-chronic treatment are summarized in (tables 4.1 and 4.2), respectively. Body weight of both the treated and control groups increased with increasing duration. As it can be seen from (figures 4.1, 4.2, 4.3 and 4.4), the body weight increase patterns of the male and female rats during the sub-chronic treatment with both of the

plants extracts seem to be normal. In the seeds extract of *Albizia gummifera* administered groups, no significant difference ($p>0.05$) was observed in the mean values of the body weights of male and female rats treated with 125mg/kg body weight and males treated with 250mg/kg body weight as compared with their respective controls. However, significant difference was observed in the mean body weight of female rats treated with 250mg/kg body weight as it decreased at the 9th week by 8.7% and further decreased by 12.8% at the 10th week as compared with the control group. Similarly, in seeds extract of *Millettia ferruginea* treated groups, the mean body weight difference between the administered and control groups were not significant ($p>0.05$), except, in the males' rats treated with 125mg/kg body weight during the 9th and 10th weeks of administration period, where the mean body weight increased by 6.5% in the 9th week and further by 4.2% during the 10th week.

Table 4.1: Effect of the subchronic administration of *Albizia gummifera* seeds extract on the body weight of male and female rats

<i>Period</i>	<i>Sex</i>	<i>Control</i>	<i>Treatment groups (mg/kg body weight/day)</i>	
			<i>125</i>	<i>250</i>
WK1	Male	166.4±1.75	168.2±3.35 (0.24)	166.8±2.49 (0.51)
	Female	164.8±2.13	167.6±3.36 (0.40)	167.8±3.67 (0.32)
WK2	Male	175.2±2.15	179.6±4.13 (0.24)	176±5.09 (0.12)
	Female	172.2±3.65	179.2±5.12 (0.53)	178.6±3.44 (0.91)
WK3	Male	191.8±2.18	191.2±4.08 (0.25)	171.6±5.29 (0.11)
	Female	186.6±2.98	189.6±3.97 (0.59)	187.2±4.25 (0.51)
WK4	Male	201.6±2.80	189.8±3.48 (0.68)	196.2± 5.90 (0.18)
	Female	197.6±3.28	196.2±4.12 (0.67)	202±4.15 (0.66)
WK5	Male	209.8±2.85	205.2±2.08 (0.56)	216.8±4.91 (0.32)
	Female	205.2±3.06	209.4±5.42 (0.29)	225.2±3.47 (0.81)
WK6	Male	216.8±2.75	209.4±1.29 (0.17)	218.8±3.65 (0.59)
	Female	213±2.76	211.6±4.27 (0.42)	217.2±2.94 (0.90)
WK7	Male	223.6±2.50	214.4±1.89 (0.59)	219.8±4.83 (0.23)
	Female	220.4±3.76	207.2±4.62 (0.70)	226.8±2.08 (0.28)
WK8	Male	231±2.51	221.4±1.80 (0.54)	220.8±3.80 (0.44)
	Female	227±4.89	210.6±4.61 (0.91)	229.8±2.39 (0.19)
WK9	Male	242.2±2.35	226.6±1.99 (0.75)	222.2±4.59 (0.22)
	Female	235.6±4.53	212.2±7.77 (0.32)	215.2±1.39 (0.23)
WK10	Male	252.6±2.42	233.8±2.15 (0.83)	234.8±4.53 (0.25)
	Female	247.8±3.65	223±6.40 (0.32)	216±7.07 * (0.04)
WK11	Male	261.6±6.77	238.8±2.35 (0.06)	237.6±3.47 (0.22)
	Female	255.2±3.76	219.4±6.52 (0.31)	220.6±2.06 (0.27)
WK12	Male	265±4.82	249.6± 3.43(0.53)	240.2±5.11 (0.43)
	Female	265.4±4.53	225.6±5.81 (0.64)	226.4±3.47 (0.62)
WK13	Male	269.2±4.22	258.8±3.53 (0.74)	248.4±3.33 (0.20)
	Female	274.4±4.00	223.8±5.99 (0.45)	232.6±2.60 (0.42)

Values are given as Mean ± S.E.M. for each male and female subgroup. The figures under the brackets indicate the calculated p values of the treatment groups as compared to the controls.

**=significant (p<0.05). The mean difference is considered significant at p< 0.05. (n=10 (5 male and 5 female in each group)).*

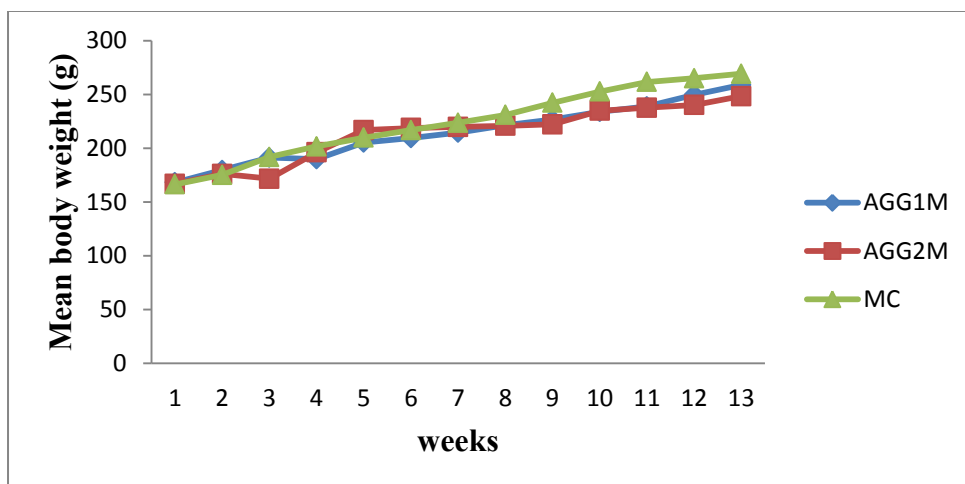


Figure 4.1: Time course and effect of seeds extract of *Albizia gummifera* on body growth pattern of male rats treated with 125mg/kg body weight and 250mg/kg body weight as compared to the controls. Each value point represents mean \pm S.E.M. Note: AGG1M= *Albizia gummifera* administered group I male rat, AGG2M= *Albizia gummifera* administered group II male rat & MC= male control rat.

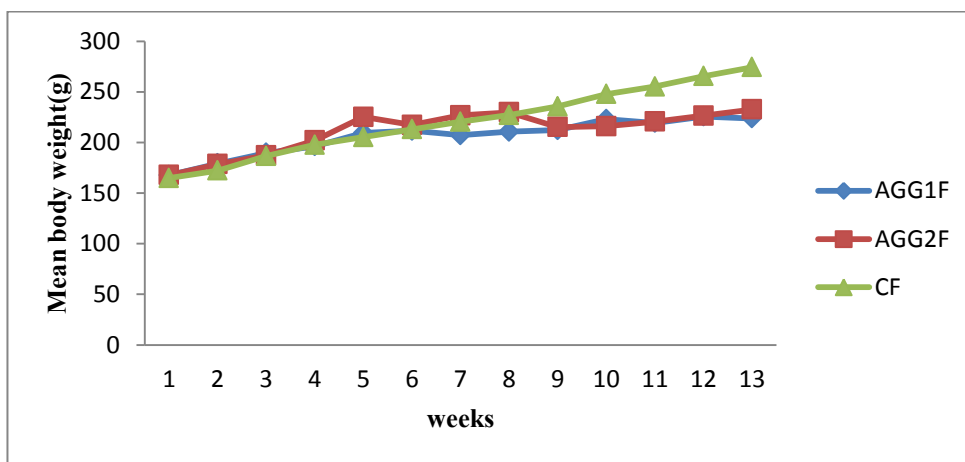


Figure 4.2: Time course and effect of seeds extract of *Albizia gummifera* on body growth pattern of female rats treated with 125mg/kg body weight and 250mg/kg body weight as compared to the controls. Each value point represents mean \pm S.E.M. Note: AGG1F= *Albizia gummifera* administered group I female rat, AGG2F= *Albizia gummifera* administered group II female rat & CF= female control rat.

Table 4.2: Effect of seed extract of *Millettia ferruginea* on the body weight of male and female rats

<i>Period</i>	<i>Sex</i>	<i>Control</i>	<i>Treatment groups (mg/kg body weight/day)</i>	
			<i>125</i>	<i>250</i>
WK1	Male	166.4±1.75	164.2±1.59 (0.86)	166.8±3.93(0.15)
	Female	164.8±2.13	163.8±1.99 (0.89)	165.6±3.52 0.36)
WK2	Male	175.2±2.15	171± 2.86 (0.59)	183.4±3.88 (0.28)
	Female	172.2±3.65	171.2±1.91 (0.24)	171.2±3.32 (0.86)
WK3	Male	191.8±2.18	184.6±2.66 (0.71)	195±3.85(0.29)
	Female	186.6±2.98	177.4±2.38 (0.67)	176.2±3.12 (0.93)
WK4	Male	201.6±2.80	200.2±1.11(0.12)	209±3.94 (0.53)
	Female	197.6±3.28	187.4±3.44 (0.93)	183.4±1.94(0.33)
WK5	Male	209.8±2.85	204.8±3.42(0.86)	220.8±5.18 (0.27)
	Female	205.2±3.06	195.4±4.89 (0.38)	186.6±3.49(0.80)
WK6	Male	216.8±2.75	212.4±5.27(0.32)	232.8±3.65 (0.59)
	Female	213±2.76	205±6.32(0.14)	199.4±3.31 (0.73)
WK7	Male	223.6±2.50	220.2±5.48(0.23)	250.6±5.50 (0.16)
	Female	220.4±3.76	212.2±3.59 (0.93)	201.6±1.81 (0.18)
WK8	Male	231±2.51	212.8±5.39(0.24)	258.2±6.24 (0.11)
	Female	227±4.89	223±4.85(0.99)	210.6±4.78 (0.97)
WK9	Male	242.2±2.35	258±11.31 * (0.01)	212.4±5.52(0.19)
	Female	235.6±4.53	230.4±6.62 (0.48)	220.4±5.81 (0.64)
WK10	Male	252.6±2.42	263.2±11.29 *(0.01)	220.2±6.82(0.11)
	Female	247.8±3.65	238.6±6.45 (0.29)	230.6±6.38 (0.30)
WK11	Male	261.6±6.77	212.8±5.73(0.68)	258.2±10.78(0.39)
	Female	255.2±3.76	246.2±6.01 (0.39)	235.4±5.32(0.52)
WK12	Male	265±4.82	211.4±6.86(0.72)	252.2±11.09 (0.51)
	Female	265.4±4.53	258.6±5.39 (0.75)	240.2±6.24 (0.55)
WK13	Male	269.2±4.22	221.8±6.52(0.86)	260±11.02(0.36)
	Female	274.4±4.00	267.2±4.69 (0.77)	242.2±7.14 (0.29)

Values are given as Mean ± S.E.M. for each male and female subgroup. The figures under the brackets indicate the calculated p values of the treatment groups as compared to the controls.

**=significant (p<0.05). The mean difference is considered significant at p< 0.05. (n=10 (5 male and 5 female in each group)).*

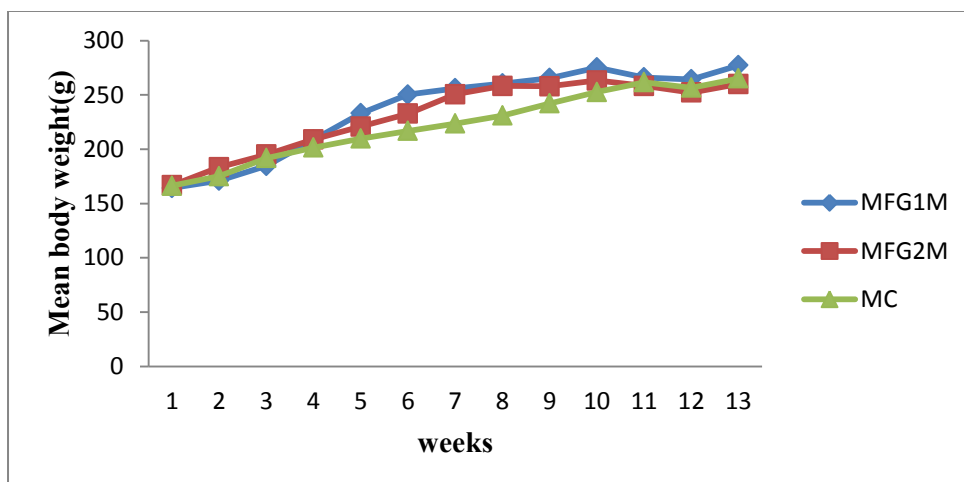


Figure 4.3: Time course and effect of seeds extract of *Millettia ferruginea* on body growth pattern of male rats treated with 125mg/kg body weight and 250mg/kg body weight as compared to the controls. Each value point represents mean \pm S.E.M. Note: MFG1M= *Millettia ferruginea* administered group I male rat, MFG2M= *Millettia ferruginea* administered group II male rat & MC= male control rats.

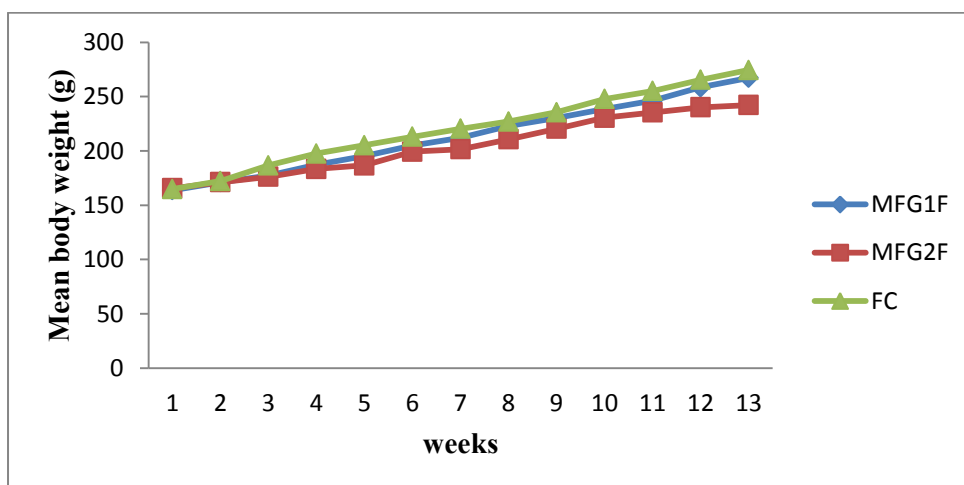


Figure 4.4: Time course and effect of seeds extract of *Millettia ferruginea* on body growth pattern of female rats treated with 125mg/kg body weight and 250mg/kg body weight as compared to the controls. Each value point represents mean \pm S.E.M. Note: MFG1F= *Millettia ferruginea* administered group I female rats, MFG2F= *Millettia ferruginea* administered group II female rat & CF= female control rat.

4.2.2. Effect of *Albizia gummifera* and *Millettia ferruginea* hydro alcohol seeds extract on hematological parameters

The effect of sub-chronic treatment of the seed extract of *Albizia gummifera* on hematological parameters of male rats as compared to the controls is illustrated in (Table 4.3). The sub-chronic treatment with both 125mg/kg and 250mg/kg body weight seed extract did not significantly affect hematological parameters except a few of the parameters seen to be significant ($p < 0.05$). Sub-chronic treatment of male rats with 125mg/kg and 250mg/kg of seed extracts produced significant change ($p < 0.05$) in the MCH as it decreased by 3.4% in the 125mg/kg administered group and further decreased by 5.9% in the 250mg/kg administered group, and RDW-CV as it increased by 7.3% in the 125mg/kg administered group and further increased by 8.6% in the 250mg/kg administered group. Besides, the seed extract induced significant change in the NEUT at 250mg/kg body weight as it increased from 15.36 ± 2.01 to 32.70 ± 2.20 .

Hematological parameters such as RBC, WBC and PLT increased in the male rats at both doses compared to the control although not significant. But, parameters such as HGB, HCT, MCV, MCH, LYMPH, EO and BASO were all found decreased in the male rats even though they were statistically not significant.

The effect of 13 weeks of sub-chronic treatment of the seed extract of this plant on hematological parameters of female rats is illustrated in (Table 4.4). The seed extract induced significant change ($p < 0.05$) at 250mg/kg body weight in the MCH as it decreased by 5.6% and in the MCHC as it decreased by 2.0% at the lowest dose and further by 2.7% at the highest dose. In addition, the seed extract affected the NEUT at 250mg/kg body weight as it increased from 12.06 ± 2.14 to 35.00 ± 1.80 . The parameters like WBC, RBC and PLT increased in the female rats compared to their controls but this increment was not significant. On the other hand hematological parameters such as EO and MCV decreased at both doses but still the values were not significant.

The effect of 13 weeks of sub-chronic treatment of the seed extract of *Millettia ferruginea* on hematological parameters of male and female rats are illustrated in tables 4.5 and 4.6, respectively. The sub-chronic administered seeds extract at both 125mg/kg and 250mg/kg body

weight did not significantly affect hematological parameters in both sexes. However, the hematological parameters MCHC and MONO of female rats administered 250mg/kg resulted in significant changes ($p < 0.05$) as it decreased by 2.2% and 66.8%, respectively. Although not statistically significant, hematological parameters like HGB, HCT, MCV, MCH and MCHC increased at both the lowest and highest doses in the male rats compared to the male control rats. Similarly, in the females rats some of the parameters like HCT and RDW-CV found increased as compared to the female control group, but this increment was not significant.

Table 4.3: Hematological parameters of male rats administered with 125 and 250mg/kg body weight of seed extract of *Albizia gummifera*.

Hematological Parameters	Control (G3)	125mg/kg body weight (G1)	250mg/kg body weight (G2)
WBC (x10 ³ /μL)	7.99± 1.08	12.24±2.18 (.19)	10.94± 1.49 (.39)
RBC (x10 ⁶ /μL)	9.94± .17	10.14±.14 (.54)	10.00± .11 (.93)
HGB (g/dL)	18.50± .25	18.26± .38 (.80)	17.53±.23 (.10)
HCT (%)	54.36± 1.12	54.30± 1.06 (.99)	52.73± .49 (.41)
MCV (fL)	54.66± .44	53.50± .36 (.25)	52.73± .66 (.06)
MCH (pg)	18.63± .06	18.00± .20 (.04) *	17.53± .14 (.004) *
MCHC (g/dL)	34.03± .33	33.63± .18 (.49)	33.23± .23 (.12)
PLT (x10 ³ /μL)	901.66± 67.49	1101.00± 81.22 (.11)	1079.33±23.53 (.15)
RDW-CV(%)	20.16± .06	21.63± .21 (.00) *	21.90±.15 (.00) *
NEUT (%)	15.36±2.01	28.30±4.93 (.05)	32.70± 2.20 (.01) *
LYMPH(%)	77.56±1.73	66.50±6.24 (.14)	64.66±1.46 (.09)
MONO(%)	5.30±1.44	4.70±1.20 (.90)	5.60± .70 (.97)
EO(%)	1.40± .95	.26±.08 (.32)	.16± .06 (.27)
BASO (%)	.36± .17	.23±.13 (.73)	.20± .10 (.63)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05.

Table 4.4: Hematological parameters of female rats administered with 125 and 250mg/kg body weight of seed extract of *Albizia gummifera*.

Hematological Parameters	Control (G3)	125mg/kg body weight (G1)	250mg/kg body weight (G2)
WBC (x10 ³ /μL)	5.27± 2.32	9.55±1.71 (.42)	10.48± 3.17 (.30)
RBC (x10 ⁶ /μL)	8.66± .31	8.99±.18 (.51)	9.32± .14 (.14)
HGB (g/dL)	16.86± .67	17.16± .55 (.88)	17.13±.06 (.90)
HCT (%)	49.13± 1.74	51.36± 1.40 (.42)	50.93± .34 (.55)
MCV (fL)	56.76± .66	57.10± .40 (.88)	54.63± .61 (.06)
MCH (pg)	19.46± .23	19.06± .23 (.42)	18.36± .23 (.02) *
MCHC (g/dL)	34.33± .14	33.63± .12 (.03) *	33.40± .20 (.01) *
PLT (x10 ³ /μL)	665.00± 218.70	1029.00± 12.34 (.18)	1222.33±92.09 (.05)
RDW-CV(%)	17.23± .98	18.60±.20 (.26)	19.43± .27 (.07)
NEUT(%)	12.06±2.14	20.90±6.96 (.32)	35.00± 1.80 (.02) *
LYMPH(%)	79.83±3.89	73.36±8.51 (.67)	58.13±4.16 (.07)
MONO(%)	4.40±.55	5.13±1.88 (.94)	6.53± 2.51 (.64)
EO(%)	3.66± 3.12	.50±.05 (.40)	.26± .12 (.36)
BASO(%)	.03± .03	.10±.05 (.48)	.06± .03 (.81)

*Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05.*

Table 4.5: Hematological parameters of male rats administered with 125 and 250mg/kg body weight of seed extract of *Millettia ferruginea*.

Hematological Parameters	Control (G3)	125mg/kg body weight (G1)	250mg/kg body weight (G2)
WBC (x10 ³ /μL)	7.99± 1.08	9.36±1.23 (.55)	7.75± .48 (.97)
RBC (x10 ⁶ /μL)	9.94± .17	10.04±.32 (.95)	9.82± .35 (.94)
HGB (g/dL)	18.50± .25	18.99± .40 (.60)	18.76± .48 (.85)
HCT (%)	54.36± 1.12	55.63± .97 (.65)	54.63± 1.18 (.97)
MCV (fL)	54.66± .44	55.46± .84 (.68)	55.63± .88 (.59)
MCH (pg)	18.63± .06	18.86± .23 (.59)	19.10± .20 (.19)
MCHC (g/dL)	34.03± .33	34.10± .11 (.97)	34.33± .24 (.62)
PLT (x10 ³ /μL)	901.66± 67.49	1001.66± 59.40 (.46)	922.66±59.91 (.96)
RDW-CV(%)	20.16± .06	20.33±.54 (.95)	20.00± .52 (.95)
NEUT(%)	15.36±2.01	14.30±.68 (.81)	13.30± 1.07 (.50)
LYMPH(%)	77.56±1.73	81.23±.43 (.15)	80.93±1.36 (.19)
MONO(%)	5.30±1.44	4.03±.53 (.62)	5.33± .94 (1.00)
EO(%)	1.40± .95	.30±.05 (.33)	.20± .10 (.28)
BASO(%)	.36± .17	.13±.03 (.32)	.23± .08 (.64)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. The mean difference is not significant (p≥0.05).

Table 4.6: Hematological parameters of female rats administered with 125 and 250mg/kg body weight of seed extract of *Millettia ferruginea*.

Hematological Parameters	Control (G3)	125mg/kg body weight (G1)	250mg/kg body weight (G2)
WBC (x10 ³ /μL)	5.27± 2.32	6.63±.49 (.72)	4.22± .02 (.81)
RBC (x10 ⁶ /μL)	8.66± .31	8.73±.07 (.95)	8.66± .16 (1.00)
HGB (g/dL)	16.86± .67	17.30± .10 (.70)	16.86±.26 (1.00)
HCT (%)	49.13± 1.74	51.53± .06 (.29)	49.53± .85 (.95)
MCV (fL)	56.76± .66	57.46± .03 (.54)	59.03± .56 (.30)
MCH (pg)	19.46± .23	19.80± .15 (.32)	19.60± .05 (.79)
MCHC (g/dL)	34.33± .14	34.20± .05 (.72)	33.56± .17 (.01)*
PLT (x10 ³ /μL)	665.00± 218.70	956.00± 95.10 (.30)	1121.66±13.92 (.09)
RDW-CV(%)	17.23± .98	17.60±.81 (.92)	18.03± .37 (.69)
NEUT(%)	12.06±2.14	12.06±2.91 (1.00)	12.83± .43 (.95)
LYMPH(%)	79.83±3.89	83.90±2.20 (.47)	84.96±.34 (.33)
MONO(%)	4.40±.55	3.16±1.01 (.38)	1.46± .14 (.03) *
EO(%)	3.66± 3.12	.76±.26 (.46)	.53± .20 (.41)
BASO(%)	.03± .03	.10±.05 (.48)	.03± .03 (1.00)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05.

4.2.3. Effect of *Albizia gummifera* and *Millettia ferruginea* hydro alcohol seeds extract on serum biochemical parameters

Effects of sub-chronic treatment with hydro-alcoholic seed extract of *Albizia gummifera* on serum biochemical parameters of male and female rats are shown in (tables 4.7 and 4.8), respectively. Except urea with male administered at 250mg/kg body weight as it increased by 28.94%, all the parameters measured were not significantly different between the control and extract administered groups at both doses. The effects of sub-chronic treatment with hydro-alcoholic seed extract of *Millettia ferruginea* on serum biochemical parameters of male and female are shown in (tables 4.9 and 4.10), respectively. All the parameters measured in male rats which received any of the doses were not significantly different from those of the controls. ALP, ALT and urea, however, were found to be significantly different in the female rats at 250mg/kg body weight as they increased by 59.40%, 27.45% and 19.56%, respectively.

Table 4.7: serum biochemical parameters of male rats administered with 125 and 250mg/kg body weight of seed extract of *Albizia gummifera*.

Biochemical Parameters	Control (G3)	125mg/kg dose (G1)	250mg/kg dose (G2)
Albumin(g/dl)	4.70± 0.22	4.79 ± 0.26(0.94)	4.45 ± 0.19(0.65)
ALP (U/L)	135.75 ± 11.69	168±12.79 (0.44)	160.75 ± 29.26(0.59)
ALT(U/L)	79.75± 2.59	94.25±5.15(0.11)	79.75± 6.21 (1.00)
AST(U/L)	179.75 ± 5.20	251.5 ± 43.65(0.34)	253.75 ± 47.42 (0.32)
Urea (mg/dL)	28.50 ± 0.96	33 ± 0.41 (0.09)	36.75 ± 2.21 (0.005) *

*Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05.*

Table 4.8: Serum biochemical parameters of female rats administered with 125 and 250mg/kg body weight of seed extract of *Albizia gummifera*

Biochemical Parameters	Control (G3)	125mg/kg dose (G1)	250mg/kg dose (G2)
Albumin(g/dl)	5.16± 0.19	5.01 ± 0.16(0.79)	4.87 ± 0.16(0.42)
ALP (U/L)	75.75 ± 12.49	88.75±8.41 (0.79)	134.75 ± 22.87(0.05)
ALT(U/L)	63.75± 5.66	74.75±4.00(0.26)	61.50± 5.33 (0.93)
AST(U/L)	194 ± 16.92	237.25 ± 62.26(0.65)	192.25 ± 13.88 (0.99)
Urea (mg/dL)	34.50 ± 2.33	38.75 ± 2.49 (0.29)	37.00 ± 1.08(0.62)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. The mean difference was not significant (p ≥ 0.05).

Table 4.9: Serum biochemical parameters of male rats administered with 125 and 250mg/kg body weight of seed extract of *Millettia ferruginea*.

Biochemical Parameters	Control (G3)	125mg/kg dose (G1)	250mg/kg dose (G2)
Albumin(g/dl)	4.70± 0.22	5.03 ± 0.20(0.39)	4.85 ± 0.10(0.82)
ALP (U/L)	135.75 ± 11.69	158.00±17.56 (0.48)	161.25 ± 13.74(0.39)
ALT(U/L)	79.75± 2.59	85.75±4.27(0.39)	80.50± 3.28 (0.98)
AST(U/L)	179.75 ± 5.20	210.25 ± 21.44(0.22)	181.25 ± 5.07 (0.99)
Urea (mg/dL)	28.50 ± 0.96	32.25 ± 1.49 (0.17)	30.00 ± 1.73(0.69)

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. The mean difference was not significant (p > 0.05).

Table 4.10: Serum biochemical parameters of female rats administered with 125 and 250mg/kg body weight of seed extract of *Millettia ferruginea*.

Biochemical Parameters	Control (G3)	125mg/kg dose (G1)	250mg/kg dose (G2)
Albumin(g/dl)	5.16± 0.19	5.34 ± 0.15(0.62)	5.15 ± 0.10(1.00)
ALP (U/L)	75.75 ± 12.49	87.00 ± 6.01(0.54)	120.75±2.05 (0.006)*
ALT(U/L)	63.75± 5.66	74.25± 4.34 (0.20)	81.25±2.28(0.03)*
AST(U/L)	194 ± 16.92	206.00 ± 20.33(0.95)	264.00 ± 46.59 (0.24)
Urea (mg/dL)	34.50 ± 2.33	34.25 ± 0.75(0.99)	41.25 ± 1.38 (0.03)*

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05.

4.2.4. Macroscopic observations and organ weights

After the period of 90 days of sub chronic-toxicity study, rats that were orally administered with the repeated doses of the extracts at both 125 and 250mg/kg body weight of both plants showed no abnormal gross findings in the liver and kidneys in the postmortem macroscopic examination. The mean organ weight of liver and kidney of the seed extract of *Albizia gummifera* administered groups and control group are shown in tables 4.11 and 4.12 for the male and female rats, respectively. No significant difference (p>0.05) was seen in the organ weights between extract treated and control rats of both sex. The mean organ weight of liver and kidney of the seed extract of *Millettia ferruginea* administered groups and control group are shown in tables 4.13 and 4.14 for the male and female rats, respectively. No significant difference (p>0.05) was noted in the organ weights between extract treated and control rats of either sex.

Table 4.11: Organ weights of male rats administered with 125 & 250 mg/kg body weight doses of the seed extracts of *Albizia gummifera*.

Group	Dose (mg/kg)	Liver (g)	Kidney (g)
I	125	9.32 ± .18 (.29)	1.05 ± .02 (.95)
II	250	9.18 ± .13 (.09)	.99 ± .05 (.60)
III	Control	9.60 ± .07	1.04 ± .04

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated *p* values of the treatment groups as compared to the control. The mean difference was not significant (*p* > 0.05).

Table 4.12: Organ weights of female rats administered with 125 & 250 mg/kg body weight doses of the seed extracts of *Albizia gummifera*.

Group	Dose (mg/kg)	Liver (g)	Kidney (g)
I	125	9.40 ± .19 (.32)	.91 ± .05 (.91)
II	250	9.42 ± .09 (.27)	1.01 ± .03 (.52)
III	Control	9.16 ± .12	.85 ± .19

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated *p* values of the treatment groups as compared to the control. The mean difference was not significant (*p* > 0.05).

Table 4.13: Organ weights of male rats administered with 125 & 250 mg/kg body weight doses of the seed extracts of *Millettia ferruginea*.

Group	Dose mg/kg	Liver (g)	Kidney (g)
I	125	9.32 ± .11 (.09)	.99 ± .04 (.96)
II	250	9.34 ± .09 (.12)	.88 ± .19 (.56)
III	Control	9.60 ± .07	1.04 ± .04

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated *p* values of the treatment groups as compared to the control. The mean difference was not significant (*p* > 0.05).

Table 4.14: Organ weights of female rats administered with 125 & 250 mg/kg body weight doses of the seed extracts of *Millettia ferruginea*.

Group	Dose (mg/kg)	Liver (g)	Kidney (g)
I	125	9.12 ±.04 (.96)	1.02 ± .04 (.49)
II	250	9.22± .12 (.87)	1.05 ±.02 (.37)
III	Control	9.16±.12	.85± .19

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control. The mean difference was not significant ($p > 0.05$).

4.2.5. Microscopic observations

4.2.5.1. Effect of hydroalcoholic seed extract of both plants on histopathology of the liver

Routine hematoxylin and eosin stained sections of liver were examined to assess the effect of the 90 days sub-chronic oral administration with 70% ethanolic seed extract of *Albizia gummifera* and *Millettia ferruginea* on this tissue. Light microscopic examination of the liver sections of 125mg/kg of both male and female rats for seed extract of *Albizia gummifera* administered group showed inflammation and congestions of blood in the central vein (figure 4.5). The 250mg/kg administered group also showed congestions of blood in the central vein and in the sinusoids, focal cellular necrosis and pyknosis (figure 4.5).

In addition, light microscopic examination of the liver sections of 125mg/kg of both male and female rats for seed extract of *Millettia ferruginea* administered group showed congestions of blood in the hepatic artery and portal vein (figure 4.6). At the 250mg/kg, beside congestions of blood in the central vein, has shown minor focal cellular necrosis and inflammation (figure 4.6).

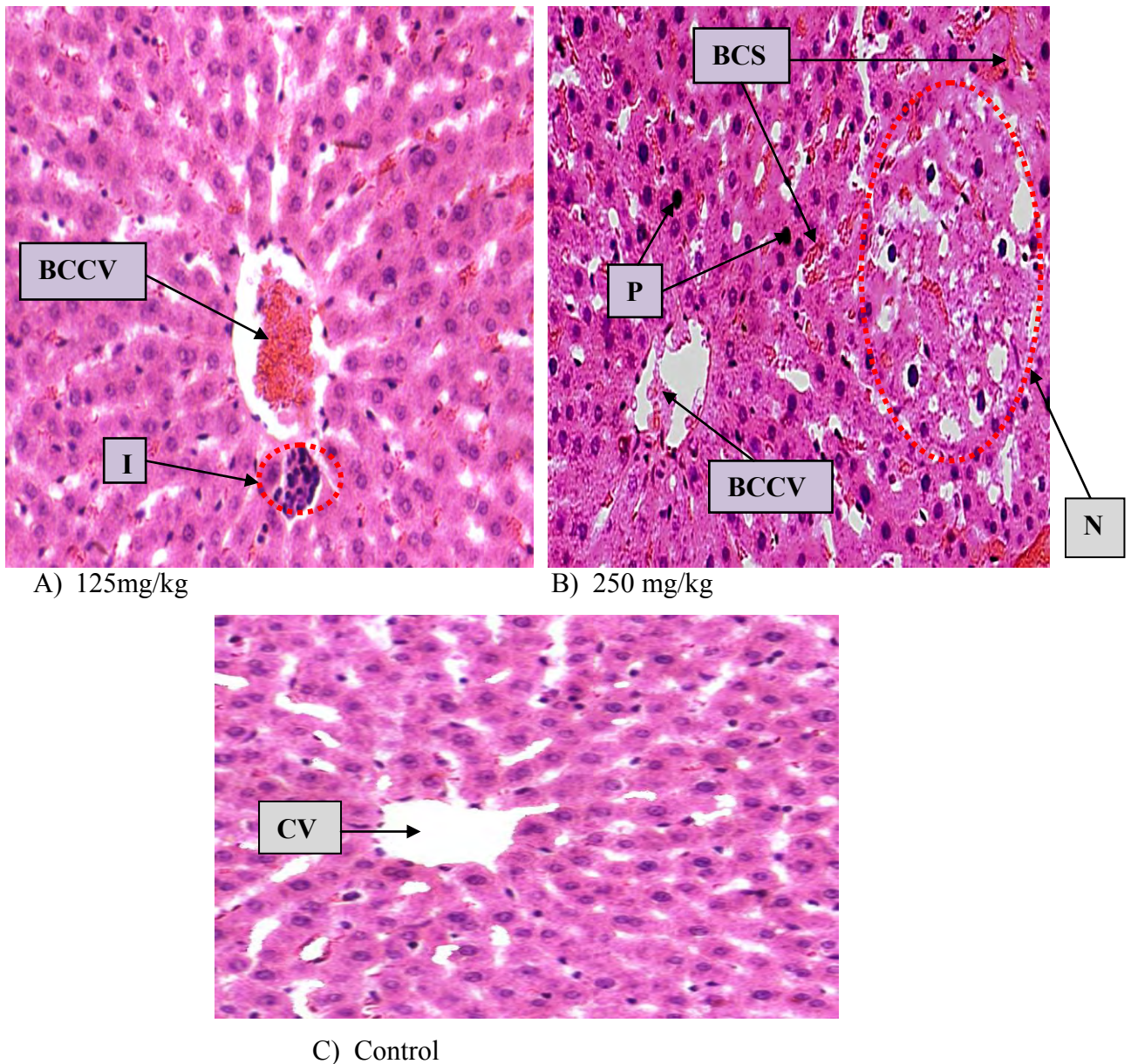


Figure 4.5. Photomicrograph of H &E stained liver sections from rats administered with hydroethanolic seeds extracts of *Albizia gummifera* at 125mg/kg body weight/day (A), 250mg/kg body weight/day (B), and control (C) rats. The following changes were observed in the sections from the hydroethanolic extract administered rat: inflammations around central vein (I) and congestion of blood in the central vein (BCCV) in rat administered at 125mg/ kg body weight/day (A); focal cellular necrosis(N), congestion of blood in the central vein (BCCV), congestion of blood in the sinusoids(BCS) and pyknosis(P) in rats administered 250mg/k body weight/day (B); While there was no histopathological changes visible in the sections of the control (C) rats. (Magnifications, all $\times 2000$).

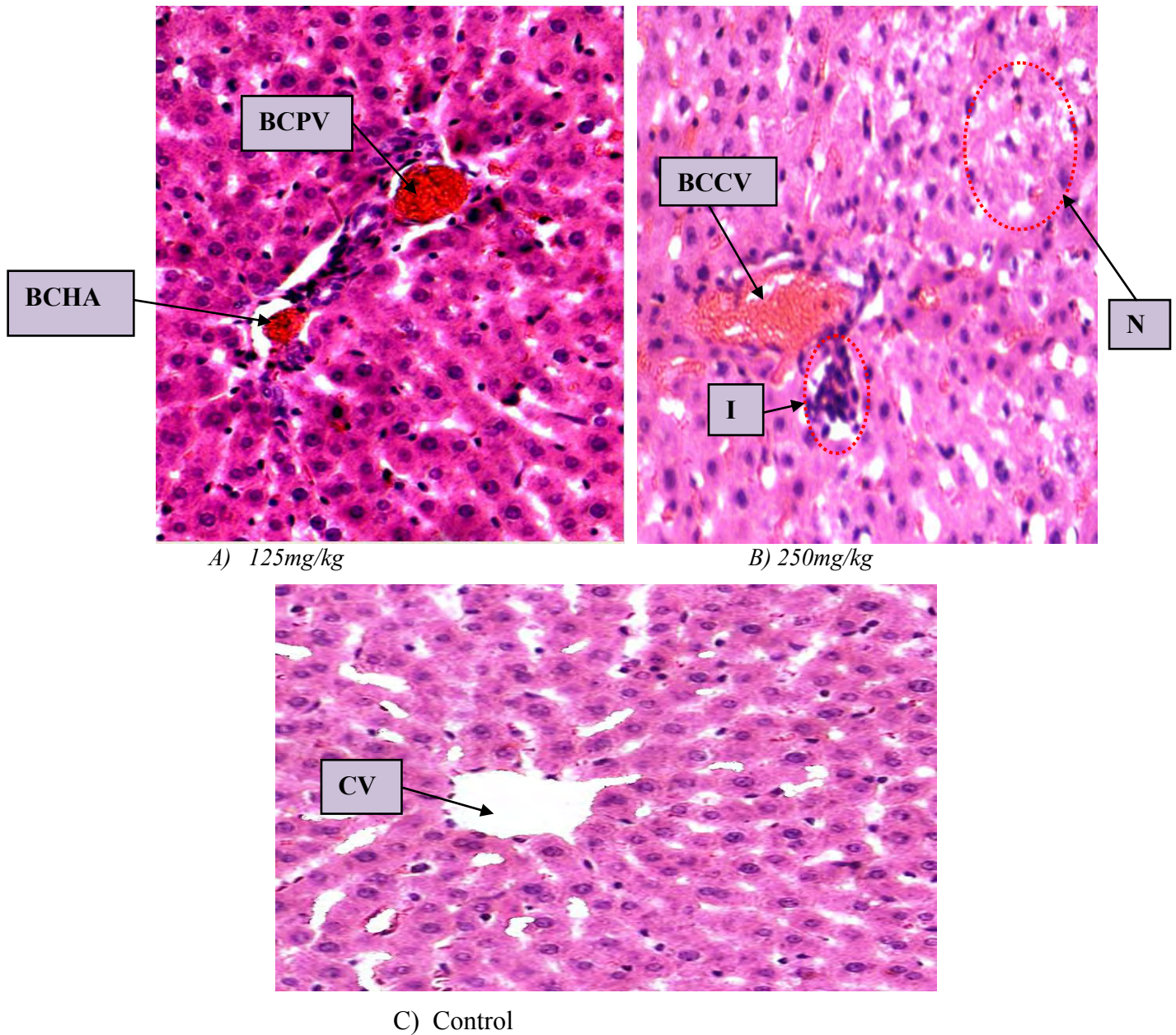
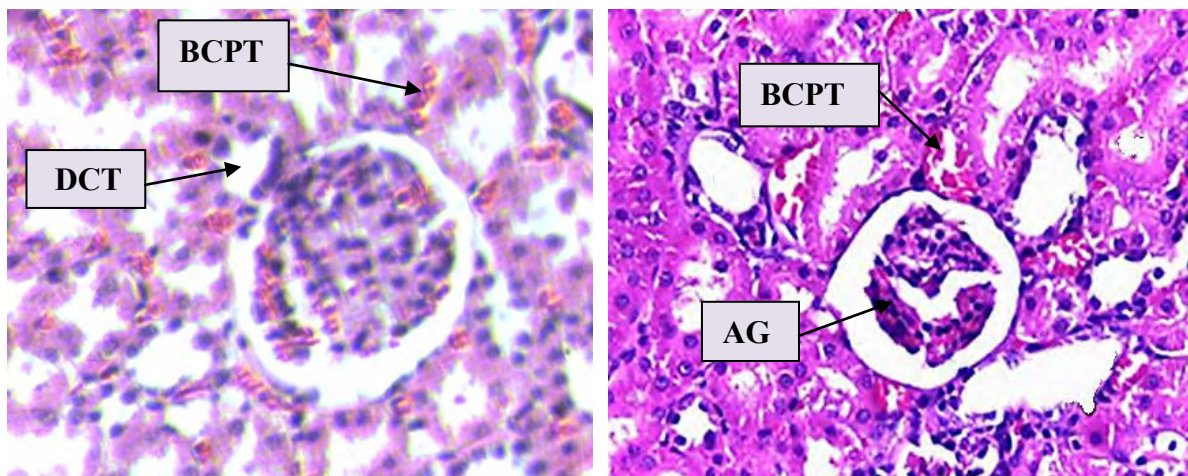


Figure 4.6. Photomicrographs of H & E stained liver sections from rats administered with hydroethanolic seeds extracts of *Millettia ferruginea* at 125mg/kg body weight/day (A), 250mg/kg body weight/day (B), and control (C) rats. The following changes were observed in the sections from the hydroethanolic extract administered rat: congestion of blood in the portal vein (BCPV) and congestion of blood in the hepatic artery (BCHA) in rat administered at 125mg/kg body weight/day (A); inflammations around central vein (I), minor focal cellular necrosis (N) and congestion of blood in the portal vein (BCCV) in rats administered 250mg/kg body weight/day (B); While there was no histopathological changes visible in the sections of the control (C) rats. (Magnifications, all $\times 2000$).

4.2.5.2. Effect of hydroalcoholic seed extract of both plants on histopathology of the kidneys

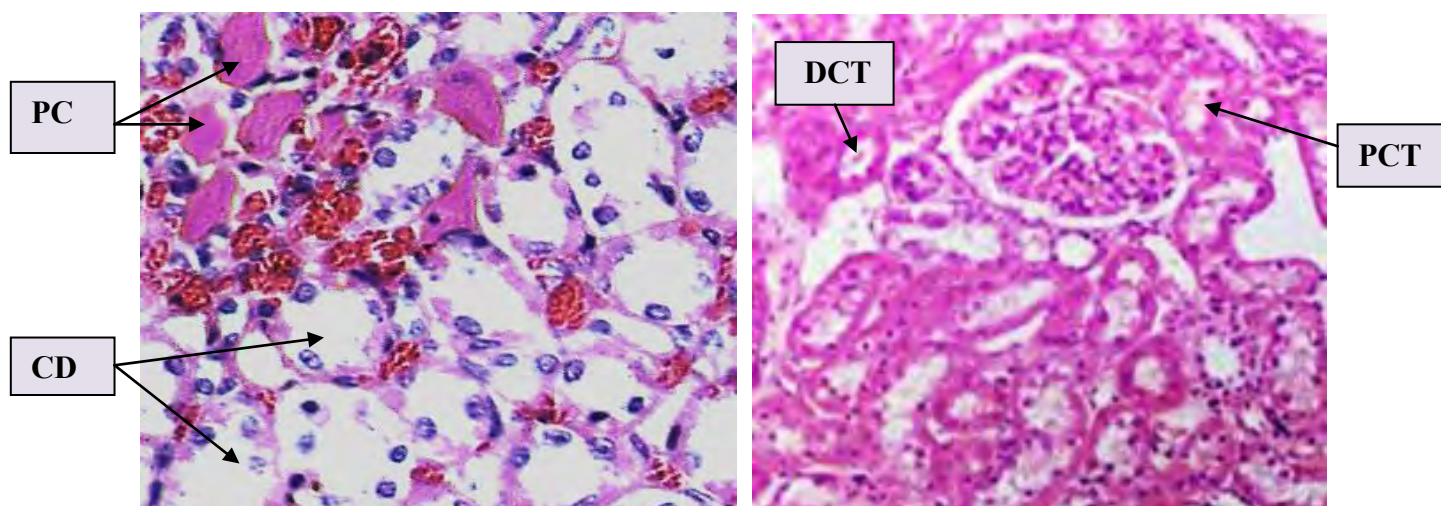
Histological examinations of sections of the kidney from rats administered with the seeds extract of *Albizia gummifera* at 125mg/kg in both male and female rats have shown peritubular blood congestions (figure 4.7). Kidney histology of both sex administered with 250 mg/kg body weight have also shown congestions, atrophy of glomeruli and formation of focal protein cast in the renal interstitium (figure 4.7).

Similarly, histological examinations of sections of the kidney from rats administered with the seed extract of *Millettia ferruginea* at 125mg/kg for both male and female rat showed atrophy of the glomeruli, widened urinary space & inflammation (figure 4.8). Kidney histology of both sex administered at 250 mg/kg also expressed minor tubular necrosis, atrophy of the glomeruli, widened urinary space and peritubular blood congestions (figure 4.8).



A) 125 mg/kg

B) 250 mg/kg

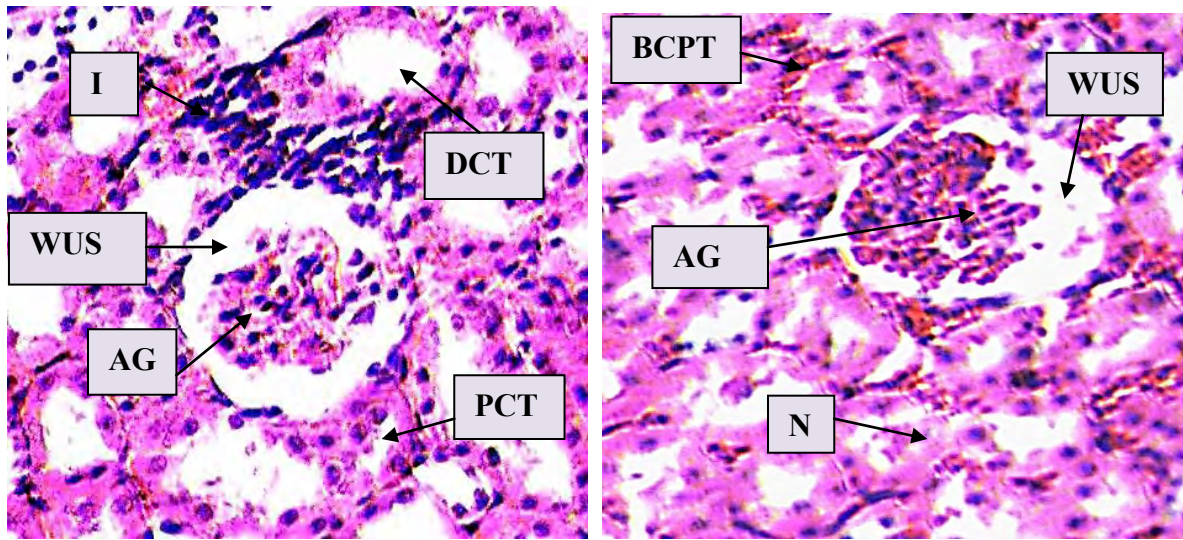


C) 250mg/kg

D) Control

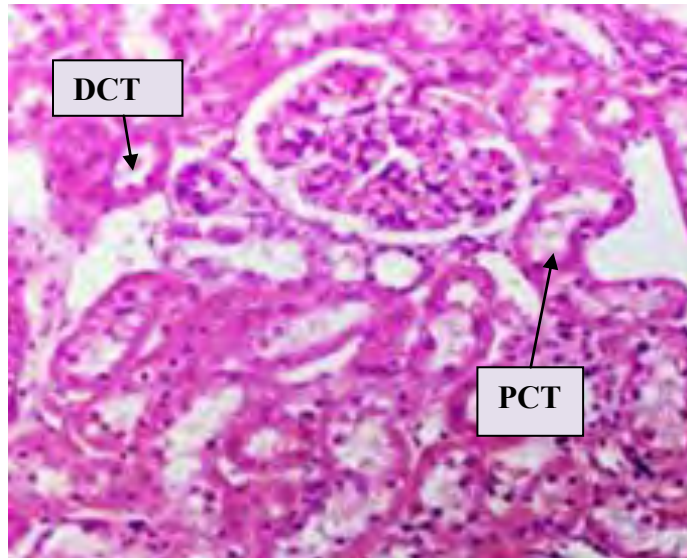
Figure 4.7. Photomicrograph of H&E stained kidney sections from rats administered with hydroethanolic seeds extracts of *Albizia gummifera* at 125mg/kg body weight/day (A), 250mg/kg body weight/day (B & C), and control (D) rats. The following changes were observed in the sections from the hydroethanolic extract administered rats: congestion of blood in the peritubular areas (BCPT) in rat administered at 125mg/ kg body weight/day (A); congestion of blood in the peritubular areas (BCPT) , atrophy the glomeruli (AG) and protein cast in the kidney interstitium (PC) in rats administered 250mg/k body weight/day (B & C); While there was no histopathological changes visible in the sections of the control rat (D). (Magnifications, all $\times 2000$).

Note: PCT=Proximal convoluted tubule, DCT=Distal convoluted tubule & CD= Collecting duct.



A) 125mg/kg

B) 250 mg/kg



C) Control

Figure 4.8. Photomicrograph of H&E stained kidney sections from rats administered with hydroethanolic seeds extracts of *Millettia ferruginea* at 125mg/kg body weight/day (A), 250mg/kg body weight/day (B), and control (C) rats. Observe the following changes in the sections from the hydroethanolic extract administered rat: widened urinary space(WUS), atrophy the glomeruli (AG) & inflammation(I) in rat administered at 125mg/ kg body weight/day (A); congestion of blood in the peritubular areas (BCPT) , widened urinary space(WUS), atrophy the glomeruli (AG), and minor tubular necrosis(N) in rats administered 250mg/k body weight/day (B); While there was no histopathological changes visible in the sections of the control rat (C). (Magnifications, all $\times 2000$).

Note: PCT= Proximal convoluted tubule, DCT= Distal convoluted tubule.

5. DISCUSSION

Acute toxicity test is carried out on laboratory animals, in this particular case on albino rats receiving different doses of the substances in question. The oral administration of the aqueous extracts of both *Millettia ferruginea* and *Albizia gummifera* seeds did not show any signs of toxicity nor did they produce lethality in rats up to 3000mg / Kg except for one animal which died with 250mg/kg for *Millettia ferruginea*. As this dose is minimal it is very unlikely to associate the death of the animal with the extract. 50 % of the animals died with 3500 mg/kg & 4000 mg/kg for *Millettia ferruginea* & *Albizia gummifera* seeds extract treated groups, respectively. This has, therefore, indicated that the LD₅₀ of these plants were found to be 3500 mg/kg & 4000 mg/kg body weight, respectively. The LD₅₀ of *Albizia gummifera* is in agreement with an earlier work on the aqueous leaf extract of *Albizia chevalieri* by Saidu *et al.*, (2007), that the extract has an LD₅₀ of greater than 3000 mg/kg body weight. In line with this finding, a previous study done by (Debella *et al.*, 2007), has indicated that in the mice the LD₅₀ of *Albizia gummifera* and *Millettia ferruginea*, whose effective dose are 125mg/kg, through the oral administration, respectively were 2300 and 2500 mg/kg body weight. As the effective doses for the plants have been found as 125 mg/kg body weight (Karunamoorthi *et al.*,2009 and Taye *et al.*, 2011), it could be suggested that the lethal dose is more than twenty times greater than the effective dose.

The results of the 90 days sub-chronic administration, in the present study, also showed that the extract of both plants were tolerable, since no mortality and abnormal signs were observed throughout the study period. However, one male rat died during the 3rd week of administration of *Millettia ferruginea* seed extract. The reason for the death of this animal is unclear for no abnormal gross necropsy of liver and kidneys were observed.

The seeds extracts of both plants had no harmful effect on body growth patterns of test groups. Body weight of both the treated and untreated animals of both sexes increased as the duration increased. However, during the 9th week, although statistically not significant, there was a slight decrease in the body weight of female rats treated with *Albizia gummifera* seeds extract in a dose dependent manner as compared with the controls. But statistically significant difference in body

weight was observed in female rats during 10th week at 250mg/kg body weight. Such changes in the body weight is in line with the results of toxicity studies following administration of aqueous extract of *Vernonia amygdalina* (Amole *et al.*, 2006) and administration of aqueous extract of *Clerodendrum myricoides* (Kebede *et al.*, 2011) which results in suppression of weight gain of the extract treated animals.

As was described by Amacher (2002), biological markers are used to recognize, characterize and monitor treatment-related responses following exposure to xenobiotic agents. Biomarkers serve three primary applications in toxicology: to confirm exposure to a deleterious agent, to provide a system for monitoring individual's susceptibility to a toxicant, and to quantitatively assess deleterious effects of a toxicant to an organism or individual. One of such biomarkers is blood profile as manifestation of abnormal changes in metabolism due to underlying disease conditions. In toxicological studies, changes in hematological as well as biochemical parameters are used as indices of toxicities (Bin-Jaliah *et al.*, 2014).

Measurement of RBC count, HCT also sometimes referred to as a PCV, and HGB can be used to determine anemia which could be due to a decrease in the total number of erythrocytes, MCV, MCH or MCHC (Hume *et al.*, 1973; Sagone *et al.*, 1973; Sysmex, 2012). More recently RDW-CV, automated parameter providing information on the degree of variation of individual red cell size, has been used in conjunction with the traditional red cell indices in order to narrow down the possible causes of anemia in an individual patient (Sysmex, 2012). The mitotic capacity of different hematopoietic cell lineages predominates in marrow at different times. On direct marrow examination, the great majority of mitoses (74% to 90%) were of erythroid lineage; only a few (0% to 10%) were granulocytic (Keinanen *et al.*, 1986). The alteration in number of RBC count and HGB content may be due to defective haematopoiesis, inhibited erythropoiesis or an increase in destruction of red blood cells (Selmanoglu *et al.*, 2001; Choudhari and Deshmukh, 2007).

In the present study of 125 and 250 mg/kg body weight/ day of *Albizia gummifera* seeds extract treated group, did not decrease total RBC count, PLT, WBC, LYMPH, MONO, EO , BASO, HCT and HGB in the male rats as compared with the male control group. This observation is in agreement with other findings in which the values of the various RBC and WBC parameters of

oral administration of saponins isolated from *Albizia lebbek* bark extract which were found to be comparable with those of the control group (Gupta *et al.*, 2005). The seeds extract of *Albizia gummifera*, however, significantly increased the NEUT at 250mg/kg as compared to the control. The seeds extract of this plant did not decrease the levels of two red blood cell indices (MCV and MCHC) but, significantly decreased the level of MCH and increased the RDW-CV at both doses as compared to the male control. This indicates the seeds extract of *Albizia gummifera* may slightly induce anemia in male rats.

The seeds extract of this plant did not decrease total RBC count, PLT, WBC, LYMPH, MONO, EO, BASO, HCT, HGB and RDW-CV in the female rats. But have significantly increased the NEUT at 250mg/kg as compared to the control. The plant seeds extract did not decrease the level of MCH but, decreased MCV at 250mg/kg administered group and MCHC at both doses (125 and 250 mg/kg body weight) in the female rats as compared to the female control. This also indicates the plant seeds extract may induce anemia. This may happen in both of male and female rats because of the seeds extract of *Albizia gummifera* may have effect on iron metabolism or/ and erythropoiesis.

In the sub-chronic oral administration of both doses of *Millettia ferruginea* seeds extract did not decrease total RBC count, PLT, WBC, LYMPH, MONO, EO, BASO, HCT and HGB in the male rats as compared to the male control group. The seeds extract of this plant did not decrease the levels of red blood cell indices MCV, MCHC, MCH and did not increase the RDW-CV at both doses as compared to the male control. In the oral administration of female rats administered 125 and 250 mg/kg body weight/ day of *Millettia ferruginea* seeds extract, there was no significant changes in the blood parameters. The results of this blood profile in both of male and female rats is in line with previous study by Onyegeme-Okerenta *et al.*, (2013) on the effect of ethanol leaf extract of *Millettia aboensis* on haematological indices of wistar albino rats at doses lower than 2000 mg/kg body weight of the extract. However, one of red blood cell index (MCHC) has decreased at 250mg/kg body weight and MONO has also decreased at 250mg/kg body weight as compared to the female control. The decrease of MONO may show that there is a sub-chronic inflammatory process indicating the migration of the monocytes to the site of the toxic insult.

Another biomarker to toxic effect is serum biochemical profile. This effect can be detected or quantified by measuring the various serum biochemical parameters. ALT is primarily localized in liver tissue, and trace amount is found in skeletal muscle and heart tissue. Its cellular localizations are cytoplasm and mitochondria. This biochemical parameter could leak out from damaged tissues because of histopathological lesion particularly during hepatocellular necrosis (Dufour *et al.*, 2000a; Dufour *et al.*, 2000b and Amacher, 2002). AST is localized in liver, heart, muscle, brain and kidney tissues. Similarly, its cellular localizations are cytoplasm and mitochondria. This may also leak out from damaged tissues mainly because of hepatocellular necrosis (Dufour *et al.*, 2000a; Dufour *et al.*, 2000b and Ozer *et al.*, 2008). ALP is localized in liver, bile duct, bone, placenta, kidney and intestine. Its cellular localization is the cell membrane. There could be over production and release in blood because of hepatobiliary injury and cholestasis (Saukkonen *et al.*, 2006 and Ramaiah, 2007). Albumin is produced and localized in liver tissue, and is released into blood plasma. Because of hepatic dysfunction it is decreased in synthesis (Thapa and Walia, 2007).

In the seeds extract of *Albizia gummifera* administered group, the serum biochemical parameters analyzed for male rats are not found to be significant. Similarly, all the biochemical parameters analyzed for female rats as compared to the control were found insignificant. This result is in agreement with the previous studies by Saidu *et al.*, (2007) on aqueous leaf extract of *Albizia chevalieri* and oral administration of saponins isolated from *Albizia lebbek* bark by Gupta *et al.*, (2005) which showed no significant effect on serum liver and kidney function and biochemical parameters. Serum urea was increased at 250mg/kg body weight as compared to the male control. This indicates the seeds extract of *Albizia gummifera* may induce renal toxicity in male rats at higher doses. This result is supported by other findings in that drug-induced nephrotoxicity may be gender related (Bennett *et al.*, 1982). The serum levels of creatinine and urea in different drug-induced nephrotoxicity were higher in males than females (Bennett *et al.*, 1982, Goodrich and Hottendorf, 1995, Laniado-Laborin and Cabrales-Vargas, 2009 and Nematbakhsh *et al.*, 2013). Usually urea is increased in acute and chronic intrinsic renal diseases, which is characterized by decrement in effective circulating blood volume within the kidney

(Feres *et al.*, 2006). This explains why urea level increase in blood is one of the good indicators for kidney damage.

For the 90 days sub-chronic oral administration of 125 and 250 mg/kg body weight/ day of *Millettia ferruginea* seeds extract, serum biochemical parameters analyzed for male rats are all found normal. Biochemical parameters (albumin and AST) analyzed for female rats as compared to the control at both doses were also normal. On the other hand, ALP, ALT and urea were statistically significant at 250mg/kg body weight compared to the female control. This shows the seeds extract of *Millettia ferruginea* may induce hepatic and renal toxicity in female rats at higher doses. Increments in these biochemical parameters in experimental animals were also observed by other researchers with aqueous extract of *Tithonia diversifolia* in rats (Oyewole *et al.*, 2007), and *Clerodendrum myricoides* in mice (Kebede *et al.*, 2011).

Analysis of the organ weight is usually employed to determine whether the size of the organ has changed, as indicator of the adverse effect of the toxicants on that organ. According to Onyeanusi *et al.*, (2009), the mean kidney weight of the male rats was higher than those of females in both African giant rats and wistar rats, although the difference was not significant. In addition to these, the mean weight of the right kidney was heavier than that of the left kidney in both African giant rats and wistar rats. In this study, both of plant seeds extracts did not produce any detectable and meaningful change in the organ weight of liver and kidneys in both male and female rats at all doses.

Analysis of the toxic potential of a therapeutic agent on target organs is incomplete without gross and histopathological assessments. It is more rationale that all functional studies in toxicology should be coupled with appropriate morphologic pathology studies. Liver and kidney microscopic pathology serve as important tools for identifying and characterizing liver and kidney injuries respectively, whether or not biochemical and macroscopic changes are also identified. Some of the main patterns of liver injury during hepatotoxicity include zonal necrosis and vascular lesions (Singh *et al.*, 2011). Similarly, general pathology of renal structures include glomeruli hyper cellularity which may result from increased intrinsic cells or from accumulation

of leukocytes in capillary lumina, tubular necrosis is elicited as manifestations of either local metabolic abnormalities or systemic processes (characterized by loss of brush border staining for proximal cells, diffuse flattening of cells with resulting dilatation of lumina, loss of individual lining cells, and sloughing off of cells into lumina, (Robbins and Cotran, 2004). It is worthwhile to note that the kidneys of African giant rats and wistar rats are reddish brown with the African giant rat having a darker red colour *in vivo* (Onyeanusu *et al.*, 2009).

The present study, rats that were orally administered with repeated doses of the extracts at both 125 and 250mg/kg body weight for both of the plants showed no abnormalities of the liver and kidneys in the postmortem macroscopic evaluation. The present study noted the presence of histopathological changes in the liver and kidney tissue of the seeds extract of *Albizia gummifera* administered rats. Histopathological changes in the liver at the lowest dose were not detected for *Albizia gummifera* except for sign of congestion and hemorrhage. But, histopathological changes in the liver at 250mg/kg body weight showed few pyknotic cells and minor focal necrosis. This result is in agreement with herbal plants like *Atractylis gummifera* and *Callilepis laureola* reported by Larrey (1997). In contrast to the investigation of Larrey (1997), rat liver, kidney and heart tissues analyzed histopathologically were normal after acute and sub-chronic administration of the aqueous leaf extract of *Albizia chevalieri* by Saidu *et al.*, (2007). Such disagreement between the results may be due to absence of compound responsible for toxic insult in the aqueous leaf extract of *Albizia chevalieri* or duration for administration periods as the aqueous leaf extract of *Albizia chevalieri* has been administered to rats for only about 28 days at a doses between 0-1500mg/kg body weight. The seeds extract of *Albizia gummifera* may possess a class of compound that has caused interluminal eosinophilic protein cast, congestion and minor necrosis in the kidney tissue. This is in agreement with previous work by Saidu *et al.*, (2007) on aqueous leaf extract of *Albizia chevalieri* that elicited congestion and eosinophilic tubular protein cast.

Histopathological changes in the liver at 250mg/kg body weight have shown minor focal necrosis cellular and inflammation around central vein. The inflammatory reactions observed in the present study may, be associated with the cellular and tissue damage caused by the extract in liver, suggesting that this organ is vulnerable to be damaged by the extract. This result is in line

with various herbal plant administrations in experimental animals (Larrey 1997, Effendy *et al.*, 2006 and Kebede *et al.*, 2011). There was glomeruli atrophy with widened urinary space in rats at both doses and minor focal tubular necrosis in rat at higher dose of the seeds extract of *Millettia ferruginea* treated groups. There were also signs of congestion and hemorrhage in the kidney of the extract treated rats. The atrophy of the glomeruli may be due to sluggish circulation in the glomeruli or tissue hypoxia. Similarly, the signs of congestion and hemorrhage may suggest, impaired out flow of venous blood from the tissue and severe vascular injury or depletion of coagulation factors, respectively. This is in line with other reports following herbal extract administrations in experimental animals (Effendy *et al.*, 2006 and Kebede *et al.*, 2011).

The presence of bioactive compounds such as alkaloids, tannins, saponins and glycosides in plants may cause histopathological lesions of organs (Nerurkar *et al.*, 2004 and Eleyinmi *et al.*, 2006). Kidney of rats injected with gentamicin which is widely used aminoglycoside antibiotic has showed tubulointerstitial nephritis as well as interluminal and/or intraluminal eosinophilic protein cast (El-fattah and El-sheikh, 2012).

6. CONCLUSION

From the present investigation, the hydro-alcoholic seeds extract of *Albizzia gummifera* has increased NEUT at 250 mg/kg body weight in both male and female rats. The seeds extract of this plant decreased MCH and increased RDW-CV at both doses in the male rats. Similarly, the extract decreased MCHC at both doses and MCV at higher dose in the female rats. Hence, the hydro alcoholic seeds extracts of *Albizzia gummifera* may induce anemia in both male and female rats. All serum chemistry analyzed in this study for the seeds extract of the plant were found normal in both male and female rats, except for the elevated serum urea in the male rats at 250mg/kg body weight. The hydro-alcoholic seeds extract of *Albizzia gummifera* has brought some histopathological alterations in liver such as inflammation at 125 mg/kg body weight and congestions, pyknosis and focal cellular necrosis at the 250mg/kg body weight. The extract has resulted in some histopathological alterations in the kidney too, such as protein cast and atrophy of glomeruli at 250 mg/kg body weight.

For the hydro-alcoholic seeds extract of *Millettia ferruginea* all the hematological parameters analyzed were found normal in both sexes except for the decreased MONO in the female rats suggesting the migration of the monocytes to the site of toxic insult to the tissue. The decreased MCHC in the female rats indicate the seeds extract of this plant may also slightly induce anemia.

All serum chemistry analyzed in this study for the seeds extract of the plant was found normal in the male rats. But the extract has increased serum ALP, ALT and urea at 250mg/kg body weight in the female rats. The hydro-alcoholic seeds extract of *Millettia ferruginea* results in some histopathological alterations in liver tissue such as congestions and focal cellular necrosis at the 250mg/kg body weight.

Finally, the extract has also resulted in some histopathological alterations in kidney such as inflammation at 125mg/kg body weight and congestions and minor tubular necrosis at 250 mg/kg body weight. Such observed changes in both of the plant seeds extract might have resulted because of the presence of some bioactive ingredients in the extract which could cause harmful effects to the body tissue. Therefore this observed change should be validated with further

research at chronic level and active ingredients responsible for toxic insult should be investigated with their mechanisms of action.

7. RECOMMENDATIONS

The present study demonstrated that evaluations on the sub-chronic toxicity of hydro-ethanolic seeds extract of *Albizia gummifera* and *Millettia ferruginea* extract in albino wistar rats. However, further studies are needed

- To identify active ingredients responsible for toxic insult.
- On the mechanism of action of the extract for the toxic effects.
- To examine the toxic effects of these plants on other organs using similar animal model.
- To assess the toxic effects of these plants on blood parameters and histopathology of internal organs on other animal models.
- To investigate if changes observed may also be same in humans

8. REFERENCES

Aarts P., Banga J., Houwelingen H., Heethaar R. and Sixma J. (1986). Increased red blood cell deformability due to isoxsuprine administration decreases platelet adherence in a perfusion chamber: A double-blind cross-over study in patients with intermittent claudication. *Blood*; 67(5), 1474-1481.

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

Afolayan AJ and Yakubu MT (2009). Effect of *Bulbine natalensis Baker* stem extract on the functional indices and histology of the liver and kidney of male wistar rats. *Journal of Medicinal Food*; 12(4), 814-820.

Ajibade VA. and Famurawa D. (2012). Histological and toxicological effects of crude saponin extract from *phyllanthus niruri* on organs in animal studies. *Global Journal of Medicinal Research*; 12(1), 31-38.

Alexander K. and Blackburn J. (2013). Overcoming barriers in evaluating outbreaks of diarrheal disease in resource poor settings: Assessment of recurrent outbreaks in Chobe District, Botswana. *Biomedcentral Public Health*; 1-15.

Available at: <http://www.biomedcentral.com/1471-2458/13/775>

Alferah MA (2012). Toxicity induced histological changes in selected organs of male (wistar) rats by *Lawsonia inermis* leaf extract. *European Journal of Medicinal Plants*; 2(2), 151-158.

Amacher DE (2002). A toxicologist's guide to biomarkers of hepatic response. *Human and Experimental Toxicology*; 21, 253- 262.

Ameh JS., Obodozie OO., Babalola CP. and Gamanieal SK (2011). Medical herbalism and herbal clinical research: A global perspective. *British Journal of Pharmaceutical Research*; 1(4), 99-123.

Amole O., Izegbu M., Onakoya J and Dada M. (2006). Toxicity studies of the aqueous extract of *Vernonia amygdalina*. *Biomedical Research*; 17, 39-40.

Banzouzi JJ., Prost A., Rajemiarimiraho M. and Ongoka P. (2008). Traditional uses of the African *Millettia* species (Fabaceae). *International Journal of Botany*; 4(4), 406-420.

Beyene G. and Tasew H. (2014). Prevalence of intestinal parasite, *Shigella* and *Salmonella* species among diarrheal children in Jimma health center, Jimma southwest Ethiopia: A cross sectional study. *Annals of Clinical Microbiology and Antimicrobials*; 1-9.

Available at: <http://www.ann-clinmicrob.com/content/13/1/10>.

Bennett WM, Parker RA, Elliott WC, Gilbert DN and Houghton DC (1982). Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. *Journal of Infectious Disease*; 145(3), 370-373.

Bin-Jalial I., Dallak M., Al-Hashem F, Nwoye I., Sakr H., Jamil A. and Al-Khateeb M. (2014). Derangement of hemopoiesis and hematological indices in Khat (*Catha edulis*) - treated rats. *African Journal of Biotechnology*; 13(2), 349-355.

Bryce J., Boschic C., Shibuya K. and Black R. (2005). Estimates of the causes of death in children. *Lancet*; 365, 1147-1152.

Builders MI., Isichie CO and Aguiyi JC. (2012). Toxicity studies of the extracts of *Parkia biglobosa* stem bark in rats. *British Journal of Pharmaceutical Research*; 2(1), 1-16.

Chappuis F., Sundar S., Hailu A., Ghalib H., Rijal S., Peeling R., Alvar J. and Boelaert M. (2007). Visceral leishmaniasis: What are the needs for diagnosis, treatment and control?. Nature Publishing Group; 5, 873-882.

Available at: www.nature.com/reviews/micro

Choudhari C. and Deshmukh P. (2007). Acute and sub-chronic toxicity study of *Semecarpus anacardium* on hemoglobin percent and RBC count of male albino rat. Journal of Herbal Medicine and Toxicology; 1, 43-45.

Cox F. (2010). History of the discovery of the malaria parasites and their vectors. Cox Parasites and Vectors; pp.1-9.

Available at: <http://www.parasitesandvectors.com/content/3/1/5>.

Debella A., Taye A., Abebe D., Mudi K., Melaku D. and Taye G. (2007). Screening of some Ethiopian medicinal plants for mosquito larvicidal effects and phytochemical constituents. Pharmacology on line; 3, 231-243.

Deribe K., Amberbir A., Getachew B., and Mussema Y.(2006). A historical overview of traditional medicine practices and policy in Ethiopia. Ethiopian Journal of Health Development; 20(2), 127-134.

Dufour R., Lott J, Nolte F, Gretch D, Koff R, and Seeff L. (2000a). Diagnosis and monitoring of hepatic injury I. Performance characteristics of laboratory tests. National Academy of Clinical Biochemistry; 46(12), 2027–2049.

Dufour R., Lott J, Nolte F, Gretch D, Koff R, and Seeff L. (2000b). Diagnosis and monitoring of hepatic injury II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. National Academy of Clinical Biochemistry; 46(12), 2050–2068.

Effendy W., Siti-Nurtahirah J. and Hussin Z. (2006). The side effects of *Kacip fatimah* extract on liver and kidney of white rats. Journal of Sustainability Science and Management; 1, 40-46.

Eleyinmi A., Adebawale Y., Oluwalana I., Ajisafe O., and Akintomide T. (2006). Effect of dietary inclusion of *Garcinia kola*, *Gongronema latifolium* and *Vernonia amygdalina* on the nutritional quality of a complementary diet. *Journal of Biological Sciences*; 1, 43-49.

El-Fattah HM and El-Sheikh NM (2012). Evaluation of chemoprotective role of N-acetylcysteine and Vitamin E on gentamicin-induced nephrotoxicity. *Australian Journal of Basic and Applied Sciences*; 6(3), 263-270.

Elhelu M. (1983). The role of macrophage in immunology. *Journal of the National Medical Association*; 75(3), 314-317.

Everett N., Simmons B. and Lasher E. (1956). Distribution of blood (Fe⁵⁹) and plasma (I¹³¹) volumes of rats determined by liquid nitrogen freezing. *Circulation Research*; 4, 419-424.

Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM, and VanStaden J. (2004). Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. *Journal of Ethnopharmacology*; 94(3), 205-217.

Feres C., Madalosso R., Rocha O., Leite J., Guimaraes T., Toedo V and Tagliati C. (2006). Acute and chronic toxicological studies of *Dimorphandra mollis* in experimental animals. *Journal of Ethnopharmacology*; 108, 450-456.

Geyid A., Abebe D., Debella A., Makonnen Z., Aberra F., Teka F., Kebede T., Urga K. Yersaw K., Biza T., Hailemariam B. and Guta M. (2005). Screening of some plants of Ethiopia for their anti-microbial properties and chemical profiles. *Journal of Ethnopharmacology*; 97, 421-427.

Guyton AC and Hall JE (2006). *Text book of Medical Physiology*, 11th edition, Elsevier Saunders. pp 307-309, 859-862.

Gupta RS, Chaudhary R, Yadav RK, Verma SK and Dobhal MP (2005). Effect of saponins of *Albizia lebbeck* (L.) Benth bark on the reproductive system of male albino rats. Journal of Ethnopharmacology; 96 (1-2), 31-36.

Goodrich JA and Hottendorf GH (1995). Tobramycin gender-related nephrotoxicity in fischer but not sprague-dawley rats. Toxicology Letters; 75(1-3), 127-131.

Hume R., Dagg J., and Goldenberg A. (1973). Refractory anemia with dysproteinemia: Long term therapy with low-dose corticosteroids. Blood; 41(1), 27-35.

Jaijoy K., Soonthorn chareonnon N., Lertprasertsuke N., Panthong A. and Sireeratawong S. (2010). Acute and chronic oral toxicity of standardized water extract from the fruit of *Phyllanthus emblica* Linn. International Journal of Applied Research in Natural Products; 3(1), 48-58.

Junqueira LC and Carneiro J (2005). *Basic Histology*, 11th edition, The McGraw-Hill Companies, Inc. pp 223-237, 323-324.

Karunamoorthi K., Bishaw D. and Mulat T. (2009). Toxic effects of traditional Ethiopian fish poisoning plant *Millettia ferruginea* (Hochst) seed extract on aquatic macro invertebrates . European Review for Medical and Pharmacological Sciences; 13, 179-185.

Kebede H., Afework M., Makonnen E., Ergete W. and Urga K. (2011). The effect of *Clerodendrum myricoides* aqueous extract on blood, liver and kidney tissues of mice. Mekele University Journal of Science; 3 (2), 48-63.

Keinanen M., Knuutila S., Bloomfield C., Elonen E. and Chapelle A. (1986). The proportion of mitoses in different cell lineages changes during short-term culture of normal human bone marrow. Blood; 67(5), 1240-1243.

Kogure K., Ishizaki M., Nemoto M., Kuwano H. and Makuuchi M. (1999). A comparative study of the anatomy of rat and human livers. Journal of Hepatobiliary Pancreatic Surgery; 6, 171-175.

Kokwaro JO (2009). Medicinal plants of east Africa. Third Edition, Kenya Literature Bureau, Nairobi. ISBN: 9966-44-190-5.

Kothai S. and Befirdu G. (2012). Ethno botany and antimicrobial activities of medicinal plants used for skin infections in Jimma, Ethiopia. *Discovery Pharmacy*; 2(4), 5-11.

Kreutz M., Krause S., Hennemann B., Rehm A. and Andreesen R. (1992). Macrophage heterogeneity and differentiation : Defined serum-free culture conditions induce different types of macrophages *in vitro*. *Research Immunology*; 143, 107-115.

Laniado-Laborin R, and Cabrales-Vargas MN (2009). Amphotericin B: Side effects and toxicity. *Revision Micology*; 26(4), 223-227.

Larrey D (1997). Hepatotoxicity of herbal remedies. *Journal of Hepatology*; 26, 47-51.

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

Martins P. and Neuhaus P. (2007). Surgical anatomy of the liver, hepatic vasculature and bile ducts in the rat. *Liver International*; ISSN1478-3223, pp 384-392.

Moore KL and Dalley AF (2006). *Clinically Oriented Anatomy*, 5th edition, Lippincott Williams and Wilkins. pp 289-311.

Myler P. and Fasel N. (2008). *Leishmania: After the Genome*. Caister Academic Press. pp 112-114.

Nematbakhsh M., Ebrahimian S., Tooyserkani M., Eshraghi-Jazi F., Talebi A. and Ashrafi F. (2013). Gender difference in cisplatin-induced nephrotoxicity in a rat model: Greater intensity of damage in male than female. *Nephrology Urology*; 5(3), 818-821.

Nerurkar P., Dragull K., and Tang C. (2004). In vitro toxicity of kava alkaloid, pipermethystime, in hep G2 cells compared to kavalactones. *Journal of Toxicological Sciences*; 79, 106-111.

Oduola T., Bello I., Idowu T., Adeosun G. and Olatubosun L. (2010). Histopathological changes in wistar albino rats exposed to aqueous extract of unripe *Carica papaya*. *North American Journal of Medical Sciences*; 2(5), 234-237.

Ogunka-Nnoka CU, Uwakwe AA and Nwachoko NC (2012). Serum enzyme and histological studies of albino rat treated with ethanol/potash extract of *Sorghum bicolor* leaf sheath. *Indian Journal of Drugs and Diseases*; 1(3), 74-78.

Onyegeme-Okerenta BM, Onyeike EN and Esialekpe FO (2013). Effect of ethanol leaf extract of *Millettia aboensis* on selected haematological indices of Wistar albino rats. *Global Advanced Research Journal of Medicinal Plants*; 2(1), 004-011.

Onyeanusi BI, Adeniyi AA, Ayo JO, Ibe CS and Onyeanusi CG (2009). A comparative study on the urinary system of the African giant rat and the wistar rat. *Pakistan Journal of Nutrition*; 8(7), 1043-1047.

Ottesen J. (1954). On the age of human white cells in peripheral blood. Zoophysiological Laboratory, Department of Biological Isotope Research, and the Institute for Theoretical Physics. University of Copenhagen; pp 76-93.

Oyewole O., Magaji J. and Awoyinka A. (2007). Biochemical and toxicological studies of aqueous extract of *Tithnia diversifolia* leaves in Wister albino rats. *Journal of Medicinal Plants Research*; 1, 030-033.

Ozer J., Ratner M., Shawc M., Bailey W. and Schomaker S. (2008). The current state of serum biomarkers of hepatotoxicity. *Toxicology*; 245, 194–205.

Panunto W., Jaijoy K., Lerdvuthisopon N., Lertprasertsuke N., Jiruntanat N., Soonthornchareonnon N. and Sireeratawong S. (2011). Acute and chronic toxicity studies of the water extract from dried fruits of *Terminalia chebula* Rezt. in rats. *International Journal of Applied Research in Natural Products*; 3(4), 36-43.

Ramaiah S. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*; 45, 1551–1557.

Reilly RF and Ellison DH (2000). Mammalian distal tubule: Physiology, Pathophysiology, and Molecular Anatomy. *Physiology Reviews*; 80(1), 277-313.

Reithinger R., Dujardin J., Louzir H., Pirmez C., Alexander B., and Brooker S. (2007). Cutaneous leishmaniasis. *Lancet Infectious Disease*; 7, 581–596.

Robbins, S and Cotran, R. (2004). *Patological Basis of Disease*. Elsevier, 7th Edition, Saunders, Philadelphia, USA, pp. 100-133.

Ross H and Pawlina W. (2011). *Histology: A Text and Atlas: With Cell Correlation and Molecular Biology*. 6th edn, Lippincott Williams and Wilkins, pp. 268-299; 628-643; 699-720.

Sagone AL, Lawrence JR and Balcerzak SP (1973). Effect of smoking on tissue oxygen supply. *Blood*; 4(6), 845-852.

Saidu Y., Bilbis LS, Lawal M., Isezuo SA, Hassan SW and Abbas AY (2007). Acute and sub-chronic toxicity studies of crude aqueous extract of *Albizzia chevalieri* Harms (Leguminosae). *Asian Journal of Biochemistry*; 2(4), 224-336.

Saukkonen J., Cohn D., Jasmer R., Schenker S., Jereb J., Nolan C., Peloquin C., Gordin F., Nunes D., Strader D., Bernardo J., Venkataramanan R. and Sterling T. (2006). An official ATS statement: Hepatotoxicity of antituberculosis therapy. *American Journal of Respiratory Critical Care Medicine*; 174, 935–952.

Schmidt L. and Mwaura L. (2010). World agro forestry tree database; Seed leaflet No.141

Schur N., Hurlimann E., Stensgaard A., Chimfwembe K., Mushinge G., Simoonga C., Kabatereine N., Kristensen T., Utzinger J. and Vounatsou P. (2013). Spatially explicit *Schistosoma* infection risk in eastern Africa using Bayesian geostatistical modeling. *Acta Tropica*; 128, 365– 377. Available at: www.elsevier.com/locate/actatropica.

Selmanoglu G., Barlas N., Songur S. and Koc s k . (2001). Carbendazim-induced haematological, biochemical and histopathological changes to the liver and kidney of male rats. *Journal of Human and Experimental Toxicology*; 20, 625-630.

Sergeev G., Roy S., Jarek M., Zapolskii V., Kaufmann D., Nandy R. and Tegge W. (2014). High-throughput screening and whole genome sequencing identifies an antimicrobially active inhibitor of *Vibrio cholera*. *Biomedicine Central Microbiology*; 1-13. Available at: <http://www.biomedcentral.com/1471-2180/14/49>.

Singh A., Bhat TK and Sharma OP (2011). Clinical biochemistry of hepatotoxicity. *Journal of Clinical Toxicology*; ISSN:2161-0495, 1-19. Available at: <http://dx.doi.org/10.4172/2161-0495.S4-001>.

Snyder GK and Sheafor BA (1999). Red Blood Cells: Centerpiece in the Evolution of the Vertebrate Circulatory System. *American. Zoology*; 39, 189-198.

Stetson R. (1927). Normal variation in white blood cells under conditions of minimal metabolism. *Archives of Internal Medicine*; 40(4), 488-495.

Sysmex H. (2012). Educational enhancement and development. Seed Hematology. Available at: www.sysmex-europe.com.

Taye B., Giday M., Animut A. and Seid J. (2011). Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*; 8, 370- 375.

Taylor J., Rabe T., McGaw L., Jager A. and van Staden J. (2001). Towards the scientific validation of traditional medicinal plants. Kluwer Academic Publishers, *Plant Growth Regulation*; 34, 23–37.

Tefera M., Geyid A. and Debella A. (2010). *In vitro* anti-*Neisseria gonorrhoeae* activity of *Albizia gummifera* and *Croton macrostachyus*. *Revista CENIC,Ciencias Biológicas*; 41, 1-11. Available in: <http://www.redalyc.org/articulo.oa?id=181220509005>.

Teshome D. (2013). An overview of the role of traditional medicine in Ethiopia. *National Monthly Refereed Journal of Research in Arts and Education*; 4(2), 34-40. Available at: www.abhinavjournal.com.

Thapa B. and Walia A. (2007). Liver function tests and their interpretation. *Indian Journal of Pediatrics*; 74 (7), 663-671.

Tortora GJ and Derrickson B (2009). *Principles of Anatomy and Physiology*, 12th edition, John Wiley & Sons, Inc. pp 114-132, 234-260.

WHO. (2002). Traditional medicine strategy 2002-2005. Geneva. Publication number WHO/EDM/TRM/ 2002.1. Available at: http://whqlibdoc.who.int/hq/2002/WHO_EDM_TRM_2002.1.pdf

WHO. (2005). National policy on traditional medicine and regulation of herbal medicines. report of a WHO global survey. Geneva. 5-8.

WHO. (2008). Traditional Medicine Fact Sheet. Geneva. pp 1-7.

Woldemicheal T., Debella A., Abebe D., Tassew G., Biru T., Mekonnen Y., Teka F. and Melaku D. (2006). Screening of some medicinal plants of Ethiopia for their molluscidal activities and phytochemical constituents. *Pharmacology Online*; 3, 245-258.

Yadav R. (2013). Medicinal plants in folk medicine system of Ethiopia. *Journal of Poisonous and Medicinal Plants Research*; 1(1), 001-007.

Zhang Z., Cheng J., Xu F., Chen Y., Du J., Yuan M., Zhu F., Xu X., and Yuan S. (2011). Red blood cell extrudes nucleus and mitochondria against oxidative stress. *International Union of Biochemistry and Molecular Biology Life*; 63(7), 560–565.

9. APPENDICES

9.1. Appendix I: Preparations of working solutions

10% Neutral Buffered Formalin

10% Formalin (90% distilled H ₂ O and 37% Formalin)	1000ml
Sodium dihydrogen phosphate monohydrate (NaH ₂ PO ₄ .H ₂ O).....	4g
Sodium monohydrogen phosphate anhydrous (Na ₂ HPO ₄)	6.5g

Harris' Hematoxylin

Hematoxylin Crystal	2.5g
Ethanol, 100%	25ml
Ammonium or Potassium Alum.....	50g
Distilled water	500ml
Mercuric oxide (red)	1.25g

Eosin

Eosin Y (Yellow)	0.5g
Ethanol, 95%	100ml
Glacial acetic acid	0.5ml

1% Acid Alcohol

Ethanol, 70%	500ml
HCl, concentrated.....	5ml

Bluing Solution

Sodium bicarbonate	2.5g
Ethanol.....	1000ml
Distilled water	500ml

9.2. Appendix II:

Tissue processing schedules to form paraffin blocks for manual technique

Fixation

Buffered formalin, 10%24 hrs

Washing

Running tap water.....24 hrs

Dehydration

Alcohol, 70% 1 hr

Alcohol, 80% 1 hr

Alcohol, 95% 1 hr

Absolute alcohol I 1 hr

Absolute alcohol II..... 1 hr

Clearing

Xylene I 1 hr

Xylene II 1 hr

Infiltration (in paraffin oven)

Paraffin wax I 56 °C 1½ hrs

Paraffin wax II 56 °C..... 1½ hrs

9.3. Appendix III:

Routine heamatoxylin and eosin (H and E) staining schedule for tissue sections.

Chemicals	Duration of Staining
Xylene I	5min
Xylene II.....	5min
Absolute alcohol I	3min
Absolute alcohol II	3min
Alcohol, 95%	3min
Alcohol, 80%	3min
Distilled water	5min
Harris hematoxylin	4min
Acid alcohol	agitate(1sec)
Bluing solution.....	1second (three dips)
Tap water	5min
Eosin G.....	1min
Alcohol, 80%	3min
Alcohol, 95%	3min
Absolute alcohol, II	3min
Absolute alcohol, I	3min
Xylene II.....	5min
Xylene I	until mounting

This is to certify that the thesis prepared by Mekonnen Debebe, entitled: *Evaluation of the sub-chronic toxicity of 70% ethanolic seed extracts of Albizia gummifera and Millettia ferruginea on blood parameters and liver and kidney tissues in Albino wistar rats* and submitted in partial fulfillment of the requirements for the degree of Master of Science in Anatomy complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

The Thesis has passed with **Very Good** remark.

Signed by the Examining Committee:

Examiner Dr. Girmai Gebru Signature _____ Date _____

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