EFFECTS OF AQUEOUS AND ETHANOL EXTRACTS OF *Ziziphus mauritiana* LEAVES ON THE LIVER OF LEAD ACETATE TREATED ADULT MALE WISTAR RATS

BY

Muniratu Ibrahim GEBI

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN HUMAN ANATOMY

DEPARTMENT OF HUMAN ANATOMY,
FACULTY OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
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ZARIA, NIGERIA

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DECLARATION PAGE

I declare that the work in this dissertation entitled EFFECTS OF AQUEOUS AND ETHANOL EXTRACTS OF *ZIZIPHUS MAURITIANA* LEAVES ON THE LIVER OF LEAD ACETATE TREATED ADULT MALE WISTAR RATShas been performed by me in the Department of Human Anatomy. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Muniratu Ibrahim GEBI _____ _____

Signature Date

CERTIFICATION PAGE

This dissertation entitled EFFECTS OF AQUEOUS AND ETHANOL EXTRACTS OF *ZIZIPHUS MAURITIANA* LEAVES ON THE LIVER OF LEAD ACETATE TREATED ADULT MALE WISTAR RATSby Muniratu Ibrahim GEBI meets the regulation governing the award of Master of Science (M.Sc.) degree in Human Anatomy of Ahmadu Bello University and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty ALLAH for His favour, guidance and protection.

To my late father (Alh. Ibrahim Gebi). May ALLAH have mercy on him and grant him eternal rest.

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ABSTRACT

Lead poisoning is known to cause a number of adverse effects including nephrophaty, infertility, liver, testis and heart damages in human and experimental animals. The toxic effects of lead are treated by chelation therapy whichalso depletes the body store of essential cations and as such there is need to look for alternative therapy to lead poisoning. The present study was aimed at evaluating the effects of Aqueous and Ethanol extracts of *Ziziphus mauritiana* leaves (AZM & EZM) on lead acetate induced liver toxicity in adult male Wistar rats. Forty (40) adult male Wistar rats were divided into 8 groups of 5 rats per group. Group 1 was administered with distilled water from 1st to 35th day, Group 2 to 7 were administered with 120mg/kg bwt of lead acetate from 1st to 21st. While the rats in Group 2 were sacrificed on the 22ndday of the administration, From the 22nd to 35th day; Group 3 was treated with 100mg/kg bwt of AZM, Group 4 was treated with 400mg/kg bwt of AZM, Group 5 was treated with 100 mg/kg bwt of EZM, Group 6 was treated with 400mg/kg bwt EZM, Group 7 was treated with 10mg/kg bwt of Succimer, Group 8 was administered with distilled water. The administrations were carried out once orally for 35 days. The rats were sacrificed and blood samples were collected via cardiac puncture for haematological and biochemical analysis. Liver tissues were removed and fixed in 10% Formalin and processed for histopathological studies using Haematoxylin and Eosin (H&E) for Histo-architecture of the Liver, Gordon and Sweets for Reticular fibre and Periodic Acid Schiff for glycogen storage. The results showed that there were increased in liver enzyme parameters AST, ALT and ALP, and the effects were reversed in rats exposed to lead acetate and treated with AZM and EZM. Oxidative stress was induced in rats exposed to lead acetate by decreasing catalase, superoxide dismutase and reduced glutathione, and the effects were ameliorated in lead exposed rats treated with AZM and EZM. There were changes in haematological indices of rats exposed to lead acetate and these changes were reversed in rats treated with AZM and

EZM. The study showed that there were increased lead accumulations in liver of rats exposed to lead acetate and these changes were ameliorated in rats treated with AZM and EZM. The study also revealed histopathological changes in liver of rats exposed to lead acetate, changes includes Vacoulations, Necrotic Hepatocytes, Distorted Sinusoids, Degeneration of Reticular fibres and Depletion of glycogen storage and the changes were ameliorated when treated with AZM and EZM. Thus, the present study has concluded that Aqueous and Ethanol extracts of *Ziziphus mauritiana* leaves at doses of 100mg/kg bwt and 400mg/kg bwt was able to ameliorate the effects of lead acetate induced toxicity and may likely be beneficial to the population in endemic areas that are exposed to lead poisoning.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The diverse deleterious health effects upon exposure to heavy metals in the environment are a matter of serious concern and a global issue. Lead is the most abundant toxic metal in the environment (Patra*et al*., 2011). Lead is a poisonous metal, which exist in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in the environment (Shalan*etal*., 2005). Both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yücebilgic*et al*., 2003).The metal (lead) is primarily found in leaded gasoline, (Tong *et al*., 2000). Automobile emissions have been an important source of lead exposure to urban residents, particularly in areas with congested traffic. The main source of adult human exposure is through contaminated food, which is believed to account for over 60% of blood levels; while air inhalation accounts for approximately 30% and water of 10% (John *et al*., 1991).Lead poisoning is also known as plumbism, colicaPictonum, saturnism, Devon colic, or painter's colic, which is a medical condition caused by increased levels of the heavy metal lead in the body (Rossi, 2008). The manifestations of lead poisoning in humans are nonspecific. They may include weight loss, anemia (Khalil-Manesh*et al*., 1994), nephropathy, infertility, liver, testis and heart damages (Patocka andCerny, 2003; Gurer-Orhan*et al*., 2004). Symptoms of lead toxicity include abdominal pain, confusion, headache, anaemia, irritability, and in severe cases seizures, coma and death (Barbosa *et al*., 2005).

It has been reported that lead exposure produces free radicals, such as, Reactive oxygen species (O_2, OH or lipid peroxyl radical), cause oxidative damage to lipids, proteins, and nucleic acids and may lead to oxidative stress, biological carcinogenesis, mutagenesis, aging,

atherosclerosis, neuro-degenerative diseases and stress-induced depression (Sapolsky, 2000; Bilici*et al*., 2001).

Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids (Chaurasia and Kar, 1997), a deleterious process solely carried out by free radicals (Halliwell and Gutteridge, 1990). Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande*et al*., 2001). Oxidative stress leads to metabolic cellular processes in which oxidative species such as super oxide radical anions, hydrogen peroxide and lipid peroxides are generated intracellularly (Chen and Buck, 2000; Lee *et al*., 2012). These reactive species, if not eliminated, may damage DNA, proteins or membrane lipids and cause oxidative cell death. Endogenous antioxidative enzymes as well as antioxidants are required for cells to survive (Semenza, 2005), while exogenous antioxidants have been shown to effectively prevent oxidative cell death in cultured cells (Kurutas, 2016).

The use of medicinal and aromatic plants for the treatment of diseases is as old as mankind. Medicinal plants receive attention from research centers because of their special importance in safety of communities (Najafi*et al*., 2010). The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Lozoya*et al*.,1989; Karthikeyan*et al.*, 2009), secondary metabolites grouped as alkaloids, glycosides, flavonoids, saponins, tannins and essential oils. The activities of these curative plants are evaluated by their chemical components. Indian Ayurveda utilizes about 2000 plants to cure different ailments (Daniel, 2006). Some of these curative plants have natural antioxidants, which neutralize free radicals, therefore are receiving more attention from nutritionist and medical researchers for their potential effects in the prevention of chronic and degenerative changes, such as cancer, cardiovascular disease and aging (Young and Woodside, 2001).

Ziziphusmauritiana leaf is an important part of jujube tree which is useful in treating many health problems. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Morton, 1987; Michel, 2002).

1.2 Statement of Research Problem

Lead remains a considerable occupational and public health problem which is known to interfere with a number of body functions and it primarily affects the central nervous, heamatopoietic, hepatic and renal systems (Kalia*et al.*, 2005). The toxic effects of lead are treated by chelation therapy which also depletes the body store of essential cations (Ruff *et al*., 1996) and as such there is need to look for an alternative solution to lead poisoning.There are reports on lead induced toxicity in experimental studies but scanty works on medicinal plants as treatment options for lead induced liver damage.

1.3 Significance of the Study

The recent increase in lead exposure in our environment, especially the cases of Zamfara and Niger states (Lucia, 2015) has placed a heavy burden on health risk in both adult and children. The incessant exposure to lead and related products require investigation on a locally available mode of treatment.

*Ziziphusmauriitana*leaves has been reported to elicit strong antioxidative activity of which the present study intends to evaluate. The present study could be of importance in identification and evaluation of available natural remedy that may possibly be used as alternatives to currently used chelation therapy to treat lead poisoning, which is costly and not free from adverse effects. Therefore, from the results of the present study, *Ziziphusmauritiana* leaves could be of help in the management of lead poisoning.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of the study is to evaluate the effects of aqueous and ethanol extracts of *Ziziphusmauritiana* leaves on lead acetate induced liver toxicity in adult male Wistar Rats.

1.4.2 Objectives of the study

The objectives of the study were:

I. to study changes in the histology of the liver using routine Haemotoxylin and Eosin stain; histochemical techniques using special stains Periodic Acid Schiff (PAS) for glycogen; and Gordon and Sweet for Reticular fibres.

II. to determine the effect of the extracts on hepatotoxicity using biochemical analysis for liver enzyme markers (Alanine amino transaminase – ALT, Alkaline Phosphotase – ALP and Aspartate amino transferase – AST).

III. to examine the effect of the extracts on lead induced alterations on biomarkers of oxidative stress: Catalase (CAT), Superoxide dismutase (SOD), Malondialdehyde (MDA) and Reduced Glutathione (GSH).

IV. to study changes in heamatological parameters (WBC, RBC, HGB, PLT, LYMP) following treatment with aqueous and ethanol extracts of *Ziziphus mauritiana* leaves on the liver of lead acetate exposed adult male Wistar rats.

V. to examine changes in liver lead concentration following treatment with aqueous and ethanol extracts of *Ziziphus mauritiana* leaves on the liver of lead acetate exposed adult male Wistar rats using atomic absorption spectrometry.

1.5 Scope of the Study

The scope of the study was limited to the study of the general histomorphological and biochemical changesfollowing treatment with aqueous and ethanol extracts of *Ziziphus mauritiana* leaves on the liver of lead acetate exposed adult male Wistar rats.

1.6 Study Hypothesis

Aqueous and Ethanol extracts of *Ziziphusmauritiana* leaves has effect on lead acetate induced changes on the liver of adult male Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Lead

Lead a soft, grey-blue heavy metal found ubiquitously, can be found in the soil crust, in mineral form as galena, anglesite, crussite, mimetite, pyromorphite, linarite, vanadinite and wulfenite (Humphreys, 1988). Lead is a poisonous metal which occurs in both organic and inorganic forms in the environment (Shalen*et al*., 2005). The most well-known organic lead compounds are the tetraethyl and triethyl lead, which were once used as octane enhancers in gasoline. Tetra-ethyl lead has been eliminated from the petrol supplies of the majority of countries, but is still used in about 9 countries (UNEP, 2012). Today organic lead compounds are used much less often- usually as drying agents for lacquers and similar materials. Organic forms of lead can be more toxic than inorganic lead because the body more readily absorbs it. Potential exposures to organic lead should be taken very seriously (ASTDR, 2012). The combustion of organic lead – when it is added to petrol as a fuel additive – results in the release of lead into the atmosphere. Inorganic lead compounds include metallic lead, lead alloys, lead oxide, and lead sulphate. A major use of inorganic lead is in the manufacture of batteries, industrial paints (red lead oxide primer) or for use in assaying, soldering, radiator repair and metal reclaiming. Lead compounds are also used in jewellery making, stained glass making and in ceramic glazes. It is the form found in old paint, soil, dust and various consumer products (ASTDR, 2012). The color varies, depending on the chemical form, and the most common forms are white lead (a lead carbonate compound), yellow lead (lead chromate, lead monoxide) or red lead (lead tetraoxide). Lead acetate has a sweetish taste. Both organic and inorganic lead is very toxic to human body, and features of poisoning differ depending on whether the agent is an organic compound or an inorganic one.

2.1.1 Sources of lead

Lead occurs naturally in the environment, it is rarely found in its elemental form but occurs in the Earth's crust primarily as the mineral galena (PbS), and to a lesser extent as anglesite ($PbSO_4$) and cerussite ($PbCO_3$). Lead minerals are found in association with zinc, copper, and iron sulfides as well as gold, silver, bismuth and antimony minerals. It also occurs as a trace element in coal, oil, and wood (Carocci*et al*., 2015).There are different sources of lead in the environment and these include natural sources, industrial sources, consumable products and diffusion and combustion sources.

Natural Sources: Lead (Pb) occurs in the earth crust (Sohair*et al*., 2010) as the end product of the radiometric decay of three naturally occurring radioactive elements: Uranium, Thorium, and Actinium. Lead, primarily in the form of lead sulfide in galena ore, constitutes approximately 10 to 17 milligrams per kilogram (mg/kg) or 0.001 to 0.002 percent of the earth's crust (ARB, 1993). A common natural means of releasing lead to the atmosphere is via windborne dusts created by weathering of deposits. Other natural sources of lead emissions are: sea and saltlake aerosols, forest fires, and volcanic erruptions (HSDB, 1995). Lead released from natural sources, such as volcanoes, windblown dust, and erosion, are minor compared with anthropogenic sources. Anthropogenic sources of lead include the mining and smelting of ore, manufacture of lead-containing products, combustion of coal and oil, and waste incineration. Many anthropogenic sources of lead, most notably leaded gasoline, leadbased paint, lead solder in food cans, lead-arsenate pesticides have been eliminated or strictly regulated due to lead's persistence and toxicity (Carocci*et al*., 2015). Since lead does not dissipate, biodegrade and decay, lead deposited into dust and from Pb paint, leaded gasoline and industrial or occupational source of lead become a long term source of lead exposure (Habal, 2002).

Industrial sources: Gasoline (petrol) used to be the major source of exposure to lead in the environment. Leaded gasoline is still widely used in many countries and contributes to elevated blood lead level especially in urban children. Lead contamination may arise from cottage industries that recycle lead.After lead in petrol, lead in paint is one of the largest sources of exposure to lead. Leaded paint can remain a source of exposure to lead and lead poisoning for many years after the paint has been applied to surfaces(Meyer *et al*., 2003). Paint industrial source may drastically increase air and soil level in part of the world where environmental controls have not been effectively implemented (Meyer *et al*., 2003).

Consumable products: Most of the daily intake of lead (>80%) is acquired through the ingestion of food, dirt and dust. The quantity of lead in food and plants depends on soil concentrations and is highest around mines and smelters. Poorly glazed pottery could result in high food lead level. The use of lead-soldered food and beverage cans may considerably increase lead content especially in the case of acidic foods or drinks. Since alcoholic drinks tend to be acidic, the use of any lead-containing products in their manufacture or distribution will raise lead levels. More so, smoking tobacco increases lead intake (Habal, 2002).

Diffusion and combustion sources: Burning of fossil fuels (petrol and gas), batteries disposal and burning lead painted wood contribute to lead exposure to man and animals (D'souza*et al*., 2011).

Lead in products: Lead is found in variety of products such as kohl eye cosmetics and toys. Lead is a problem in toys for two reasons: (1) The toy may be painted with leaded paint; and (2) The toy itself is made of lead.It is also found in vinyl mini-blinds and commonly incorporated into herbal remedies such as Indian ayuverdic preparations and remedies of Chinese origin. Cosmetics such as lipstick and lead-based paints are the most common high dose sources of lead exposure (00, 2002).

2.1.2 Exposure routes of lead

Exposure to lead occurs through three (3) main ways: ingestion, inhalation and dermal contact.

Oral exposure: Consumption of contaminated food, water or alcohol is an important mode of exposure. Ingestion of soil and house dust is a major pathway for the exposure of young children to lead, due to "hand-to-mouth" activity, these appears to be a more significant pathway than inhalation for young children (D'souza*et al*., 2011). Old lead pipes can slowly dissolve in some soft and acidic waters, there by contaminating the water with lead(D'souza*et al*., 2011). Food is a major source of lead intake for adult population; produce can be contaminated from airborne deposition and lead-rich soil (Lead facts, 2005). Lead can be ingested through fruits and vegetables contaminated by high levels of lead in the soil. Soil is contaminated due to the lead in pipes, lead dust from old paints and residual lead from gasoline (D'souza*et al*., 2011)

Inhalation exposure: This is the second major pathway of exposure, especially for workers in lead-related occupations. Almost all inhaled lead is absorbed into the body at the rate of 20-70% and usually more in children than adults. Approxiemately 30-40% of inhaled lead is absorbed into the blood stream (Phillip *et al*., 1994). Inhalation usually occurs when people are exposed to lead in particulate matter less than 10μm in diameter (PM10). Severe cases of lead poisoning following inhalation of contaminated dust have been documented (Amitai*et al*., 1991). The inhalation of lead could be from industrial action of fossil fuel powder odour (EPA, 2002; Hu *et al*., 2007), inhalation of tobacco smoke and dust and in many occupational settings such as pigments and batteries production, galvanization and recycling of electrical tools (Saper*et al*., 2004).

Dermal exposure: This plays a role for exposure to organic lead among workers, but is not considered a significant pathway for the general population. Organic lead may be absorbed

directly through the skin, organic lead (tetramethyl lead) is more likely to be absorbed through the skin than inorganic lead and dermal exposure is most likely among people who work with lead (ASTDR, 2010). The rate of skin absorption is also low for inorganic lead via the use of lead ointments or cosmetic which may not result in disease condition (WHO, 2015).

Endogenous exposure: In addition to the above 3 main ways, exposure to lead can occur endogenously. Endogenous exposure to lead may also contribute significantly to an individual's current blood lead level, and of particular risk to the developing fetus. Once absorbed into the body, lead may be stored for long periods in mineralizing tissue (teeth and bones). The stored lead may be released again into the bloodstream, especially in times of calcium stress (pregnancy, lactation, osteoporosis), or calcium deficiency (ASTDR, 2010).

2.1.3 Toxicokinetics of lead acetate

Absorption of lead: Lead gain access into the body through inhalation, ingestion or dermal (skin) contact; it can be transferred to the fetus through the placenta (Goyer, 1996). Inhalation and dermal contact are routes of exposure more typical of occupational settings where as the primary route of exposure for general population is ingestion from minor amounts in food and hand-to-mouth activity particularly in children(Goyer, 1996). Adults absorb approximately 5-10% of ingested lead into the circulation, of this amount, less than 5% is retained in the body (Goyer, 1996). Young children can absorb considerably more (30-40%) of ingested lead; this explains their enhanced susceptibility to the potential effects of lead (Goyer, 1996).

Inorganic lead is absorbed from the respiratory or gastrointestinal tract but not through the skin. Approximately 90% of the total body burden is stored in bone and the remainder is in blood stream and soft tissue (Philip and Gerson, 1994). Gastrointestinal absorption varies depending on nutritional status and age. Iron is believed to impair lead uptake in the gut,

while iron deficiency is associated with increased blood lead concentrations in children. Lead exposure in pregnant animals usually occurs through the oral route. It is known that absorption of this metal increase during pregnancy. Lead crosses the placenta and it accumulates in the fetus. Accumulation of lead occurs in the fetal brain owing to lack of blood-brain barrier (BBB). Lead also accumulates in the placenta in times of fetal stress (Gupta*et al*., 2012).

After absorption of Pb from the gastrointestinal tract or the lungs, it enters the blood stream. At first, lead attaches to proteins in the blood and carried to different tissues or organ systems in the body. It has been reported that most of the lead present in the blood is bound to the red blood cells (Brian and Fred, 1995).

Distribution of lead: Absorbed lead that is not excreted is exchanged primarily among 3 body compartments; blood, soft tissues (liver, kidney, lungs, brain, spleen, muscles and heart) and mineralizing tissues (bone and teeths)(Rabinowitz, 1991). Organic lead compounds are metabolized in the liver while inorganic lead, the most common form of lead, is not metabolized in the liver. The body accumulates lead over a lifetime and normally releases it very slowly. Mobilization of lead from maternal bone is particularly relevant during pregnancy and lactation and may also occur in persons with osteoporosis (ATSDR, 2010).

Research has established that about 95% of the blood lead concentration is bound to the erythrocyte membrane and is distributed in this form in the organism. In the body, the lead is present as a displaceable and an irreversibly bound fraction (Brian and Fred, 2005). The rapid exchange pool represents the biologically effective body burden which correlates with the blood lead concentration i.e the lead concentration in soft tissues (Elias *et al*., 2014). Lead readily passes the placental barrier, and foetal blood therefore has more or less the same lead concentration as the maternal blood (Gulson*et al*., 2003; Ettinger*et al*., 2009). Lead passes the blood-brain barrier, but is not thought to accumulate in the brain (Brian and Fred, 2005).

Human hair is one of the important areas where lead is stored and many authors have reported that human hair mineral analysis is a good maker of environmental pollution. Therefore, using scalp hair as an indicator of the environmental exposure to several trace elements has become a common practice (Shah *et al*., 2005; Ozden*et al*., 2007). The advantage of hair is that it is a storage tissue and retains trace elements over an extended period of time (Foo *et al*., 1993; Izabela*et al*., 2014). Metal body burden of lead is better reflected in hair than in blood because hair gives a record of relatively long periods, while blood shows momentary levels that fluctuate with time. Furthermore, hair is inert and easier to sample than blood and can be stored without technical problems (Mehra and Juneja, 2004). The investigation of Nowak and Chmielnicka, (2000) revealed that among the investigated keratinous matrices (hair, nail, tooth), lead content in hair was the mostly meaningful environmental marker of exposure to this metal in the human organism and depended on sex and age.

Excretion of Lead: Lead is excreted primarily through the urine (65-75%) and feces (25-30%) with smaller amounts excreted in sweat, hair, nails and breast milk (Park *et al*., 2008). However, the rate of elimination is relatively slow. It is very difficult to give a biological half-life because of the constantly decreasing availability of the depot in the bones. The "Task Group on Metal Accumulation" gives a half-life for lead in human bones of about 10years (Kaste*et al*., 2003). Therefore, many people (and most occupationally exposed workers) are unable to get rid of as much lead as they take in. That is why the "body burden" of lead increases over decades. Until later in life, most persons are steadily getting more and more lead in their tissues. Only among the elderly, for example those 70 to 80 years old, does the body lead burden begins to get less (Brian and Fred, 2005).

2.1.4 Effects of lead

Lead has no known physiologically relevant role in the body (Manay, 2008), and its harmful effects are myriad. The effects of lead toxicity are systemic involving multiple organs. Numerous studies regarding the toxicology of lead have shown it to be a potent neurotoxicant, especially during nervous system development (Feldman *et al*., 1980). High levels lead exposure in adults and children has been associated with deficits in memory and in intellectual functioning (Arvig*et al*., 1980; Baghurst*et al*., 1992; Baker 1982; Baker *et al*., 1984), attention and concentration (Arvig*et al*., 1980; Stollery*et al*., 1989), speed and psychomotor performance (Arvig*et al*., 1980; Stollery*et al*., 1991). Lead has also well-known effects on the cardiovascular (Vaziri, 2002), renal (Gonick, 2002), reproductive (Bellinger, 2005) and immune (Dietert and Piepenbrink, 2006) systems, as well as bones and teeth (Hu *et al*., 1998) and it has been also identified as a probable human carcinogen (Silbergeld, 2003; van Wijngaarden and Dosemeci, 2006). Epidemiologic studies suggest an association of inorganic lead exposure to lung, stomach and, to a lesser extent, kidney and brain cancer (Steenland and Boffetta, 2000; IARC 2006).

Lead is known to disrupt dopaminergic function in experimental studies; it seems also to induce oxidative stress (Ercal*et al*., 2001), which is a candidate hypothesis for the etiology of Parkinson, Alzheimer and other neurodegenerative age-related diseases.

Effects of lead on nervous system: Lead affects the peripheral nervous system (especially motor nerves) and the central nervous system (Dart *et al*., 2004). Peripheral nervous system effects are more prominent in adults and central nervous system effects are more prominent in children (Bellinger, 2004). Lead causes the axons of nerve cells to degenerate and lose their myelin coats (Dart, 2004). The brain is the most sensitive organ to lead exposure (Cleveland *et al*., 2008). In a child's developing brain, synapse formation is greatly affected in the cerebral cortex by lead. Lead also interferes with the development of neurochemicals,

including neurotransmitters, and organisation of ion channels (Casarett*et al*., 2007). Lead poisoning also causes loss of neuron myelin sheath, reduction in the number of neurons, it interferes with neurotransmission and decreases neuronal growth (Pearson and Schonfeld, 2003).

In the central nervous system, lead poisoning is a potential factor in brain damage, mental impairment and severe behavioural anomalies, neuromuscular weakness, decreased hearing and impaired cognitive functions in experimental animals (Liuji*et al*., 2002; Verina*et al*., 2007; Flora *et al*., 2007). Oxidative stress, oxidative damage to cellular components and activation of the oxidant sensitive transcription factor could, in part, underlie some of the toxic effects of Pb. The deleterious effects of Pb can involve both reactive oxygen and nitrogen species (Gurer and Ercal, 2000).

It has been understood, during the past decade how much the nervous system is affected by lead. That means, earlier recommendations on "safe" amounts of lead in blood were dangerously close to levels now considered very likely to cause mental retardation in children (Brian and Fred, 2005). Elevated levels of lead (Pb^{2+}) have been shown to induce cognitive and behavioural deficits in adults and children with different brain targets by inhibiting the N-methyl-D-aspartate receptor (NMDAR) or VGCCs. Inhibition of NMDAR or VGCCs result in a significant reduction of Ca^{2+} entry into the cell which alter pathways involved in synaptic development and neurotransmitter release in brain (Toscano and Guilarte, 2005; Bouchard *et al*., 2011).

Effects of Lead on Renal System: A number of studies found low level environmental lead exposure to be associated with accelerated deterioration of chronic renal insufficiency (Yu *et al*., 2003). Even at levels far below the limits of normal ranges in the general population, both blood lead level and blood lead burden were found to be increased, predicting accelerated progression of chronic renal disease. Wedeen*et al*. (1975) studied the effects of occupational

lead hazards on patients. They observed tubular dysfunctions in patients who underwent biopsy. Such studies suggest that lead nephropathy may be an important occupational hazard. Excretion of the waste product urate was reported in lead poisoning, suggesting gout, in which urate builds up in the body (Wright *et al*., 1984; Ekong*et al.*, 2006; Rubin and Strayer, 2008).

In rats, proximal tubular injury involves the convulated and straight portions of the tubule (Dieter *et al*., 1993) with greater severity, at least initially, in the straight (S3) segment (Murakami *et al*., 1983). Typical histological features include, in the acute phase, the formation of intranuclear inclusion bodies in proximal tubule cells, abnormal morphology of proximal tubular mitochondria (Fowler *et al*., 1980). Karyomegaly, cytomegaly and cellular necrosis at sufficiently high dosage. These changes appear to progress, in the chronic phase of toxicity and with sufficient dosage, to tubular atrophy and interstitial fibrosis (Khalil-Manesh*et al*., 1992 a and b). Glomerular sclerosis has also been reported (Khalil-Manesh*et al*., 1992a). Adenocacinomas of the kidney have been observed in long-term studies in rodents in which animals also developed proximal tubular nephropathy (Koller, 1985; Goyer, 1993).

Effects of lead on reproduction system: Lead causes a number of adverse effects on the reproductive system in both men and women. Common effects seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone. Women on the other hand, are more susceptible to infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery (Flora *et al*., 2011).

Furthermore, direct effect of lead on the progressive stages of the fetus was also studied during the gestation period (Saleh*et al*., 2009). Experimental findings revealed that animals exposed to lead exhibited lower levels of plasma luteinizing hormone (LH) after stimulation

with gonadotropin-releasing hormone as compared to controls as well as reduction in inhibin/follicle-stimulating hormone (FSH) ratio (Foster *et al*., 1993). The rats treated with lead at a dose of 30 mg/kg BW presented considerable decrease in T3, T4 and TSH levels in serum, and histopathological findings exhibited an enlarged thyroid follicles associated with flattened epithelium as compared with negative control animals (El-Mehi and Amin, 2012).

Effects of lead on blood: Lead may be rapidly absorbed and reached considerable amount in the blood (Haque*et al*., 2006). Once absorbed, 99% of blood lead is transported to the erythrocytes as lead diphosphate (Freeman, 1970). Increment of blood lead level -following lead acetate and lead nitrate administration was demonstrated in the experimental animals (Ferguson *et al*., 1998; Sharma *et al*., 2011d). Some reports suggested that this element is strongly bound to macromolecules in the intracellular compartment because lead binding proteins have been isolated from kidney, liver, blood and brain (Moussa*et al*., 2001). The half- life of lead differs for each of the compartment, ranging from 25 to 40 days in erythrocytes, 40 days in soft tissues and as many as 28 years in bone (Sharma *et al*., 2011d).Studies have shown that Pb intoxication has destructive effects on blood (Elias *et al*., 2014). Mugahi *et al*., (2003) evaluated the effects of chronic lead acetate intoxication on blood indices of animals by exposing male adult rats to 1% lead acetate in 0.4% acetic acid. A decrease in red blood cell count and increase in leukocyte count were observed. In addition, the rats also had monocytosis, eosinopenia, neutrophilia and thrombocytosis (Mugahi*et al*., 2003). Similarly, Okediran*et al*., (2010) evaluated the toxic effect of lead on blood cells in animals by administering lead at 0.5, 1.0, 1.5, 2.0 mg/kg bwt to rats for 14days and observed higher blood lead concentration, decreased packed cell volume, decreased haemoglobin concentration and decreased red blood cell count. There were also leukocytosis, monocytosis and neutrophilia in rats with higher doses of lead.Lead decreases heme biosynthesis by inhibiting Aminolevulinic Acid Dehydratase (ALAD) and ferrochelatase

activity. Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is quite sensitive to lead (Suradkhar, *et al*., 2009). It is also reported that, lead also inhibit the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in haemoglobin production and shortened life-span of erythrocytes (Klassen, 2001).Progressive destruction of RBCs due to binding of lead with RBCs, leading to increase in fragility and destruction could be another reason for decrease hematological values. Analysis of total leukocytes count and differential leukocytes count revealed leucopenia and lymphopenia in higher dose group animals. This might be due to direct toxic action of lead on leucopoiesis in lymphoid organs, (Suradkhar, *et al*., 2009).Study has established that the significant reduction in hematological parameters of RBC, Hb, HCT and an increase in platelet count and WBC was induced by lead poisoning. This is possibly as a result of marrow infiltration by lead with a reactionary increase in some parameters (Nwokocha*et al*., 2011).It has also been shown that lead toxicity facilitates conversion of Hb into met-Hb. During Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in erythrocyte cell membrane (Vargas *et al*., 2003). As a result, lead might induce generation of reactive oxygen species (ROS) by interacting with oxy-Hb, leading to peroxidative damage of erythrocyte membranes (Lyn-Patrick, 2006; Sharma *et al*., 2011).

Lead- induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande and Flora, 2002; Auman*et al*., 2007; Waters *et al*., 2008). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasani*et al*., 2001) and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system (Adanaylo*et al*., 1999).

2.1.5Molecular mechanism of lead toxicity

Oxidative stress which plays an important role in lead poisoning is caused by an imbalance in the production of free radicals and compromising the system's ability to readily detoxify the body of those radials resulted in cellular damage (Flora, 2011). It starts with the onset of oxidative stress that has been proposed occurring in two simultaneous pathways; first, the generation of ROS such as hydro peroxides (HO_2•), singlet oxygen, and hydrogen peroxide (H_2O_2). Second, the depletion of natural-occurring-antioxidants reserves (Flora, 2002). The body's antioxidant enzymatic system works to neutralize the ROS generation, with the cell's most important enzyme which is the GSH. GSH is a tripeptide (containing sulfhydryl groups) found in mammalian tissues which destroy free radicals (Mates, 2000). GSH exists in two forms; reduced (GSH) and oxidized GSH dismutase (GSSG). In reduced state, GSH donates reducing equivalents (H+ + e–) from its thiol groups in cysteine residues to ROS, thus making them stable. After donating its electron, it then readily combines with another molecule of its own to the form GSH disulfide (GSSG) in the presence of GPx. The reverse/opposite reaction generates GSH GSSG in the presence of GSH reductase (GR). Normally, only about 10% of GSH exists in the oxidized form (GSSG), the rest exist in reduced form as GSH. However, GSSG concentration is much higher than that of GSH during oxidative stress.

Lead shows a strong electron sharing property which aids in the formation of covalent bonds. These covalent bonds are formed between the lead moiety and the sulfhydryl groups in the antioxidant enzymes, making the enzymes most susceptible targets for lead, eventually rendering them inactive. On the other hand, lead inactivates GSH by binding with its sulfhydryl. This process gives rise to the synthesis of GSH from cysteine via the γ-glutamyl cycle, but would normally not be effective enough to replenish the supply of GSH (Hultberg*et al*., 2001). Furthermore, in addition, lead inactivates δ-ALAD, GR, GPx, and

GSH-S-transferase enzymes, and further depresses the levels of GSH (Ahamed and Siddiqui, 2007). Others are; SOD and CAT. Decreased concentration of SOD reduces the clearance of superoxide radical, while reduced CAT impairs the superoxide radical (O2 – •) scavenging. Lead is also able to replace the zinc ions which serve as important co-factors for these antioxidant enzymes in trying to inactivate the enzymes (Flora *et al*., 2007), this is apart from targeting their sulfhydryl groups.

Lipid peroxidation is considered as a biomarker to oxidative stress and is one of the most investigated consequences of ROS on lipid membranes. The ROS takes electrons from the cell membranes and damages the cell by denaturing lipid that formed the membrane. Apart from lipid peroxidation, lead induces oxidation to Hb, resulting to hemolysis of the RBC. This happens due to ALAD inhibition and caused increased in the concentration of ALA substrate in blood, as well as urine. An elevated level of ALA, in turn, generates hydrogen peroxide and superoxide radical which goes to interact with oxy-Hb, resulting in the generation of hydroxyl radicals (Patrick, 2006). The aforementioned mechanism makes the cell highly vulnerable to oxidative stress and may also lead to cell death.

Bivalent cations such as Ca2+, Mg2+, Fe2+ and monovalent cations such as Na+, could also be substituted by Pb, thereby affecting various fundamental biological processes in what is called as an ionic mechanism of lead action (Lidisky and Schneider, 2003). Fundamental cellular processes such as intra and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ionic transportation, enzyme regulation,release of neurotransmitters have been significantly affected by the aforementioned ionic mechanism (Garza *et al*., 2006). This mechanism contributes principally to neurological deficits after lead replaces calcium ions; it becomes competent to cross the blood-brain barrier (BBB) at an appreciable rate. The lead then accumulates in astroglial cells (containing lead-binding proteins). The effects of lead toxicity are more pronounced in immature astroglial cells (developing nervous system)

that lack lead-binding proteins. It (lead) easily damages the astroglial cells and obstructs the formation of myelin sheath, which is both involved in the development of BBB.

Key neurotransmitters such as protein kinase C can be affected by such lead replacement of calcium as picomolarconcentration, protein kinase C regulates long-term neural excitation, and memory storage. Lead also affects the sodium ion concentration, which is responsible for enormous biological activities that are vital such as generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine, and GABA), and regulation of uptake and retention of calcium by synaptosomes. This interaction between lead and sodium seriously impairs the normal functioning of the aforementioned sodium-dependent processes (Bressler*et al*., 1999).

2.2 *Ziziphus mauritiana*

2.2.1 General description of *Ziziphusmauritiana* plant

Ziziphusmauritiana belongs to the family Rhamnaceae (Michel, 2002). *Ziziphusmauritiana* commonly known as Indian Jujube or 'Ber' is a tropical fruit found in many parts of the world especially in Pakistan, India and Africa (Gupta *et al*., 2012). *Ziziphusmauritiana* is popularly known as *Magarya* in Hausa (Saalu, 2016). *Ziziphusmauritiana* is a fast growing small to medium-sized, single or multi-stemmed, spiny shrub or tree, which is almost evergreen but is deciduous during the dry season. It can reach up to 12 m tall and 30 cm diameter at breast height but is highly variable in size and general appearance. The bark is dark grey, dull black or reddish with long vertical fissures, reddish and fibrous inside. The branches are spreading and droop at the ends (Saalu, 2016). Branches typically have a leaf and a thorn at each angle. Leaves are ovoid, glossy green above and nearly white underneath. The major veins in the leaves are nearly parallel. Over time, the leaves typically become infested with a fungus, causing the leaves to have a yellow-mottled appearance above and to turn black below. Flowers of the Indian jujube are small and inconspicuous, greenish-white,

and emit an unpleasant odor. The Indian jujube fruits are initially green, but have a yellow to pale orange color when ripe. Seeds are enclosed within a woody endocarp (Brent, 2014).



Figure 2.1: Photograph of *Ziziphusmauritiana*leaves and fruits (Saalu, 2016)

2.2.2 Scientific classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Roasles

Family : Rhamnacae

Genus :*Ziziphus*

Species : *mauritiana*

Binomial name: *Ziziphusmauritiana*.

(Surendra*et al*., 2012)

2.2.3 Phytochemical constituents of *Ziziphusmauritiana*

Ziziphusmauritiana plant contains flavonoids, alkaloids, cardiac glycosides, saponins, resins, polyphenols, mucilage and vitamins. The leaves of plants of this genus contain betulic and ceanothic acids, various flavonoids, saponins, erols, and triterpenes (Ali and Hammed, 2006: Glombitza*et al*., 1994). The plant has been studied by (Ikram*et al*., 1976; Aynehchi and Mahoodian 1973) and its chemical composition is well-known (Younes*et al*., 1996; Mahran*etal*., 1996). The main constituents of the essential oil were alpha-terpineol (16.4%) and linalool (11.5%). The main neutral hydrocarbons were n-pentacosane forms (81%). Methyl esters isolated from leaves included methyl palmitate, methyl stearate and methyl myristate. beta-Sitosterol, oleanolic acid and maslinic acid were the main aglycones of the glycosides present in leaves. Sugars present in leaves included lactose, glucose, galactose, arabinose, xylose and rhamnose. The plant also contains four saponin glycosides (Mahran*et al*., 1996). The highest flavonoid content was found in the leaves (0.66%). The fruits are good source of vitamin C, sugars and contain various minerals. The pulp contains moisture, protein, fat, carbohydrates, calcium, phosphorous, iron, carotene, thiamine, riboflavin and

fluoride (WOI, 2004). Fruits also contain tannins, flavonoids, saponins, mucilage, reducing suger, organic acids (ascorbic, tartaric acid and citric acid). *Ziziphus* species has revealed the presence of polysaccharides, pectin composed of D-galacturonic acid, L-rhamnose, D-galacturonic acid as methyl ester and O-acetyl groups, cyclopeptides, peptide alkaloids, flavonoids, dodecaacetylprodelphinidin B3, Ziziphine N, O, P and Q, saponins and fatty acids (Jarald*et al*., 2009; Bhatia and Tulica, 2010; Rahman, 2012). Bark contains tanins, leukocynidine, leucopalargonidine, betulinic acid, ziziphinic acid, resin, zizogenin, and alkaloids. The petroleum ether extract of the *Ziziphusmauritiana* bark shows the presence of glycosides and sterols and methanolic extract of bark shows the presence of alkaloids, flavonoids, glycosides, phenols, Lignin, Sterols, saponins (Jain *et al*., 2012).

2.2.4 Medicinal uses of*Ziziphusmauritiana*

Traditionally it has been used in so many diseases and cure of the body system, the fruits are applied on cuts and ulcers and are employed in pulmonary ailments and fevers, and in gastrointestinal disorders, the dried ripe fruit is a mild laxative (Perumal*et al*., 2012). The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. Mixed with oil, they are rubbed on rheumatic areas. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Rahman, 2012). *Ziziphusmauritiana*fruits have highly useful contents quantity that is useful for human health (Parmar*etal*.,2012).

In a study by Criss*et al*., (2000), the aqueous extract from the leaves of *Ziziphusmauritiana* was studied. It was observed a striking decrease of the hyperglycemic arrow ($p < 0.05$), with 300 mg/kg administrated 90 minutes before starting the test. The results obtained with a dose

of 300 mg/kg once or twice a day were identical as those with glibenclamide at 0.2 mg/kg per day. So, the antidiabetic activity of *Ziziphusmauritiana* was experimentally born out but it has to be standardized for common use.Hassan *et al*., (2010), also worked on the anticancer activity by using *Ziziphusmauritiana* leaves extract and gives the result that decrease in serum MCP-1 and IL10 activity as compared to control and on the other hand it shows that EAC bearing mice showed significant increase in MCP-1, VEGF and IL10 activity as compared to both control and *Ziziphus* extract administered mice. When the phytochemical screening was performed and the results has recorded as it possess highest antioxidant levels, especially betulinic acid which is a known anticancer agent. The phytochemical analysis also tells about the presence of tannins and saponins which are responsible for antitumor act.

Dahiruland Obidoa (2007), investigated the hepatoprotective activity of aqueous extract of *Ziziphusmauritiana* leaves by alcohol induced liver damage. 40% of alcohol solution is administrated orally in rats which can produce toxicity to the liver. In the experiment, rats that receive alcohol only showed elevated level of ALT, AST, bilirubin, and hepatic lipid peroxidation while reduced glutathione, total antioxidant status and body weight significantly decreased compared to control rats. Administration of *Ziziphusmauritiana* extract prior to alcohol ingestion significantly resulted in increased levels of reduced glutathione and total antioxidant status compared to the group that received alcohol only. Depression of ALT, AST, bilirubin and lipid peroxidation levels compared to the group exposed to alcohol only. According to the results we can say that *Ziziphusmauritiana* contain very good heapatoprotective property and can prevent the liver damage.

2.3 Standard Drug: Succimer (Meso-2,3-dimercaptosuccinic acid)

Meso-2,3-dimercaptosuccinic acid (DMSA), is a water soluble sulfhydrl containing compound which is an effective oral chelator of heavy metals (Aposhian, 1983). It is a dithiol and an analogue of dimercaprol a lipid-soluble compound also used for metal chelation. Oral dosing with DMSA creates a distinct advantage over dimercaprol, which has a small therapeutic index and must be administered in an oil solution via painful, deep intramuscular injection (Muckter*et al.*, 1997). On the other hand, DMSA has a large therapeutic window and is the least toxic of the dithiol compounds (Grazizno, 1986), and has been used since the 1950s as an antidote for lead poisoning in Russia, Japan, and the People's Republic of China (Muckter*et al*., 1997).

Studies in the past have shown DMSA to be a safe and effective chelator of lead, reducing blood levels significantly (Aposhian, 1983; Graziano, 1986). At a dose of 10mg/kg for five days in adult males, DMSA lowered blood lead levels 35.5% and at a more aggressive approach utilizing 30mg/kg lowered blood lead 72.5% with corresponding improvement in the clinical symptoms and biochemical indices of lead toxicity (Graziano, 1986).

An animal study indicated DMSA is an effective chelator of lead in soft tissue, but it may not chelate lead from bone (Cory-Sleeha*et al*., 2010). In a study of lead's pro-oxidant activity and effect of thiol substances as antioxidants, five weeks of lead exposure in mice depleted hepatic and brain glutathione (GS) levels, and increased malondialdehyde (MDA), a marker of lipid peroxidation. A subsequent DMSA administration for seven days resulted in a reduction in blood, liver and brain lead levels (Alan and Miller, 1998).

It has been observed that N-acetylcysteine (NAC) supplementation decreased MDA levels, indicating that it ameliorates oxidative stress but has no effect on lead levels (Alan and Miller, 1998). A suggested protocol for lead toxicity is to identify and remove the environmental exposure, and use DMSA 10mg/kg three times a day for the first five days,

followed by 14days at 10mg/kg twice a day (Alan and Miller, 1998), to alleviate symptoms including those arising from nerve cell damage.

2.4 Liver

The liver is the largest gland in the body and, after the skin, the largest single organ. It weighs approximately 1500 g and accounts for approximately 2.5% of adult body weight. In a mature fetus in which it also serves as a hematopoietic organ it is proportionately twice as large (5% of body weight). Except for fat, all nutrients absorbed from the gastrointestinal tract are initially conveyed first to the liver by the portal venous system (Moore, 2008). The liver is the most important organ which detoxifies or removes foreign substances or toxins, especially from the gut. It is made up parenchymal cells (Hepatocytes) and mainly four different types of nonparenchymal cells namely sinusoid lining endothelial cells, Kupffer cells (liver macrophages), stellate cells (fat storing cells) and pit cells (liver associated natural killer cells) (Blomhoff and Wake, 1991).

2.4.1 Morphology of the liver

Externally, the liver is divided into two topographical (anatomical) lobes and two accessory lobes by the reflections of peritoneum from its surface, the fissures formed in relation to those reflections, and the vessels serving the liver and gallbladder. The essentially midline plane defined by the attachment of the falciform ligament and the left sagittal fissure separates a large right lobe from a much smaller left lobe. On the slanted visceral surface, the right and left sagittal fissures surround and the transverse portahepatis demarcates two accessory lobes (parts of the anatomic right lobe): the quadrate lobe anteriorly and inferiorly and the caudate lobe posteriorly and superiorly (Moore, 2008).

2.4.2 Functional division of the liver

Although not distinctly demarcated internally, where the parenchyma appears continuous, the liver has functionally independent right and left livers (parts or portal lobes) that are much more equal in size than the anatomical lobes; however, the right liver is still somewhat larger(Moore, 2008). The liver can be further subdivided into four divisions and then into eight surgically resectable hepatic segments (Moore, 2008).

2.4.3 Histology of liver

The liver contains approximately, 100, 000 lobules that serve as the structural and functional unit of liver (Mader, 2001). In sections through the liver, the substance of the organs appears to be hexagonal areas with portal spaces at the periphery and a vein, called the central or centrolobular vein, in the centre. These portal spaces which are the regions located in the corners of the lobules, contain connective tissue, bile ducts, lymphatics, nerves, and blood vessels. Hence, each liver lobule is meshly networked with vessels. The basic structural component of the liver is the liver cell, or hepatocyte. The hepatocytes in the liver lobule are radially disposed and are arranged like the bricks of a wall. These cellular plates are directed from the periphery of the lobule to its center and anastomose freely, forming a labyrinthine and spongelike structure (Junqueira and Caneiro, 2005).The space between these plates contains capillaries, the liver sinusoids. The sinusoids contain macrophages known as Kupffer cells, these cells are found on the luminal surface of the endothelial cells, within the sinusoids (Junqueira and Caneiro, 2005). In human liver, the connective tissue is scanty and the lobules often appear to merge with another. In transverse sections each lobule appears to be made up of cords of liver cells that are separated by sinusoids (Singh, 2006).

Portal triad: This consists of three main structures. The largest is a terminal branch of the hepatic portal vein (Terminal portal venule). It has a very dense wall lined by flattened endothelial cells. The hepatic artery is smaller in diameter; it is thick walled. Bile collecting

ducts which are of variable sizes and are lined by simple cuboidal or columnar epithelium, these comprise the canal herrings. Short channels drain the canaliculli into bile ductulesuaually located at the periphery of the tract which merge to form larger, more centrally located trabecular ducts into the right and left hepatic ducts and hence to the duodenum via the common bile duct. Lymphatics are also present in the portal tract but since their walls are delicate and often less seen (Burkitt*et al*., 2000).

Surrounding the portal tracts are anastomosing plates of hepatocytes. Between them are hepatic sinusoids receiving blood from both hepatic portal vein and hepatic arterial systems(Burkitt*et al*., 2000). The layer of the hepatocytes immediately bordering the portal tracts is known as the limiting plate. A network of bile canaliculi is located within each plate of hepatocytes (Burkitt*et al*., 2000).

Liver lobule: In the human liver lobule, the irregular hexagonal boundary of lobule is defined by portal triad and sparse collagenous tissue; sinusoids originate at the lobule margin and course between plates or hepatic venule(Burkitt*et al*., 2000). The plates of hepatocytes are usually one cell thick and each hepatocyte is exposed to blood on at least two sides. The plates of hepatocytes branch and anastomose to form a three dimensional structure like a sponge (Burkitt*et al*., 2000).

Liver parenchyma: The hepatocytes here form anastomosing plates usually one cell thick between which blood passes sluggishly towards the terminal hepatic venule. The sinusoids are lined by a discontinuous layer of cells which do not rest on a basement membrane and which are separated from hepatocytes by a narrow space (Disse Space) this drains into the lymphatics of the portal tracts (Burkitt*et al*., 2000).

Hepatocytes: these are largely polyhedral cells which have large round nuclei with peripherally dispersed chromatic and prominent nucleoli. The nuclei vary greatly in size,

reflecting an unusual feature, occasional binucleated cell are usually seen although up to 25% of hepatocytes are actually binucleated (Burkitt*et al*., 2000).

Sinusoid lining cells: The sinusoid lining cells include at least three types. The majority are endothelial cells with black darkly stained nuclei and thin fenestrated cytoplasm. Scattered among the endothelial cells are large plump phagocytic cell with ovoid nucleus known as kupffer cells. The cells form part of the monocyte microphage defense system. Along with spleen, they participate in the removal of spent erythrocytes and other particulate debris from the circulation. Occasional fibroblasts with the capacity to store lipid are found in the Disse space between the sinusoid lining cells and the hepatocytes (Burkitt*et al*., 2000).

Bile canaliculum: The canaliculi have no discrete structure of their own but consist merely of fine channels formed by the plasma membrane of adjacent hepatocyte plate which merge to form canals of herring before draining into the bile ductules of the portal triads. Within each hepatocyte plate the canaliculi form a regular hexagonal network, each hexagon enclosing a single hepatocyte (Burkitt*et al*., 2000).

Hepatic vasculature and biliary system: The hepatic portal vein and hepatic artery branch repeatedly within the liver, their terminal branches running within the portal tracts. Blood from both systems then circulate between the branching plates of hepatocytes in the sinusoids, which converge to drain into a terminal hepatic (centrilobular) venule. These drain to intercalated veins and hence to the hepatic vein which drains into the inferior vena cava (Burkitt*et al*., 2000).

Bile is secreted into a network of minute bile canaliculi situated between the plasma membranes of adjacent hepatocytes. The canaliculi network then drains into a system of bile collecting ducts located in the portal tracts but coursing in the opposite direction to the two blood supply system. The bile collecting system then drains to the common bile duct and ultimately to the duodenum (Burkitt*et al*., 2000).

2.4.4 Blood Supply to liver

The liver is a highly vascular organ and at rest receives up to 25% of total cardiac output, more than any other organ. Its dual blood supply is uniquely divided between the hepatic artery, which contributes 25% to 30% of the blood supply, and the portal vein, which is responsible for the remaining 70% to 75%. The arterial and portal blood ultimately mixes within the hepatic sinusoids before draining into the systemic circulation via the hepatic venous system (Blungart and Belghiti, 2007).

2.4.5 Liver function test

The function of liver has been evaluated based on various parameters. These parameters are categorized as follows (Sumanth, 2007):

Non-invasive functional methods:

Ascorbic acid content in urine- It is reported as a non-invasive test for screening hepatoprotective drugs against CCl4-induced hepatotoxicity in rats (Prakash*et al*., 2008).

Pentobarbitone induced sleeping time- Pentobarbitone is metabolized by liver. When liver architecture is disturbed, the pentobarbitone does not get metabolized and sleeping time increases in mice, and rats (Janbaz*et al*., 2004).

Bromosulphthaline clearance test- Liver clears bromosulphthalein (BSP) dye from the blood. The abnormal function of liver increases the retention of BSP in blood (Woodman and Evants, 1996).

Biochemical analysis of blood plasma and serum (Reichling and Kaplan, 1988, Sallie *et al*., 1991)

Aspartate transaminase (AST) is a mitochondrial enzyme present in the tissues of liver, kidney, heart, and skeletal muscles. It reversibly catalyses transfer of amino group from aspartate to α-ketoglutarate. Its normal serum level is 5-40 IU/L.

Alkaline transaminase (ALT) is a cytosolic enzyme primarily present in the liver and reversibly catalyses amino group transfer from alanine to α-ketoglutarate. Its normal serum level is 7-56 IU/L in human.

Alkaline phosphatase (ALP) Serum alkaline phosphatase is produced in many tissues, especially in bone, liver, intestine, and placenta. It is excreted in the bile. In the absence of pregnancy and bone disease, an elevated serum alkaline phosphatase level mostly reflects hepatobiliary disease. The elevation ALP levels in hepatic disorders may be due to abnormal hepatic excretion or increased production by hepatic parenchymal cells. Its normal value in adult is 30-120 IU/L. Principle involved in estimation of alkaline phosphatase:ALP hydrolyses substrate P-nitrophenyl phosphate with the formation of P-nitrophenol and liberation of phosphate ion (Kind and King, 1954).

Serum Bilirubin- Bilirubin is a metabolic product of heme (porphyrin ring). Normal value of conjugated bilirubin is 0.25 mg/dl in the blood of an adult. Its level rises in diseases of hepatocytes, obstruction to biliary excretion, increase in hemolysis and defects of hepatic uptake.

Serum Protein- Liver cells is the main site for synthesis of plasma proteins namely albumin, fibrinogen, prothrombin, alpha-L-antitrypsin, transferrin, alpha foetoproteins, acute phase reactant proteins etc. The blood levels of these plasma proteins are decreased in extensive liver damage (Harsh, 2000).

Aspartate transaminase (AST or SGOT) and alanine transaminase (ALT or SGPT) are sensitive markers of hepatocellular injury. Injury to liver cells results in leakage of these enzymes into the circulation and serum levels of these enzymes increases.

2.4.6 Antioxidant levels in liver

Our body is protected by various endogenous antioxidants, which acts and scavenges free radicals generated in various biological processes. These free radicals include lipid peroxides,

reactive oxygen species (ROS), reactive nitrogen species (RNS). These free radicals are generated by the biochemical process inside mitochondria and cytosols. In certain condition like in toxicities of xenobiotics or in disease state, the formation of free radicals increases and starts damaging cellular and cytosolic membranes.

Glutathione (GSH): is a tripeptide of glycine, glutamic acid, and cysteine is a naturally occurring antioxidant. It neutralizes free radicals and ROS, and maintains exogenous antioxidants such as vitamins C and E reduced (active) forms. It is a substrate in both conjugation reactions and reduction reactionsthat are catalyze by glutathione S-transferase enzymes in cytosol, microsomes, and mitochondria. It is also capable of non-enzymatic conjugation with some chemicals like paracetamol. Glutathione prevents oxidative stress in most cells by trapping free radicals that can damage DNA and RNA. Therefore, its level is critically importance in tissue injury caused by toxic substances.

Lipid peroxidation: is one of the most widely used indicators of free radical formation. Oxidative deterioration of lipids such as various unsaturated fatty acids, cholesterol, phospholipids, glycolipids of membrane, results in formation of lipid hydroperoxides. These are formed in enzymatic or non-enzymatic reactions involving free radical. Lipid peroxides are unstable and decompose to form a complex series of compounds, like reactive carbonyl compounds, such as malondialdehyde (MDA) and 4-hydroxynonenal. MDA is a key marker for estimation of lipid peroxidation.

Catalase: is catalyses hydrogen peroxide formed by superoxide dismutase and other processes into water and molecular oxygen.

Superoxide dismutase (SOD): is a chief cellular defense enzyme present in most of the cell, which dismutates superoxide radical to hydrogen peroxide and oxygen. Dismutation is a reaction in which a single reactant, converted into two different products.

2.4.7 Liver disorders

Jaundice, hepatitis and cirrhosis are three life-threatening diseases that affect the entire liver and hinder its ability to repair itself (Mader, 2001). Hepatic failure occurs when a large number of hepatocytes are destroyed; this is due to accumulation of waste products in blood (due to lack of detoxification by the liver) ultimately leads to unconsciousness (hepatic coma) and death (Singh, 2006). Hepatomegaly, metastatic carcinoma and portal hypertension are also serious liver disorders (Moore, 2008).

2.5 Blood

The composition of the blood is a reflection of the state of health, nutrition and metabolism of the body (Toporek, 1975). Blood is the circulating connective tissue of the body. The fluid and its suspended formed elements are circulated through the heart, arteries, capillaries and veins. It is the means by which oxygen and nutritive materials are transported to the tissues and cabondioxide and various metabolic products are removed for excretion, It consists of a pale yellow or straw yellow fluid, blood cells – leukocytes, erythrocytes and thrombocytes (Ganong, 2005).

The cellular elements of the blood – white blood cells, red blood cells and platelets – are suspended in the plasma. The normal total circulating blood volume is about 8% of the body weight (5600ml in a 70kg man). About 55% of this volume is plasma (Ganong, 2005).

2.5.1 Functions of blood

Blood plays vital important functions which include:

Nutritive function

Respiratory function

Excretory function

Transport of hormones and enzymes

Regulation of water balance

Regulation of acid base balance

Regulation of body temperature

Storage function

Defensive function (Sembulingam, 2006).

2.5.2 Hematological indices

These are simply the normal values of blood in which deviations indicate anomaly and/or disease state (Ganong, 2005). These indices are used to monitor the progress of treatment. They include the Packed cell volume (PCV) or Hematocrit, Red blood cell count (RBC count), Total and Differential white blood cell count, Erythrocyte Sedimentation Rate (ESR), hemoglobin content though further information concerning the red blood cells can be deduced from the above parameters which include: Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Colour Index (CI) (Ganong, 2005).

2.6 Oxidative Stress

Oxidative stress is a general term used to describe a serious imbalance between ROS production and the levels of antioxidant defenses that cause oxidative damage in a cell, tissue or organ (Sies, 1991) and in the cytoplasm via the xanthine oxidase and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase (NOX) pathways (Gao*et al*., 2003). In addition, oxidative stress may result from exposure to a variety of agents present in the environment. External sources include radiation, UV light, chemical reagents, pollution, cigarette smoke, drugs of abuse, and ethanol (Zadak*et al*., 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Materials used in the study included the followings; experimental animals, lead acetate, fresh leaves of *Ziziphusmauritiana*, digital weighing balance, cages, water bottles, dissecting set, orogastric tubes, beakers, syringes, plastic pipette, glass slides, normal saline, etc.

3.1.1 Experimental rats

Forty (40) adult male Wistar rats were obtained from Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria-Nigeria. The rats were acclimatized to experimental condition for a period of two weeks and feeding with rat chow and water was allowed *ad libitum.*

3.1.2 Source of lead acetate

Laboratory grade of Lead acetate of 99% to 103% purity manufactured by BDH Chemical Ltd England was purchased from Cardinal Scientific, No.11/12 Sokoto Road, Opposite Longman Nig. Plc. Samaru-Zaria. The chemical was taken to the Department of Chemistry, Ahmadu Bello University Zaria for identification.

3.1.3 Source and identification of *Ziziphusmauritiana* leaves

Fresh leaves of *Ziziphusmauritiana* were obtained from a private farmland in Government Reservered Area Zaria, Zaria local Government, Kaduna state of Nigeria. The leaves were identified and authenticated with a voucher number of 3253 by MallamNamadiSanusi at Department of Botany, Ahmadu Bello University, Zaria.

3.1.4 Preparation of extract

Extraction of plant (jujube leaf) was done in the Department of Pharmacognosy and drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Aqueous extraction of *Ziziphusmauritiana* leaves: Aqueous extraction of jujube leaf was prepared according to the procedure of Ofodile*et al*., (2013). In this procedure, fresh leaves of jujube were collected, washed thoroughly, shade-dried and pulverized using a mechanical grinder. The powder obtained was macerated with distilled water for 72 hours at 30±4°C. The mixture was filtered and the filtrate was concentrated in water bath at 50°C. Finally, the concentrated crude aqueous extract was subjected to drying to solid mass. The testing samples were prepared by dissolving the solid mass with distilled water to obtain a final concentration used for the experiment.

Ethanol extraction of *Ziziphusmauritiana* leaves: The leaves samples were washed then air dried at room temperature and blended to a mesh size of 1mm. The blended samples (1kg) put in 4 liters of 70% ethanol for 48 hours filtered and concentrated to dryness using rotary evaporator. The ethanolic extract was kept in the refrigerator until usage (Japon-Lujan and Luque de Castro, 2006).

3.1.5Determination of LD_{50} of animals

Thirteen (13) wistar rats were used; they were kept in separate cages. Nine rats were divided into 3 groups for the first phase of the experiment, while for the second phase; the remaining 4 rats were used according to Lorke method (Appendix I).

3.1.6 Phytochemical screening

After the extraction, the extracts were filtered and were subjected to Preliminary phytochemicalscreening (Appendix II).

3.2 Experimental Procedure

3.2.1 Dosage determination

After establishing the LD_{50} of the leaves extracts of aqueous and ethanol*Ziziphusmauritiana* to be>5000mg/kg bw, (using Lorke's method) doses of 100mg/kg bwt and 400mg/kg bwt were used in this study for both aqueous and ethanol extracts.

Based on the reported oral LD_{50} of Lead acetate which was 600 mg/kg bwt for Wistar rats (Sujatha *et al*., 2011), 20% of the LD_{50} (120 mg of lead acetate/kg bwt) was used in this study. The dose of Succimer used was 10 mg/kg bwt according to Alan and Miller, (1998).

3.2.2 Experimental design

Forty (40) adult male Wistar rats were divided into 8 groups of 5 rats per group.

Group 1;(Control) received 1 ml of distilled water for 35 days.

Group 2; Administered with 120mg/kg bwt of lead acetate for 21 days and then the rats were sacrificed on day 22 of the administration to study the direct effect of lead acetate.

Group 3; Administered with 120 mg/kg bwt of lead acetate for 21 days and then administered with 100mg/kg bwtof aqueous extract of *Ziziphusmauritiana* leaves from day 22 to day 35 of the administration.

Group 4; Administered with 120 mg/kg bwt of lead acetate for 21 days and then treated with 400 mg/kg bwt of aqueous extract of *Ziziphusmauritiana* leaves from day 22 to day 35 of the administration.

Group 5; Administered with 120 mg/kg bwt of lead acetate for 21 days and then treated with 100 mg/kg bwt of ethanol extract of *Ziziphusmauritiana* leaves from day 22 to day 35 of the administration.

Group 6;Administered with 120 mg/kg bwt of lead acetate for 21 days and then treated with 400 mg/kg bwt of ethanol extract of *Ziziphusmauritiana* leaves from day 22 to day 35 of the administration.

Group 7;Administered with 120mg/kg bwt of lead acetate for 21 days and treated with 10 mg/kg bwt of Succimer as standard treatment drug from day 22 to day 35 of the administration. All the administrations were carried out orally once per day for 35 days.

Group 8; Administered with 120 mg/kg bwt of lead acetate for 21 days and then administered with distilled water from day 22 to day 35 of the administration. This was to

study if there will be any natural recovery that would take place after the administration of lead acetate when compared to Group 2.

All the administrations were carried out orally once per day.

Table 3.1: Grouping of animals and their treatment

Groups	Administration(dosage/kg/bwt)	Duration
1	Distilled water	**Day 1-35**
2	120mg lead acetate and then Sacrificed on day 22	**Day 1-21**
3	120mgleadacetate	**Day 1-21**
	+	
	100mg ZAE	**Day 22-35**
4	120mg lead accetate	**Day 1-21**
	+	
	400mg ZAE	**Day 22-35**
5	120mg lead acetate	**Day 1-21**
	+	
	100mg ZEE	**Day 22-35**
6	120mg lead acetate	**Day 1-21**
	+	
	400mg ZEE	**Day 22-35**
7	120mg lead accetate	**Day 1-21**
	+	
	10mg succimer	**Day 22-35**
8	120mg lead acetate	**Day 1-21**
	+	
	distilled water	**Day 22-35**

N=5; ZAE:aqueous extract of *Ziziphusmauritiana,***ZEE:** Ethanol extract of *Ziziphusmauritiana*.

3.2.3 Termination of experiment

After the last administration, the rats were fasted for 24 hours before they were humanely anaesthesized by using ketamineat a dose of 75mg/kg IP (PARP, 2013). Blood samples were collected via heart puncture from each rat and the blood was divided into two (2). One half was put in Tripotassium salt of Ethylenediamine tetra acetic acid (K3 EDTA) as anticoagulant and blood was used for haematological estimation while the other half in plain bottles, anticoagulant free blood, was allowed to coagulate and serum was harvested for biochemical estimation.

Incision was made through the abdomen and the abdomen was opened and the liver of the rats were removed and fixed in 10% formalin solution. The tissues were taken to the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria for tissue processing.

3.3 Tissue Processing

The tissues were fixed in 10% formalin for 48 hours for proper fixation. The tissues were processed, sectioned at 5μm thick, stained using Haematoxylin and Eosin (H and E) technique and special stain using Gordon and Sweets for demonstration of Reticular fibres and Periodic Acid Schiff (PAS) for glycogen.

3.3.1 Haematoxylin and Eosin (H and E) staining method

The methods of H and E staining were carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90% and 70%) for three minutes each, then stained in Harris haematoxylin for ten minutes, and washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott‟s tap water for five minutes and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then

dehydrated in ascending grades of alcohol and cleared in xylene. The sections were then cover-sliped using a media (Freida, 2007). Sections of the tissues were viewed under light microscope and photomicrographs were made using digital Am scope (MD 900) microscope.

3.3.2 Special stain using Gordon and Sweet

Frozen sections were deparaffinized and hydrated to distilled water. Sections were oxidized in 1% potassium permanganate solution for 5 minutes, and rinsed in water for 2 minutes. Sections were bleached in 1% oxalic acid for 2 minutes. Sections were then sensitized in 2.5% ferric ammonium sulfate for 15 minutes and washed in several changes of distilled water. Sections were impregnated with silver solution for 2 minutes. Sections were rinsed well with distilled water and then reduced in 10% formalin solution for 2 minutes. Sections were then washed in tap water for 3 minutes and tone in 0.2% gold chloride solution for 10 minutes. Sections were washed in tap water for 2 minutes and slides placed in 5% sodium \kjbn 9---pthiosulfate for 1 minute. The sections were rinsed in tap water and then dehydrated in ascending grades of alcohol and cleared in xylene and mounted with a synthetic resin. Sections of the tissues were viewed under light microscope and photomicrographs were made using digital Amscope (MD900) microscope.

3.3.3 Special stain using Periodic Acid Schiff (PAS)

Frozen sections were deparaffinized and hydrated to distilled water. Slides were placed into 0.5% periodic acid for 5 minutes. Sections were rinsed in distilled water and covered with Schiff's reagent for 5-15 minutes and washed in running tap water for 5-10 minutes. Sections were then counter stained in hematoxylin for 3 minutes. Sections were washed in tap water and blue hematoxylin. The sections were rinsed in tap water and then dehydrated in ascending grades of alcohol and cleared in xylene and mounted with a synthetic resin. Sections of the tissues were viewed under light microscope and photomicrographs were made using digital Amscope (MD900) microscope.

3.4 Determination of Lead Acetate Concentration

The concentration of lead acetate in the sample was analysed using the fast sequential atomic absorption spectrophotometer (Model AA240S, Varian technologies, USA) following the method modified by APHA (1999). The instrument's setting and conditions of operation was carried out in accordance with manufacturer's specifications by calibrating with analytical grade of metal standard stock solution. One (1g) of each liver sample was excised and homogenized in 5mls of normal saline. Acid digestion was done using 10mls of conc HNO3 at room temperature. This was heated to almost dryness and topped up to 50ml with distilled water. The digested samples were filtered to remove any insoluble materials that could clog the atomizer. The filtrate was then analysed for heavy metals content using the AAS.

3.5 Biochemical Assay

Anticoagulant free bottles were used to collect blood samples for the analysis of biochemical parameters such as Alkaline aminotransferases (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP), using the serum, by the method of Bergmeyer and Harder (1986): Alkaline phosphatase (ALP) activity was measured at 405nm using the method of Varley., *et al* (1980). Using Biomerieux kits (Randox laboratories ltd, Diamond road, Crumlin co, Antrium, United Kingdom BT294QY.

3.6 Determination of Haematological Parameters

The whole blood samples collected into anticoagulated (EDTA) specimen bottle were analysedwithin 6 hours of collection using systemex XE-2100 Automated Haematology Blood Analyser according to the method of Monica, (2000). Each blood sample was well mixed at room temperature using a blood rotor machine for five minutes. The authomatedanalyser was put on about 30 minutes for the system to warm. A 10 µl of blood sample was aspirated through tube of the machine. Haematological parameters such as White

blood cell (WBC) count, red blood cell (RBC) count, haemoglobin concentration (Hb) and Platelets count were analysed.

3.7 Determination of Oxidative Stress Parameters

3.7.1 Determination of catalase activity

Catalase activity was determined using the method described by Sinha(1972). In this procedure, 5 % Potassium heptaoxochromate (VI) $K_2Cr_2O_7$ was mixed with glacial acetic acid in the ratio 1:3, and stored in brown bottle at room temperature, after which 0.9 ml of distilled water was added to 0.1 ml of sera and mixed thoroughly. A 2.5 ml of phosphate buffer was put in a small conical flask; 0.5 ml of sera was added; and 2.0 ml of H_2O_2 added as well, starting the stop watch. The reaction mixture was thoroughly mixed and the reaction was stopped after every 60 seconds for 3 minutes with Dichromate/Acetic acid solution. This was heated in water bath for 10 minutes at 80°C. Absorbance was read at 570 nm. Standard curve was obtained using absorbance obtained at various H_2O_2.

Calculations: the quantity of H_2O_2 consumed was obtained from the graph of the catalase standard curve, this determined the catalase activity.

3.7.2 Determination of Superoxide dismutase activity

Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1989). Based on this method, 0.1 ml of sera was diluted in 0.9 ml of distilled water to make 1:10 dilution of sera. An aliquant mixture of 0.20 ml of the diluted sera was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction started with the addition of 0.3m1 of 0.3 mM Adrenaline. The reference mixture contains 2.5 ml of 0.05 M Carbonate buffer. A 0.3 m1 of 0.3 mM Adrenaline and 0.20 ml of distilled water were used.

Absorbance was measured over 30 s up to 150 s at 480 nm.

Calculations: Increase in absorbance per minute = (A5 - A1)/2.5

% inhibition = 100 - Increase in absorbance for substrate x 100 Increase in absorbance of blank 1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute

3.7.3 Assessment of lipid peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968). In this method, to 150 µl of sera, (O.25M sucrose solution) were treated with 2m1 of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and place in water bath for l hr at 90°C. The mixture was cooled and centrifuged at 3000 rpm for 5 mm at 4°C. The absorbance of the pink supernatant 2.0 ml was measured against a reference blank using spectrophotometer at 535 nm.

Calculations: The MDA was calculated using the molar extinction coefficient of 1.56 x 10 cmM MDA conc. = absorbance/ 1.56 x105cm-1M-1 x l

3.7.4 Assay of reduced Glutathione concentration

Reduced glutathione (GSH) concentration measurements were done according to the method of Ellman, (1959). In this procedure, 0.2M phosphate buffer: 8.40 g of NaH2PO4 and 9.94 of Na2HPO4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask; the buffer was then adjusted to pH 8.

To 150 µl of serum (in phosphate -saline PH 7.4), 1.5 m1 of 10% TCA was added and centrifuged at 1 500 g for 5mm. A 1 ml of supernatant was treated with 0.5 ml of Ellman‟s reagent (19.8 mg of 5, 5‟-dithiobis (nitro benzoic acid) (DNTB) in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, p1-I 8). The absorbance was read at 412 nm.

3.8 Data Analysis

Data obtained were expressed as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to compare the mean differences followed by Tukey’s post-hoc

test. P-value less than to 0.05 was considered to be statistically significant. All the results were analyzed using the Statistical Package for Social Sciences (SPSS version 16).

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening

Percentage yield for ethanol extraction of *Ziziphus mauritiana*was 9.42% (94.21g) and for aqueous extraction was 10.39% (83.14g).Test form in appendix 1.

Table 4.1: Phytochemical Screening of ethanol and aqueous extract of *Ziziphus mauritiana*

S/NO	CONSTITUENTS & TESTS	AQUEOUS	ETHANOL
1	Carbohydrates- Molisch Test	+	+
2	Anthraquinone – Bontragers Test	-	-
3	Cardiac Glycosides- Kella-Killani Test	+	+
4	Glycosides- Fehling Test	+	+
5	Saponin – Froth Test	+	+
6	Steroid – Liberman Buchard Test	-	+
7	Triterpene – Liberman Buchard Test	+	+
8	Tannins – Ferric chloride Test	+	+
9	Flavonoids – Shinoda Test	+	+
10	Alkaloids – Dragendorff Test	+	+

+ = Present, - = absent

Phytochemical screeing revealed the presence of the following phytochemical constituents;

Aqueous Ziziphus mauritiana leaves extract-Carbohydrates, Cardiac Glycosides, Saponin, Tripterpene, Flavonoids, Tannins, and Alkaloids.

Ethanol Ziziphus mauritiana leaves extract-Carbohydrates, Cardiac Glycosides, Saponin, Steriod, Triterpene,Flavonoids, Tannins, and Alkaloids.

4.1 Change in Body Weight

Fig. 4.1 Showed that weight was affected by the Lead Acetate treatment as the weight gain was significantly lower ($p < 0.05$) when compared to control group. However, treatment with extract at low and high doses resulted in significant weight gain higher than the Positive control ($p < 0.05$) and no significant change ($p < 0.05$) was observed between Aqueous and Ethanol extract at both high and low dose, but there was a significant difference between the high and low dose in both extract. Likewise, a significant difference was not observed between the recovery group and the Positive control group.

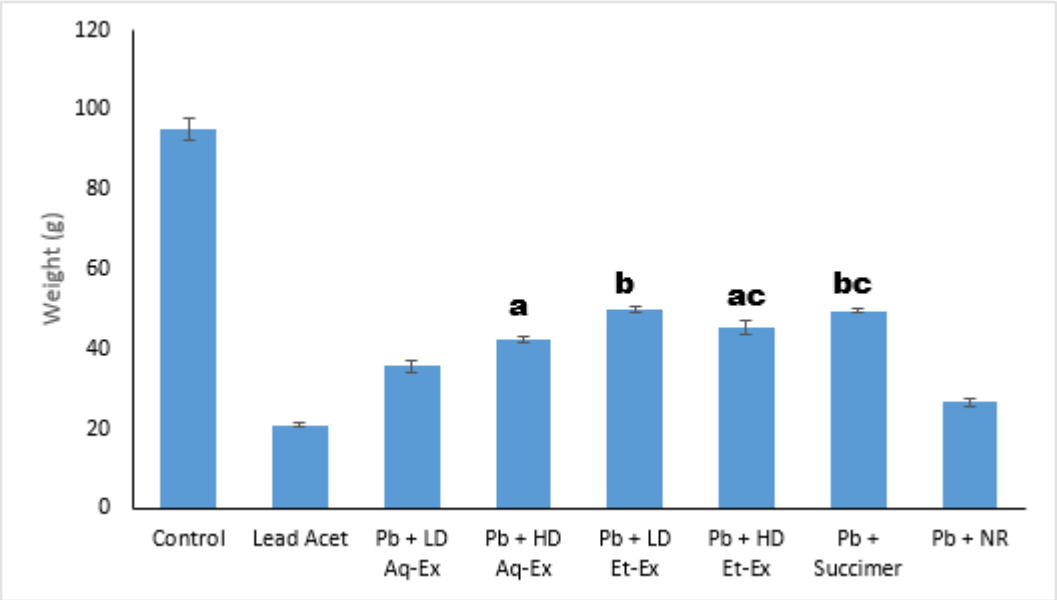


Figure 4.1: Change in body weight in Wistar rats following the administration of lead acetate and *Ziziphus* mauritiana extracts. Bars sharing the same alphabet are not significantly (p>0.05) different

4.2 Change in Organ-Body Weight Ratio

Figure4.2 A significant increased ($p < 0.05$) in organ body weight ratio was observed in the positive control group compare with both the negative group and the entire treatment group. A significant difference was not ($p < 0.05$) observed between each of the treatment group compared with the control group. However, the natural recovery group had an insignificant gain ($p > 0.05$) in organ body weight ratio compared with the entire treatment group and an insignificant ($p > 0.05$) decreased in organ body weight ratio between the recovery group and the positive control group.

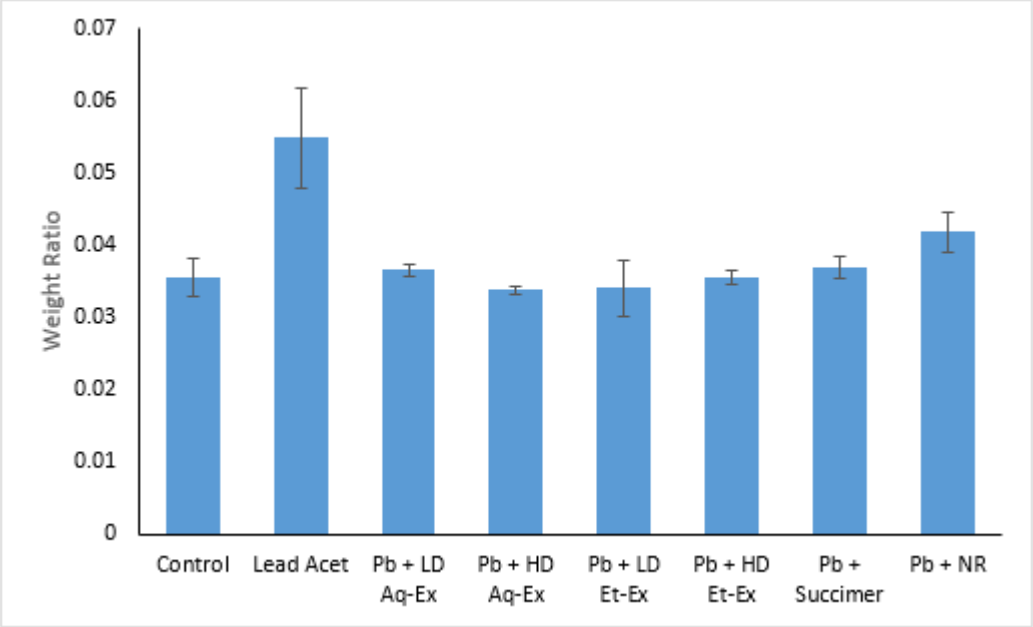


Figure 4.2: Change in organ-body weight ratio in Wistar rats following the administration of lead acetate and *Ziziphus mauritiana* extracts.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test..

4.3 Liver Lead Concentration in Wistar Rats

The result reveals that there is no significant different of lead concentration in Group 3 (0.74±0.03) and Group 5 (0.72±0.03) and Group 7 (0.72±0.06), Group 4 (0.59±0.03) and Group 6 (0.52±0.03), also in Group 5 and Group 7 (Fig 4.3).

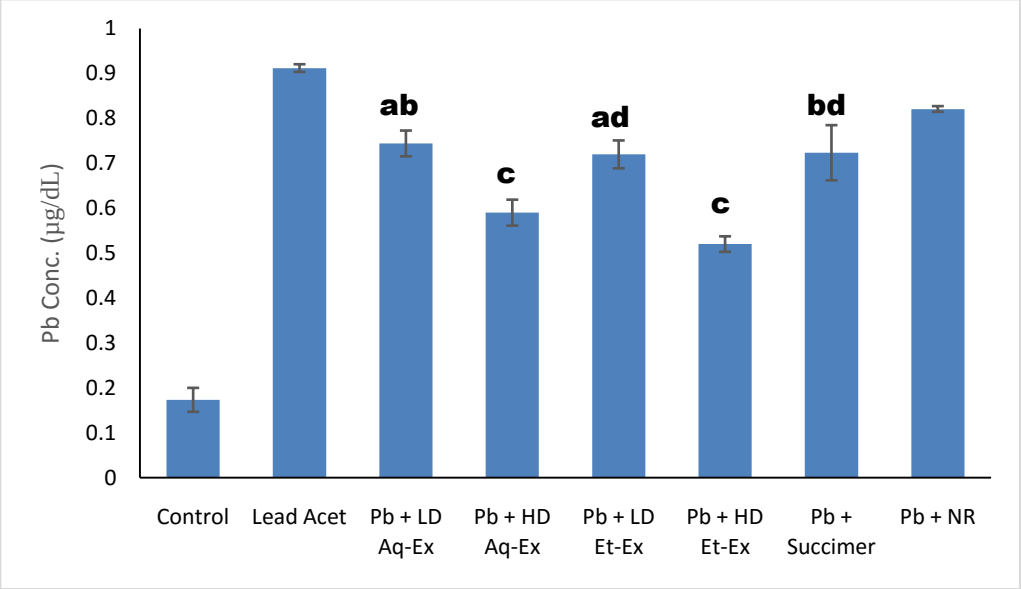


Figure 4.3: Lead Acetate Concentration in Wistar rats following the administration of lead acetate and *Ziziphus mauritiana* extracts.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same column with different superscripts a,b,c,d and e are significantly different ($p < 0.05$).

4.4 Oxidative Stress Marker

Figure 4.4, 4.6 and 4.7 showed a significant decreased ($p \leq 0.05$) in oxidative stress indicators (SOD, GSH and CAT) in the positive control group compared with the control group and the entire treatment group while a significant increased ($p \leq 0.05$) in oxidative stress indicator (MDA) (Figure 4.5) in the positive control group compared with the control group and the entire treatment group. There was therapeutic increased in oxidative stress indicator (SOD, GSH, and CAT) in the extract (Aqueous and Ethanol) treated group compared with the Positive control and Succimer treated group. The low dose of the extract and the natural recovery treated group shows no significant change in the oxidative stress indicator compare with the positive control group. The high dose of the extract proves to be therapeutic effective than the low dose of the extract.

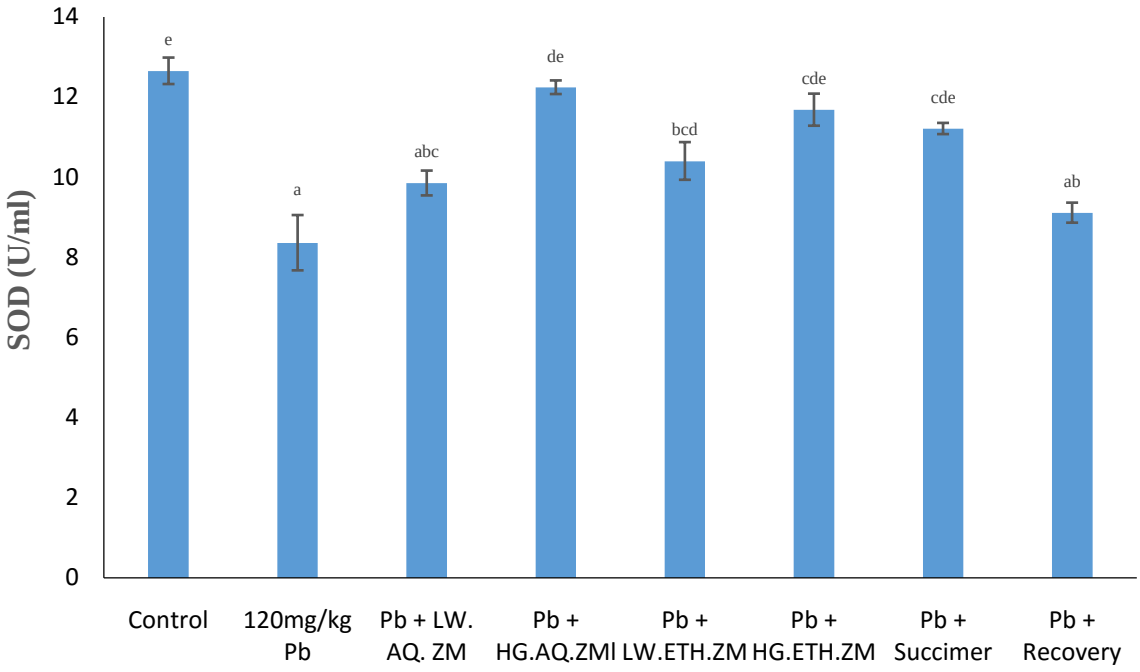


Figure 4.4: Serum levels of Superoxide Dismutase (SOD) of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same column with different superscripts a,b,c,d and e are significantly different ($p < 0.05$).

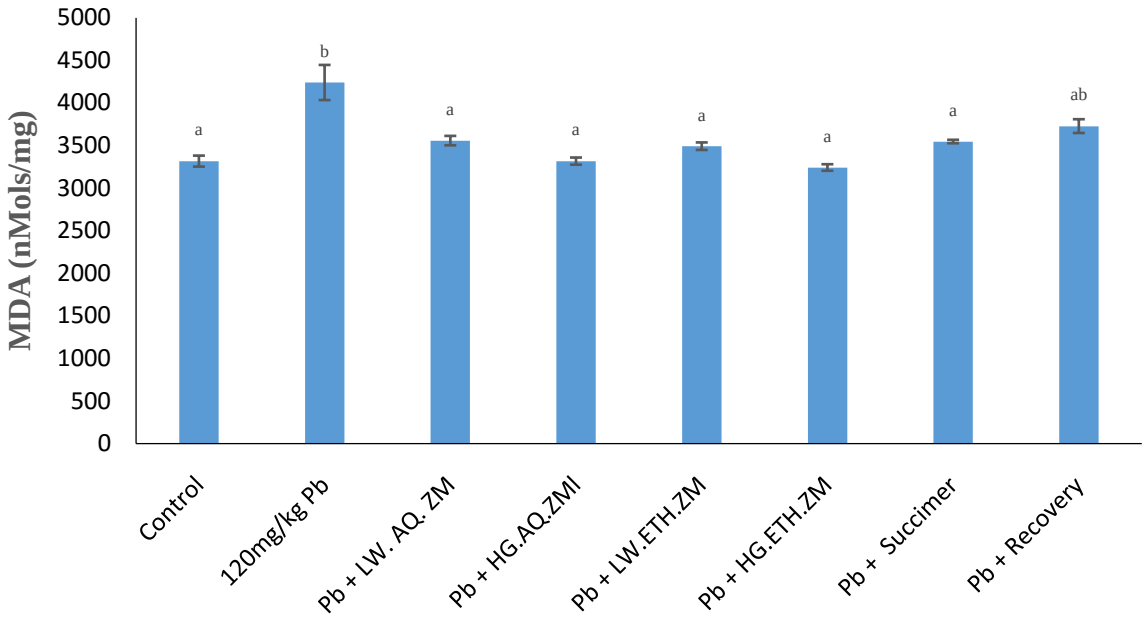


Figure 4.5: Serum levels of Malondialdehyde (MDA) of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same column with different superscripts [a] and [b] are significantly different ($p < 0.05$).

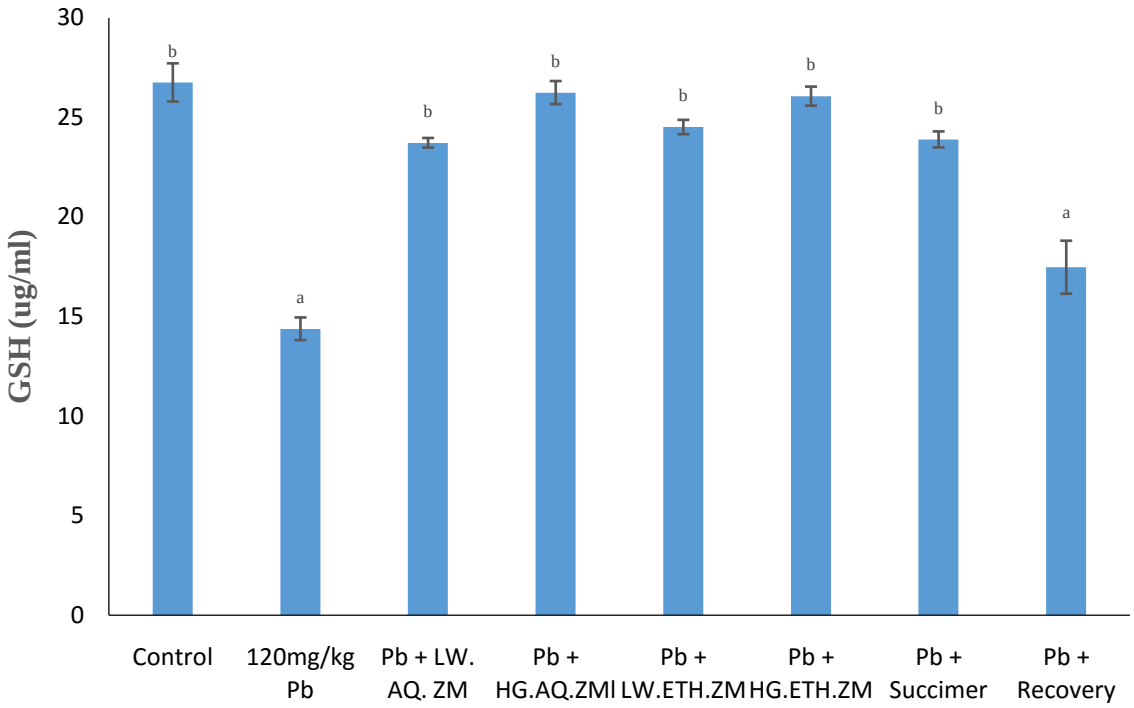


Figure 4.6: Serum levels of Reduced Glutathione (GSH) of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same column with different superscripts [a]and [b] are significantly different ($p < 0.05$).

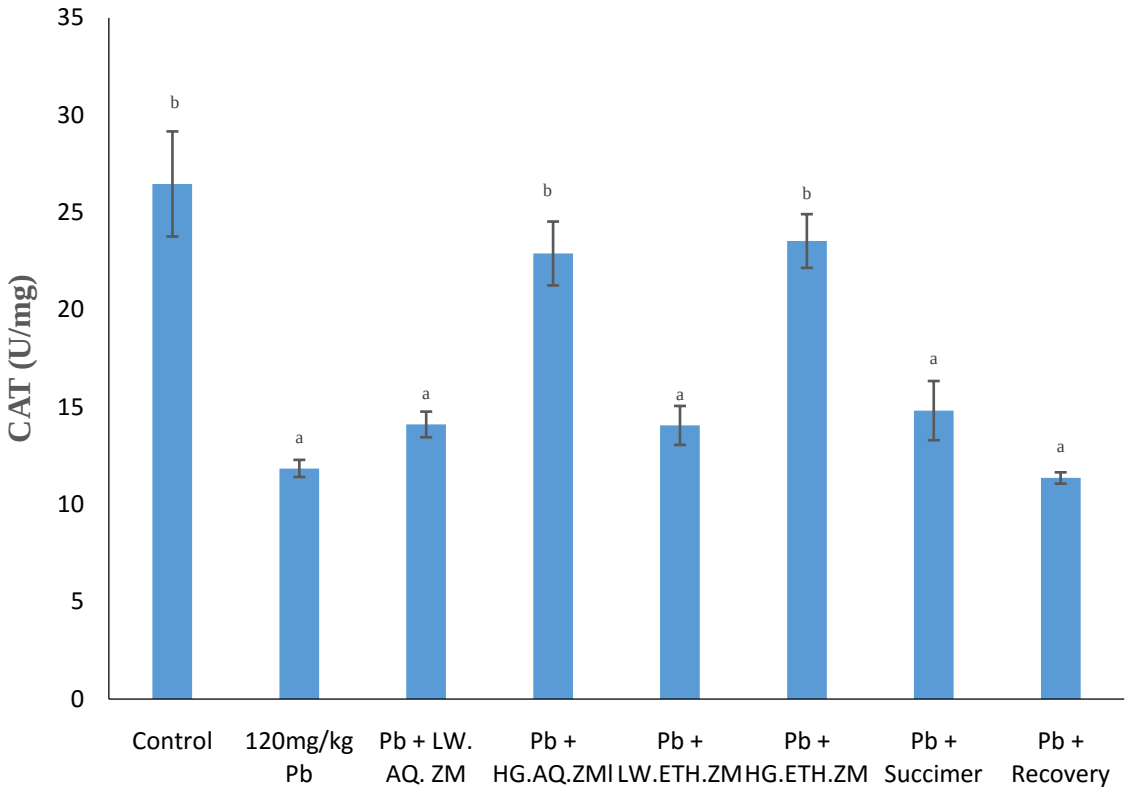


Figure 4.7: Serum levels of Catalase (CAT) of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by Tukey multiple comparison post hoc test. Values along the same column with different superscripts [a]and [b] are significantly different ($p < 0.05$).

4.5 Liver Enzymes activities

Figure 4.8, 4.9 and 4.10 showed a significant Increased ($p \leq 0.05$) in serum liver enzymes parameters (AST, ALT and ALP respectively) in the positive control group compared with the control group and the entire treatment group while There was therapeutic decreased in serum liver enzymes parameters (AST, ALT, and ALP) in the extract (Aqueous and Ethanol) treated group compared with the Positive control and lead treated group. The low dose of the extract and the natural recovery treated group shows no significant change in the oxidative serum liver enzymes parameters (AST, ALT and ALP) compare with the positive control group. The high dose of the extract proves to be therapeutic effective than the low dose of both the aqueous and the ethanol extract.

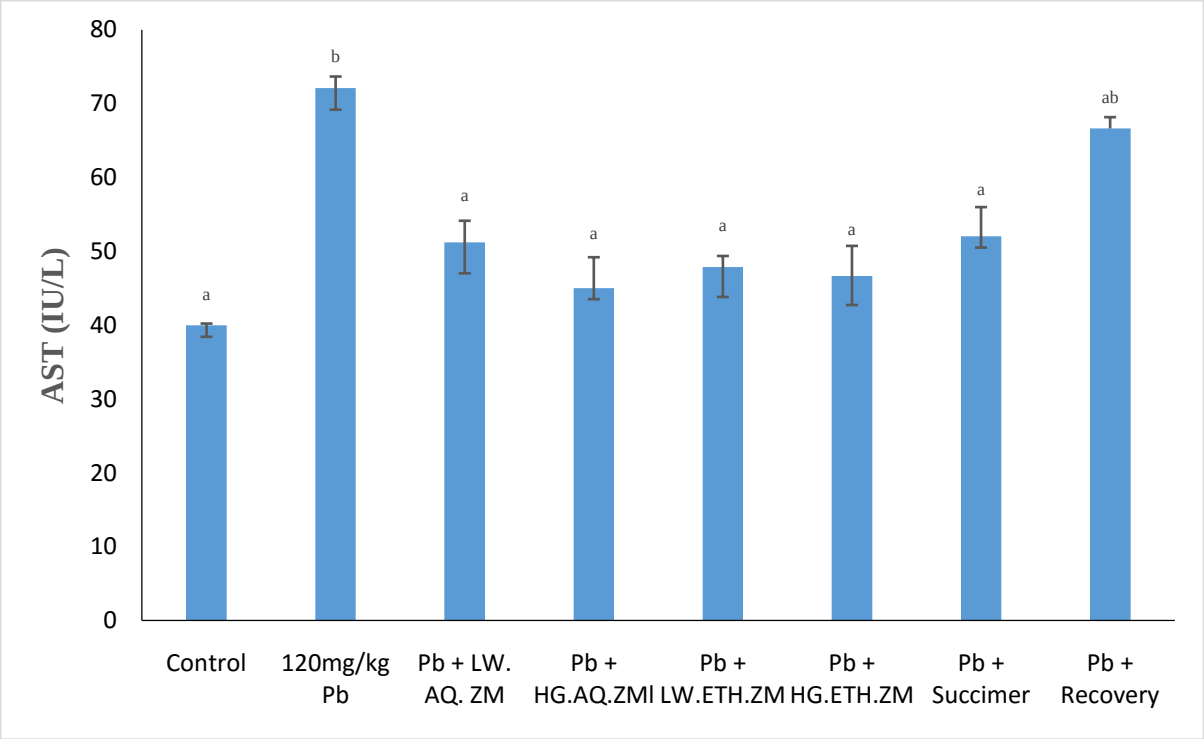


Figure 4.8: Serum levels of AST of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same column with different superscripts a,b,c,d and e are significantly different ($p < 0.05$).

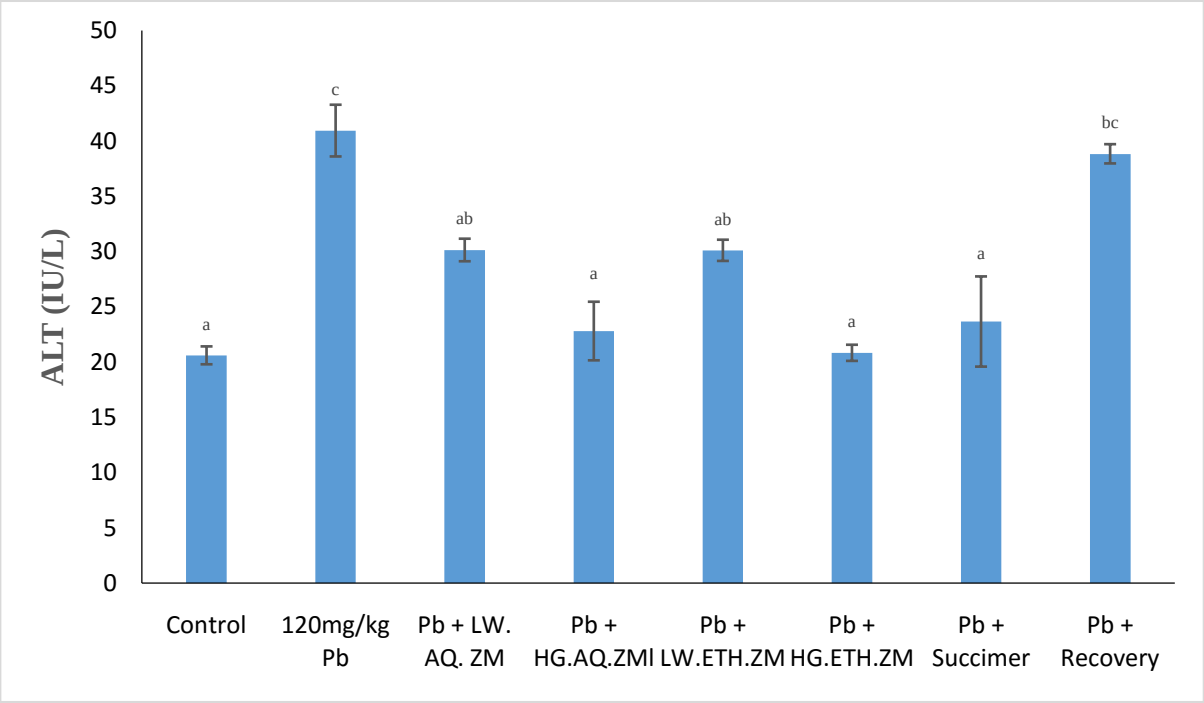


Figure 4.9: Serum levels of ALT of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values with different superscripts a, b and c are significantly different ($p < 0.05$).

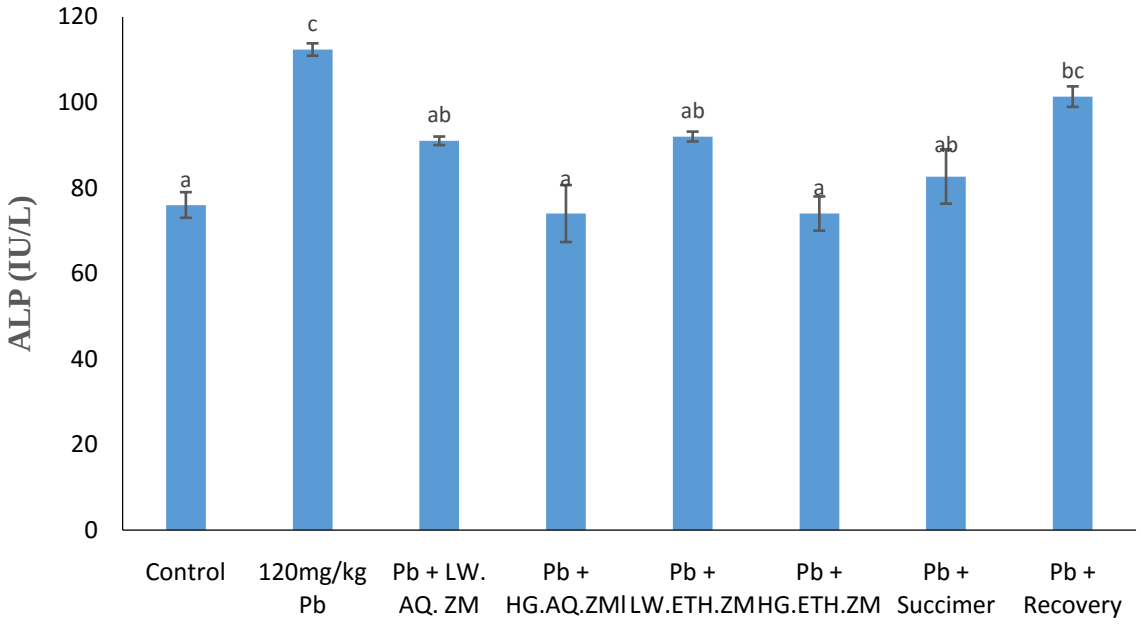


Figure 4.10: Serum levels of ALP of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer.

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values with different superscripts a, b and c are significantly different ($p < 0.05$).

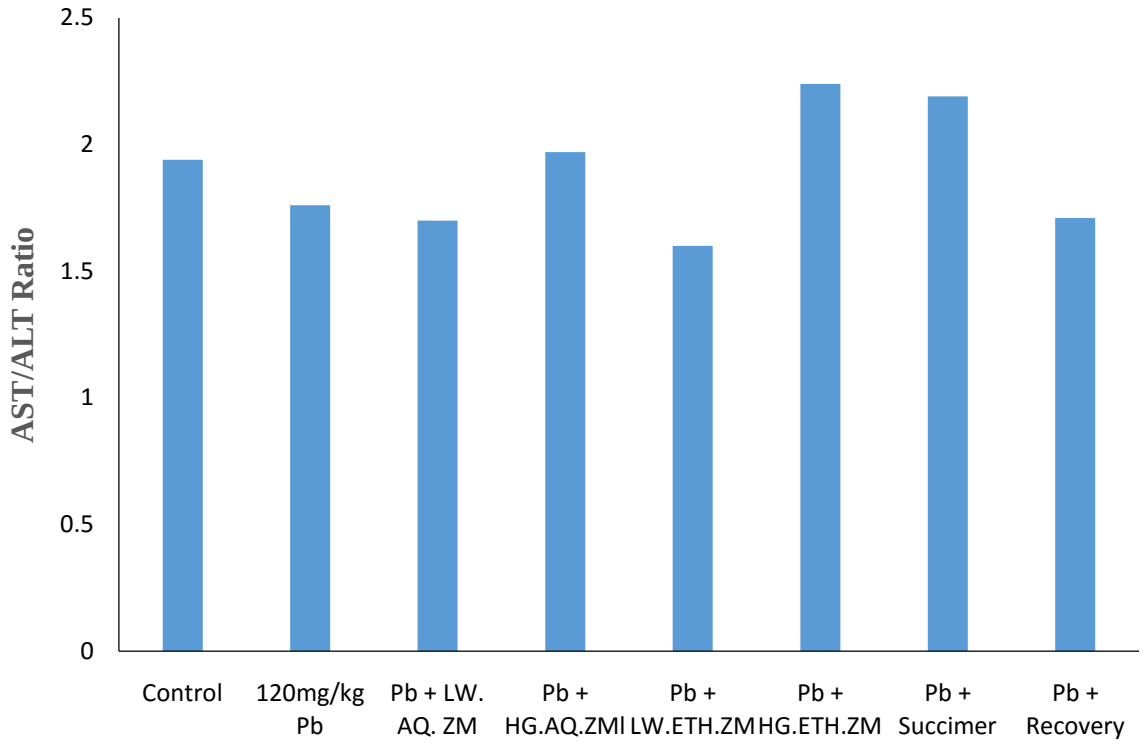


Figure 4.11: AST/ALT Ratio of Lead Acetate induced induced Liver toxicity on experimental rats treated with *Z. mauritiana* and Succimer

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values with different superscripts a, b and c are significantly different ($p < 0.05$).

4.4 Hematological Parameters

The result indicates that the WBC in Group 1 (4.07±0.47) was not significantly different from Group 3 (6.40±1.00), Group 4 (5.00±0.75), Group 5 (5.33±1.15) and Group 6 (4.07±0.32). RBC in Group 1 (8.08±0.32) was not significantly different from Group 4 (7.19±0.08) Group 6 (7.93±0.50) while the HGb in Group 3 (117.67±1.20) was not significantly different from Group 5 (120.00±0.58) and Group 7 (122.67±4.41) and Group 4 (132.67±1.45) was not significantly different from Group 6 (134.00±1.00).

Table 4.2: The Haematological Parameter (WBC, RBC, Hb, PLT and LYMP) of Lead Acetate induced Liver toxicity on experimental rats treated with*Z. mauritiana*and Succimer.

	WBC	RBC	HGB	PLT	LYMP
Control	4.07±0.47^{abcd}	8.08±0.32^{abc}	155.00±1.16	630.00±40.42	3.85±0.03
Lead Acet	12.87±1.14	3.66±0.40^{d}	100.00±2.89	598.33±76.29	8.63±1.30
Pb + LD Aq-Ex	6.40±1.00^{ah}	6.38±0.40	117.67±1.20^{ab}	581.33±55.64	5.33±2.38
Pb + HD Aq-Ex	5.00±0.75^{bg}	7.19±0.08^{a}	132.67±1.45^{c}	554.33±82.11	4.77±0.26
Pb + LD Et-Ex	5.33±1.15^{cf}	5.81±0.36	120.00±0.58^{a}	448.67±90.29	3.9±0.79
Pb + HD Et-Ex	4.07±0.32^{d}	7.93±0.50^{b}	134.00±1.00^{c}	662.67±30.63	2.63±0.18
Pb + Succimer	7.17±1.04^{e}	7.92±0.27^{c}	122.67±1.45^{b}	643.00±60.47	4.93±1.22
Pb + NR	7.58±1.40^{efgh}	3.75±0.34^{d}	108.33±4.41	447.00±50.23	4.15±0.14
F	8.708	26.496	63.776	1.704	2.594
P	<0.001	<0.001	<0.001	0.178	0.159

Values along the same column with different superscripts ^{a,b,c} and ^{d} are not significantly different ($p<0.05$).

4.7 Histopathology of the Liver

The histopathological findings obtained in this study showed that Lead Acetate was hepatotoxic as seen below in the liver section characterized by lose of cellular membrane and glycogen, due to fatty degeneration necrotic changes and a decreased in the distribution of recticular fibers (Plate II, X and XVII). Extract when administered therapeutically to rats attenuated the changes associated with Lead Acetate toxicity by reducing fatty generation, vacuolation of the hepatocytes and necrotic changes thereby restoring almost normal architecture of the liver. This was dose-dependent as treatment with High Dose showed better result than the Low dose of the Extract both aqueous and ethanol treated group as shown in the Plates of tissue slide below and the extract treatment group is more effective than the standard drugs treatment group. There was no therapeutic effectiveness in the liver tissue cytoarchtecture in all the stains in the natural recovery group compared with the lead acetate group.

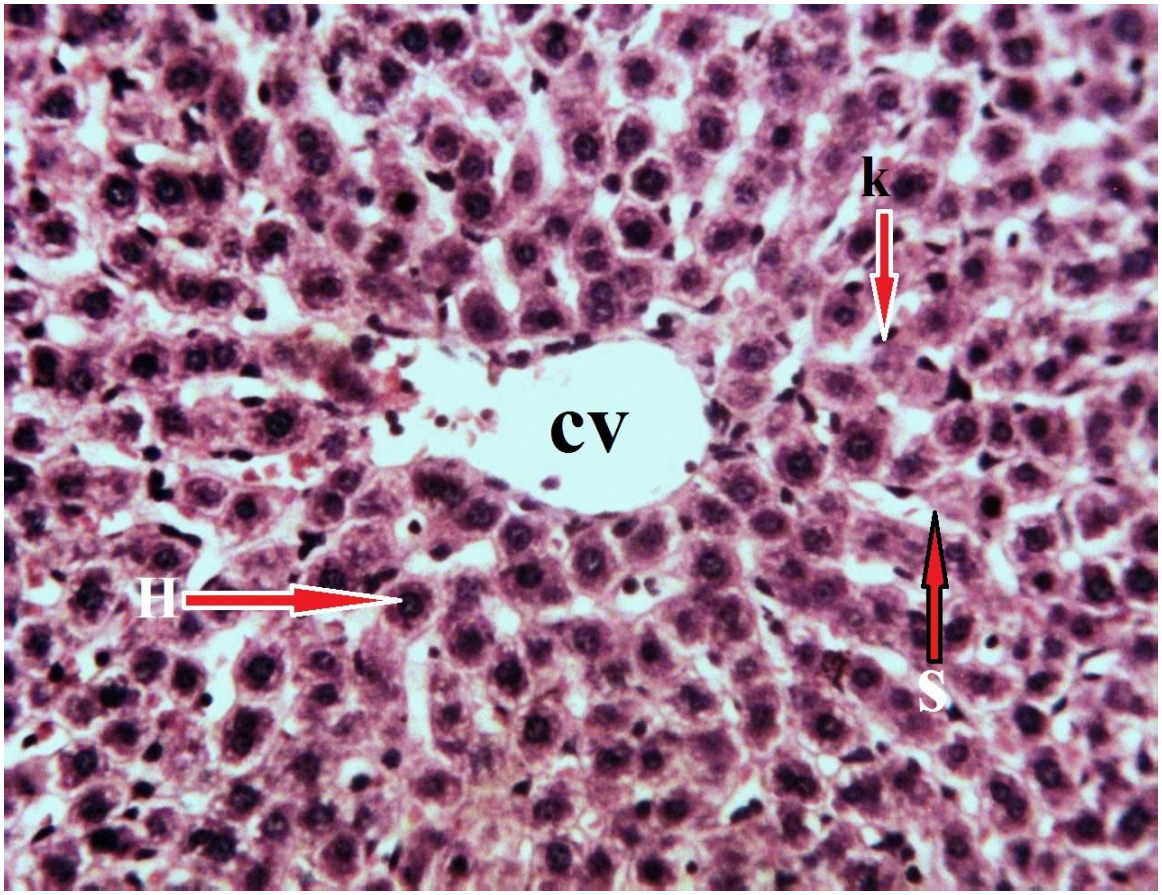
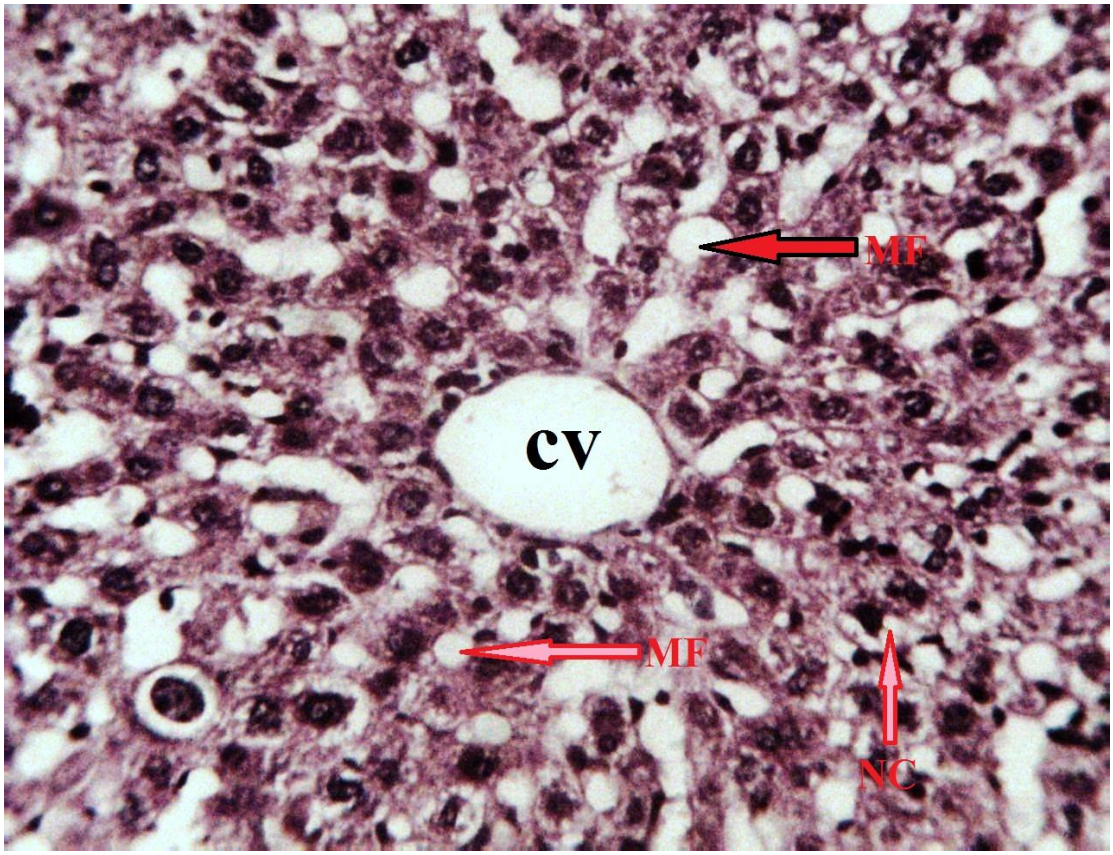


Plate I: Section of Liver tissue from Control group showing H: Hepatocytes CV: Central Vein, S: Sinusoid and K: Kupffer Cells (H and E Stain x250)



Plates II: Section of the liver tissue treated 120 mg/kg lead acetate showing both Macro and Microvascular fatty droplet (MF), loss of cellular boundaries (NC)and distorted sinusoids (H and E Stain x250)

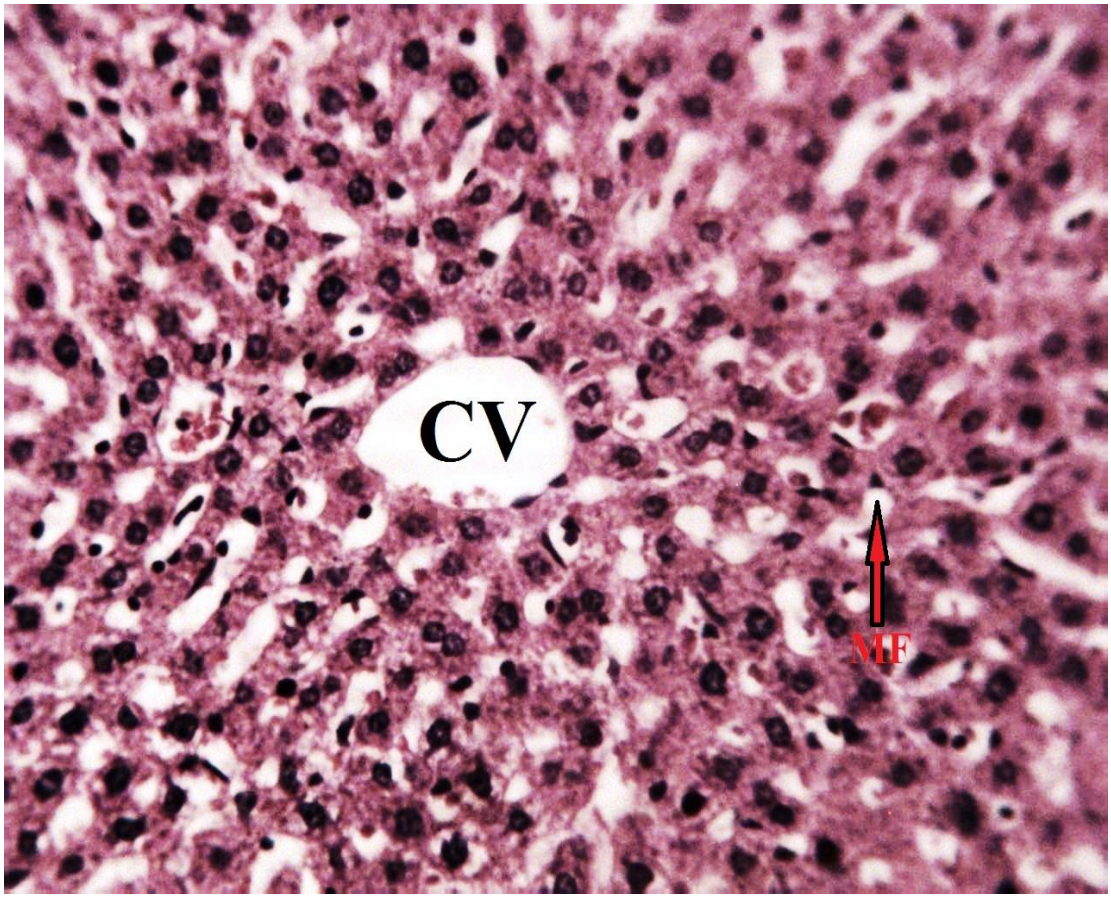


Plate III: Section of the liver treated 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Aqueous Extract) showing slightly restored liver with scanty microvascular Fatty droplet (MF) (H and E stain X250)

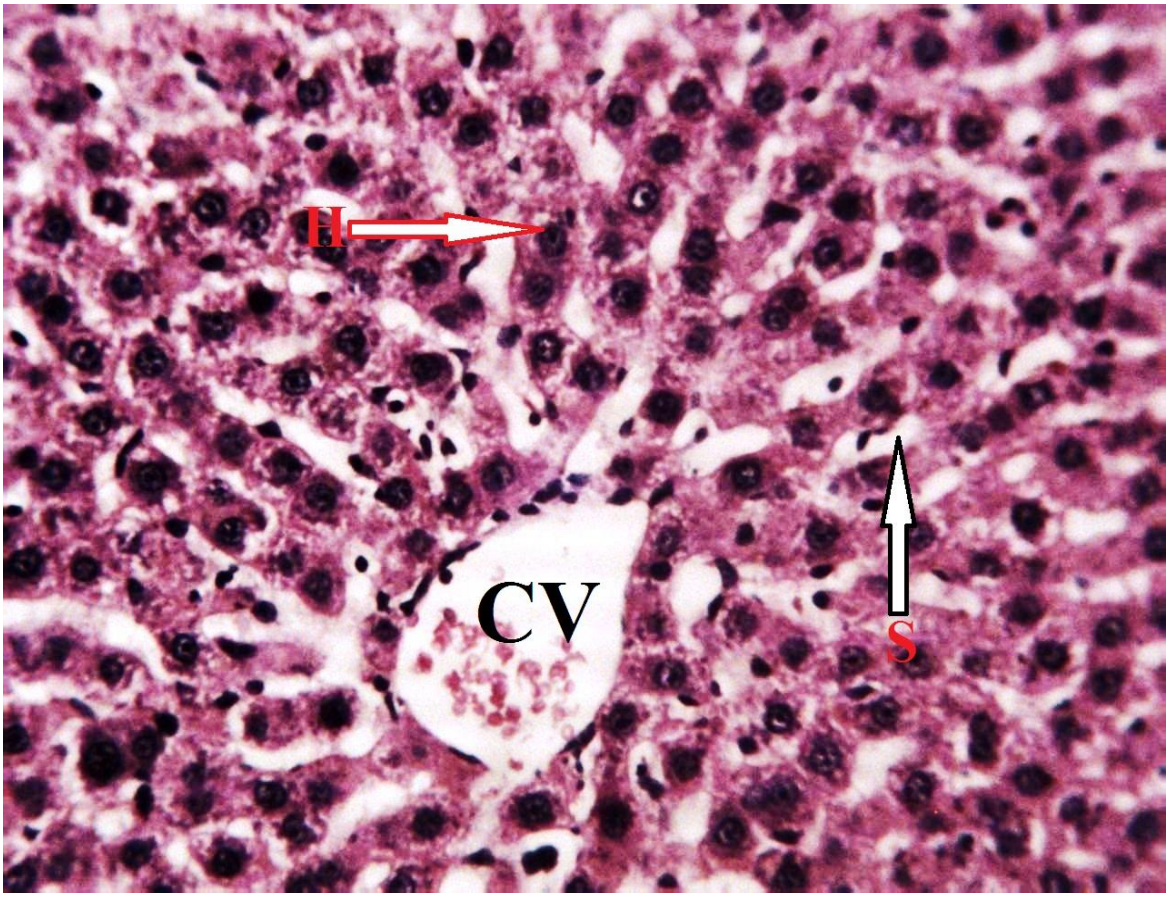


Plate IV: Section of the liver tissue treated with 120 mg/kg lead Acetate + High Dose of *Z. mauritiana* (Aqueous Extract) showing improved liver cytoarchitecture with scanty distorted Sinusoid (S) (H and E Stain, X250).

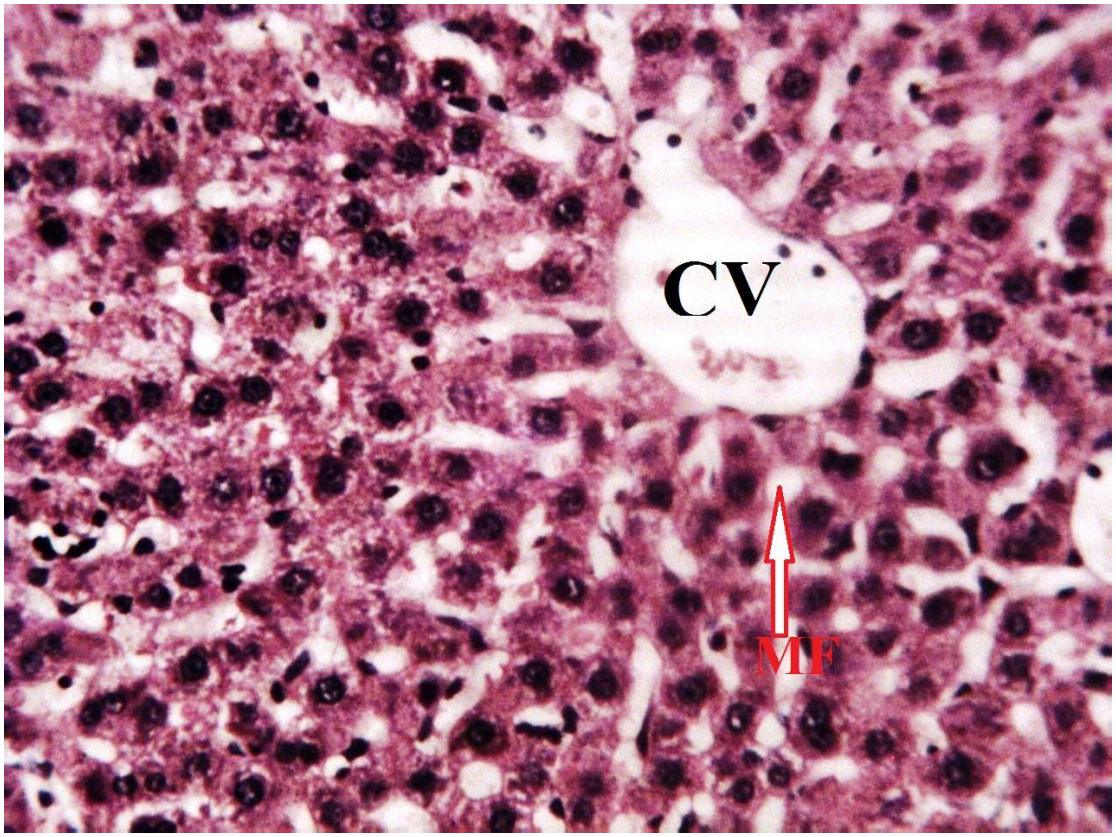


Plate V: Section of the liver treated with 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Ethanolic Extract) improved Liver Cytoarchitecture with small amount of Fatty droplet (MF) (H and E Stain, X 250).

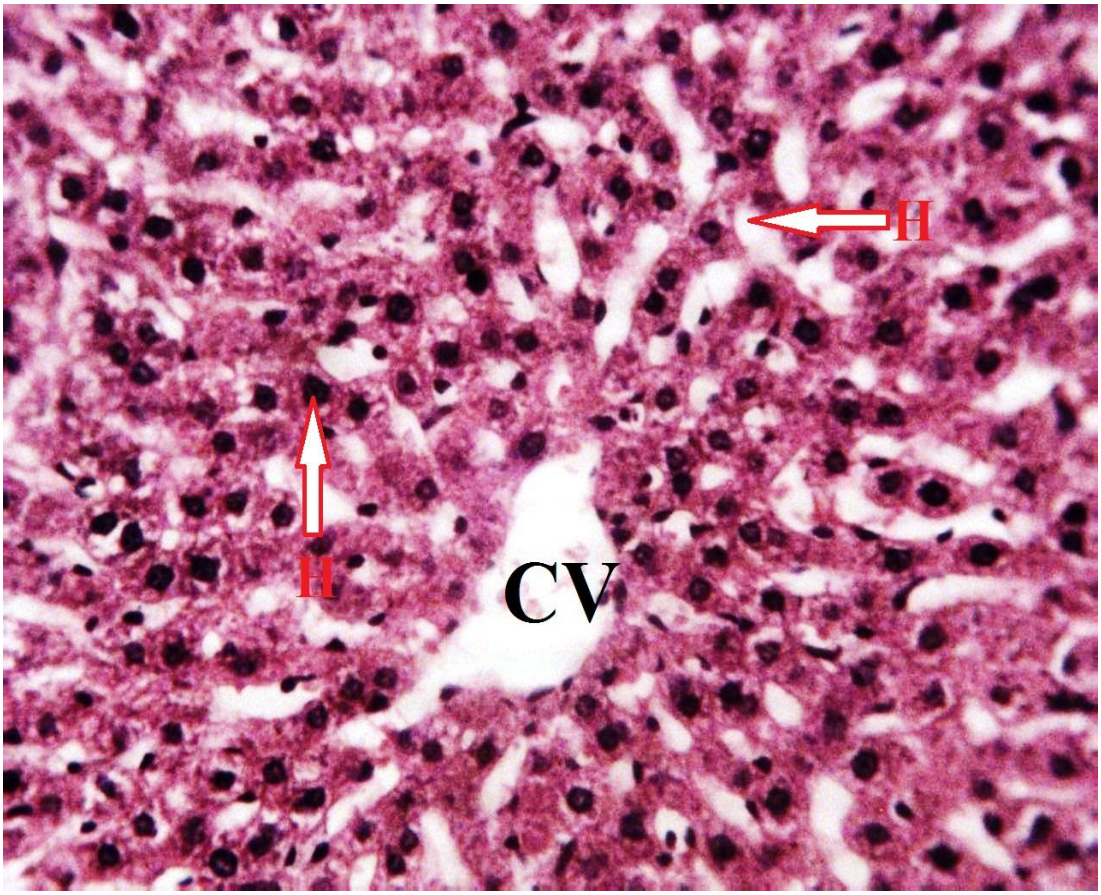


Plate VI: Section of the liver tissue treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Ethanol Extract) showing improved liver cytoarchitecture with scanty dilated Hepatocyte (H) (H and E Stain, X250).

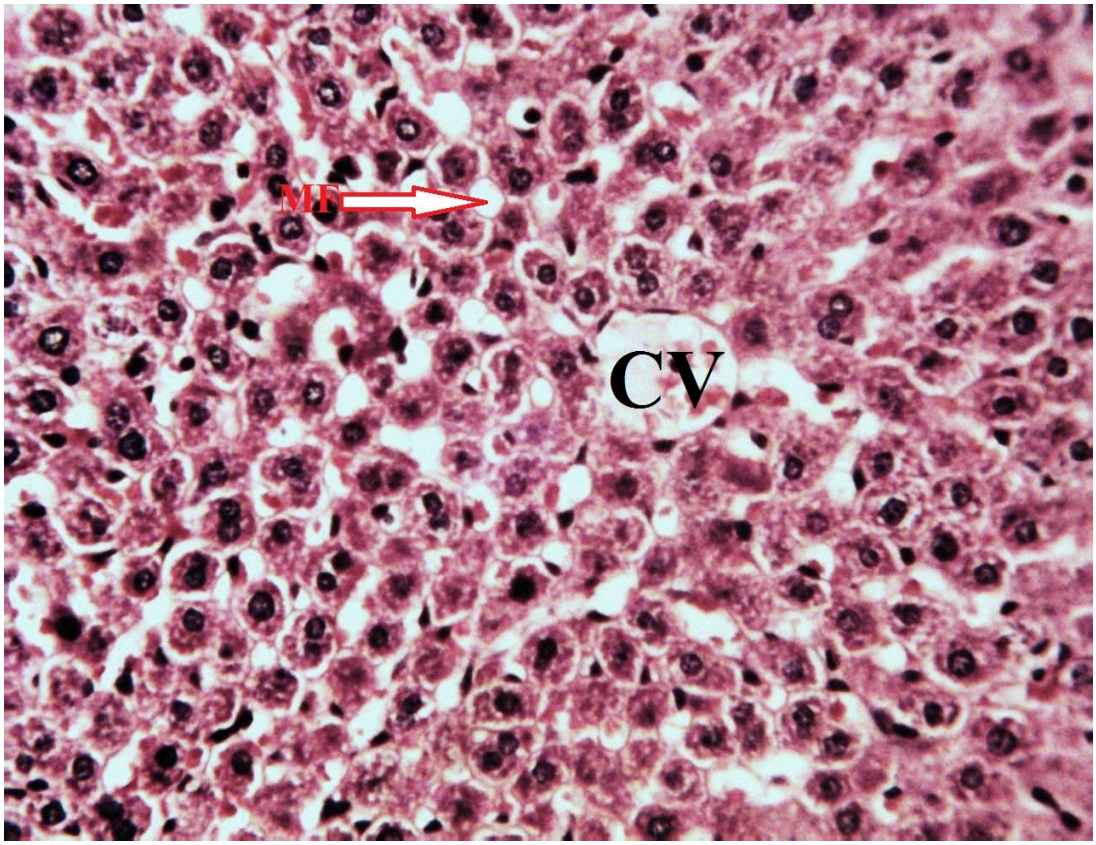
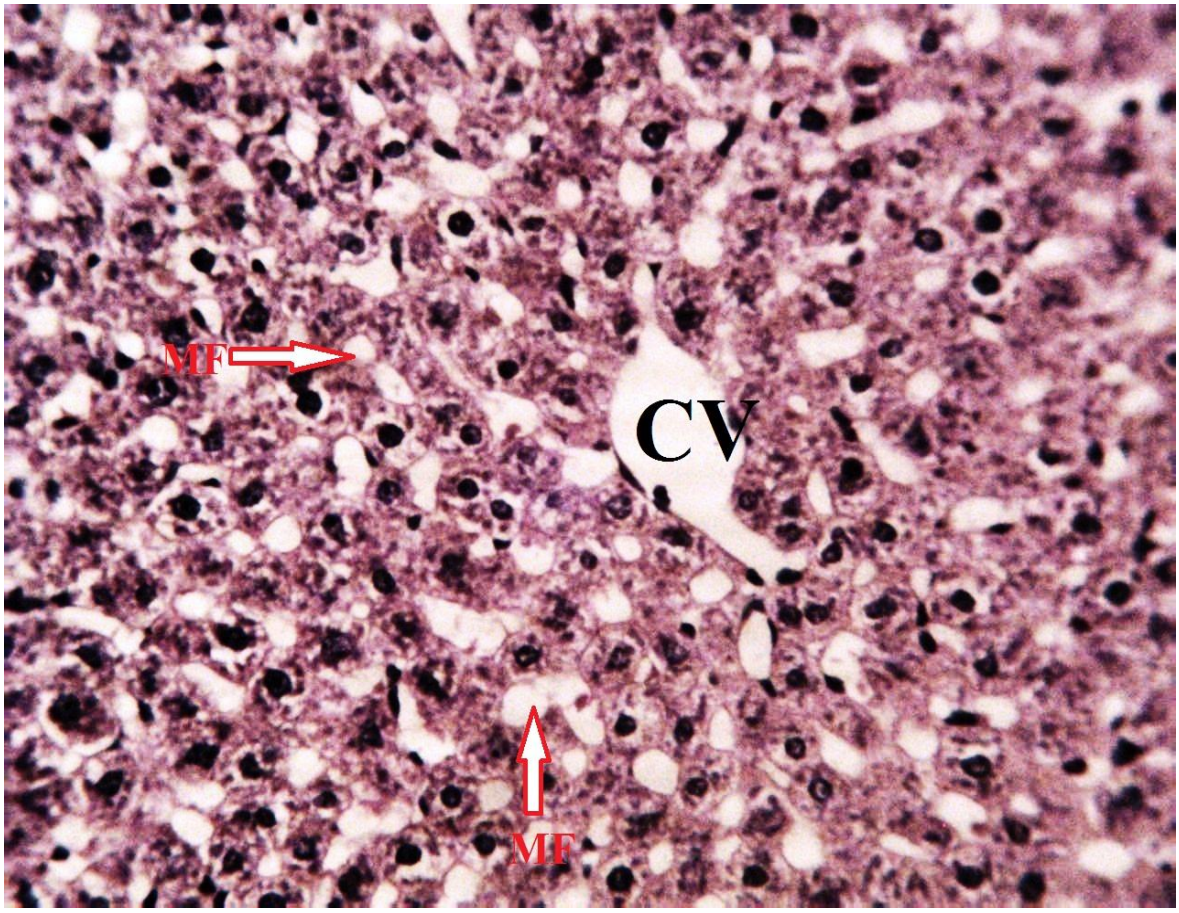


Plate VII: Section of the liver tissue treated with 120 mg/kgLead Acetate + 10 mg/kg Succimer showing Fatty Droplet (MF) (H and E Stain, X250).



Plates VIII: Section of the liver tissue treated 120 mg/kgLead Acetate + Distill Water showing macro and microvascular fatty droplet (MF), loss of cellular boundaries (H and E Stain X250)

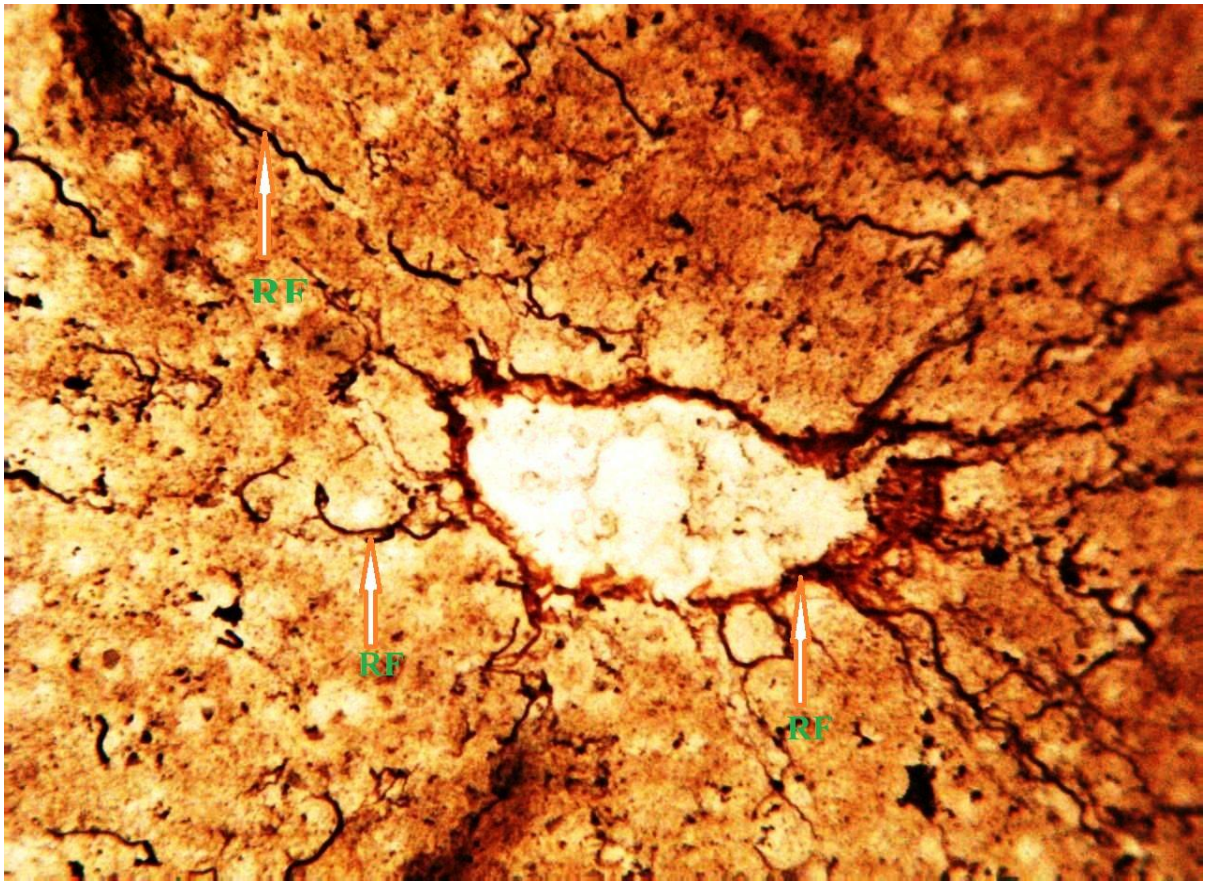


Plate IX: Microscopic section of the liver of control group. RF:Reticularfibres (Gordon Stain, ×250).

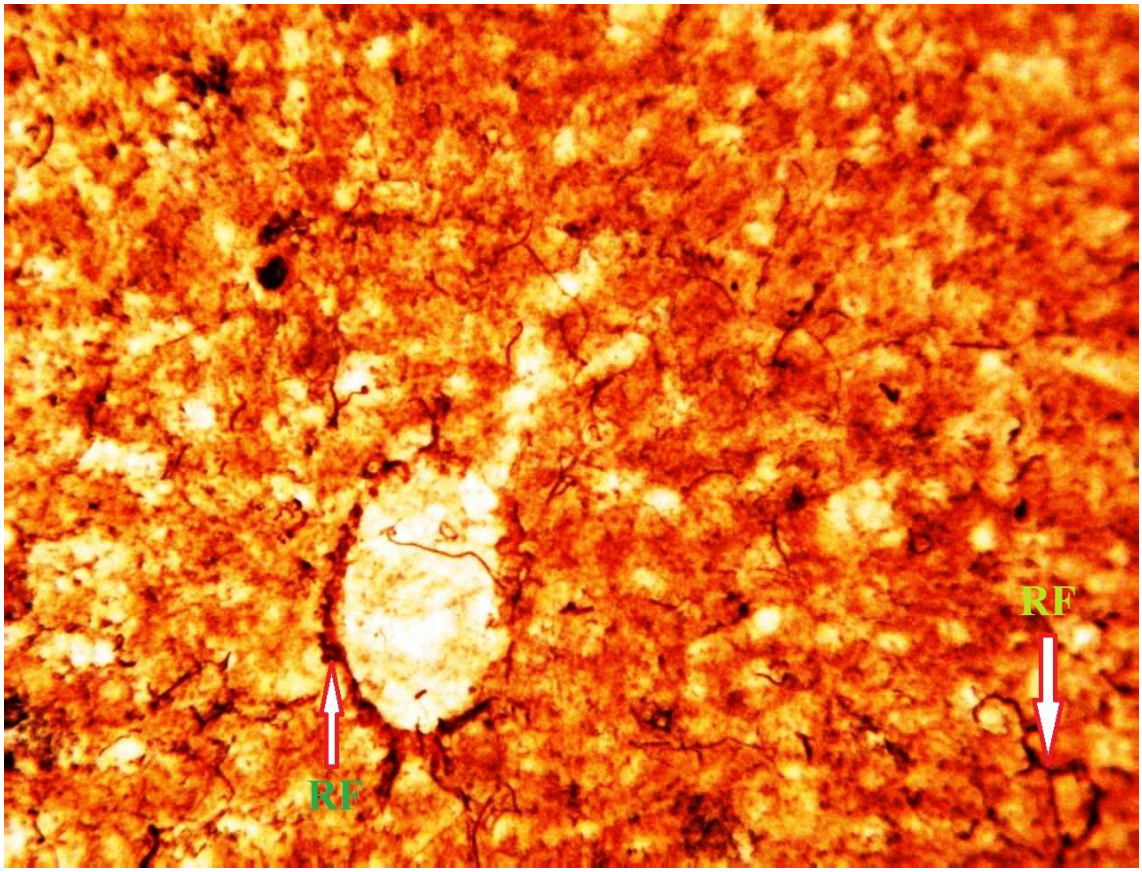


Plate X: Microscopic section of the liver of 120 mg/kgLead Acetate. RF:Reticular fibres (Gordon ×250).

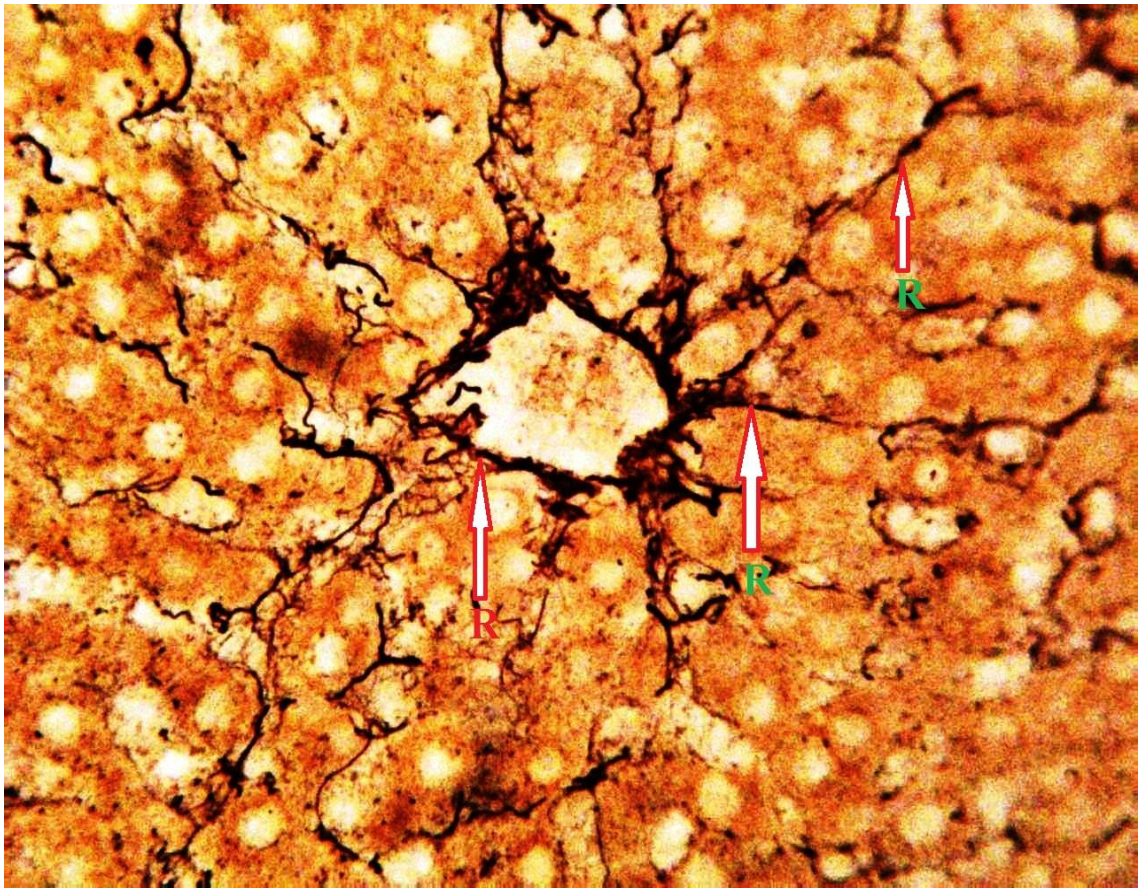


Plate XI: Micoscopic section of the liver with treated 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Aqueous Extract). RF: Reticular fibres (Gordon Stain, ×250)

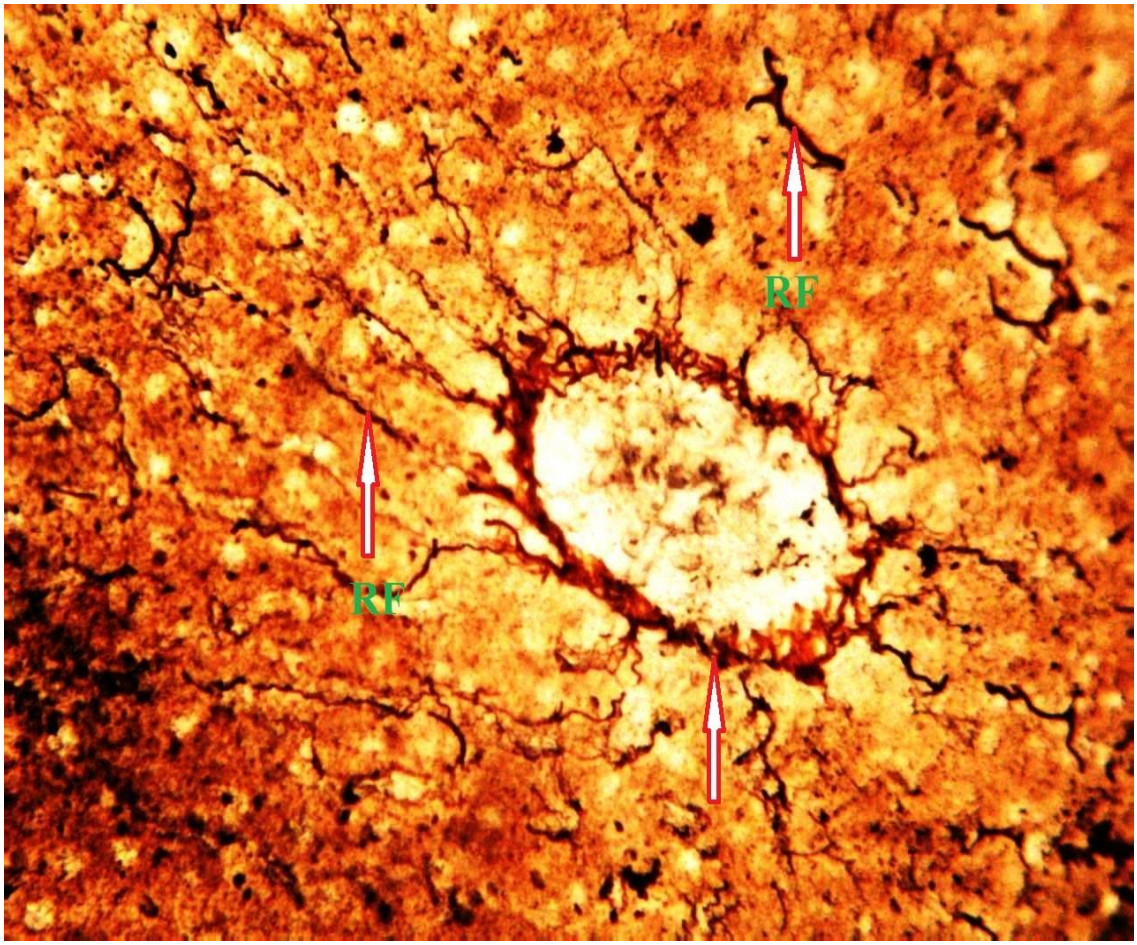


Plate XII: Microscopic section of the liver treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Aqueous Extract). RF: Reticular fibres (Gordon Stain ×250)

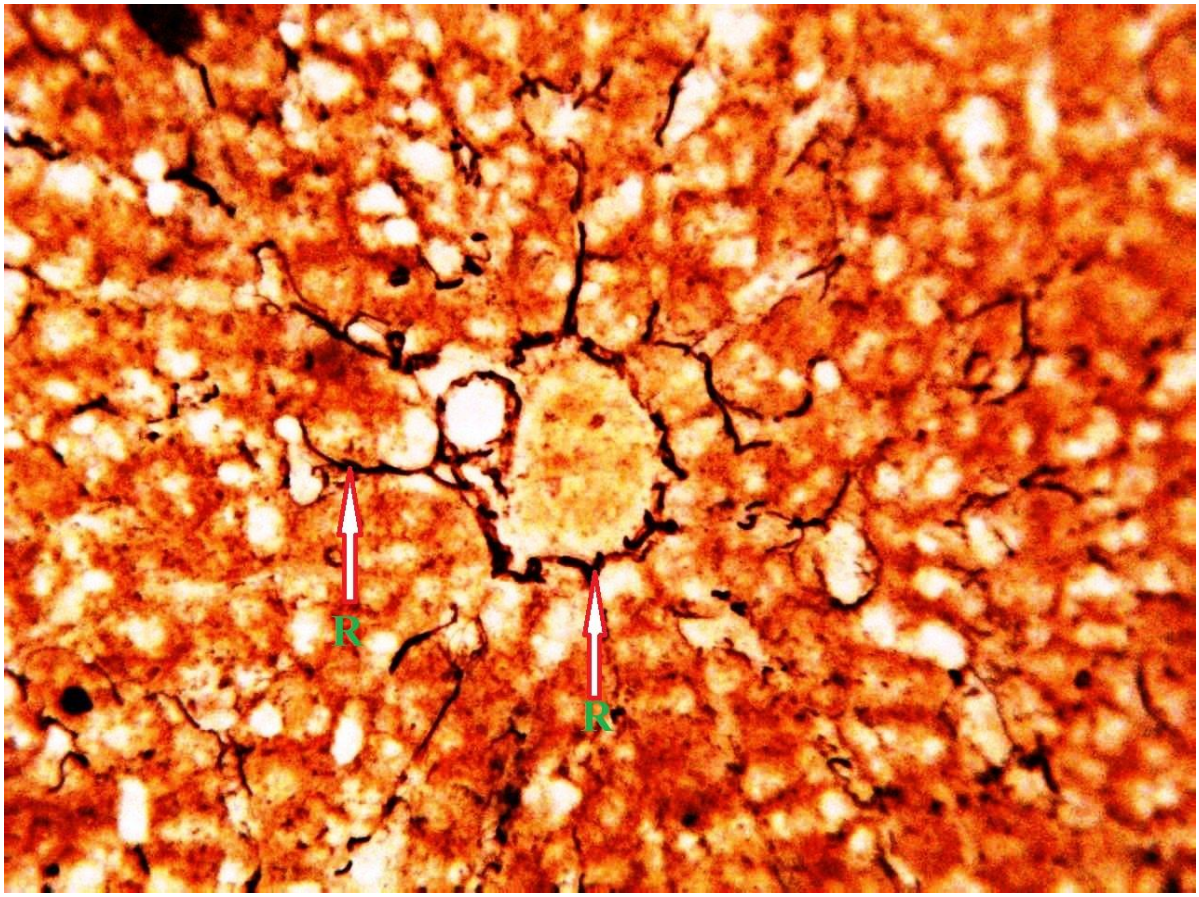


Plate XIII: Micoscopic section of the liver with treated 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Ethanolic Extract).Rf: Reticular fibres (Gordon Stain, ×250).

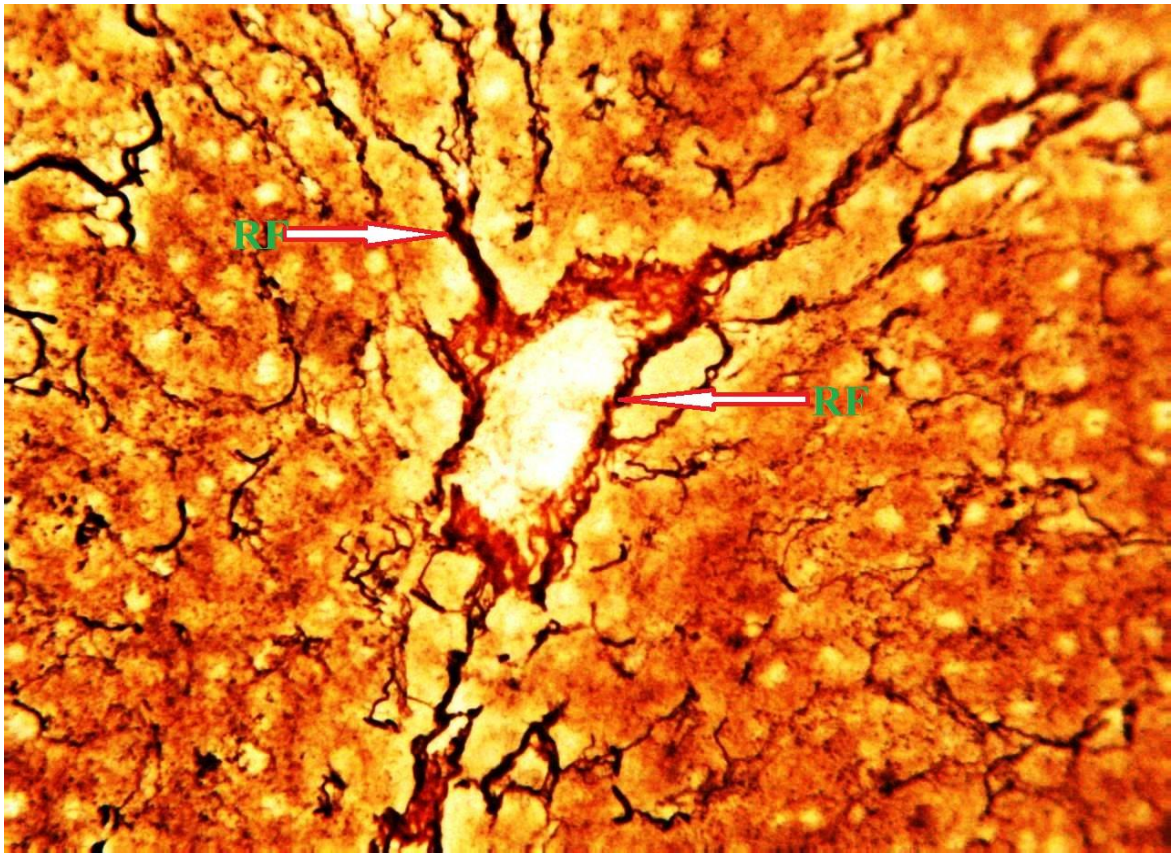


Plate XIV: Microscopic section of the liver treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Ethanolic Extract)RF: Reticular fibres (Gordon Stain ×250).

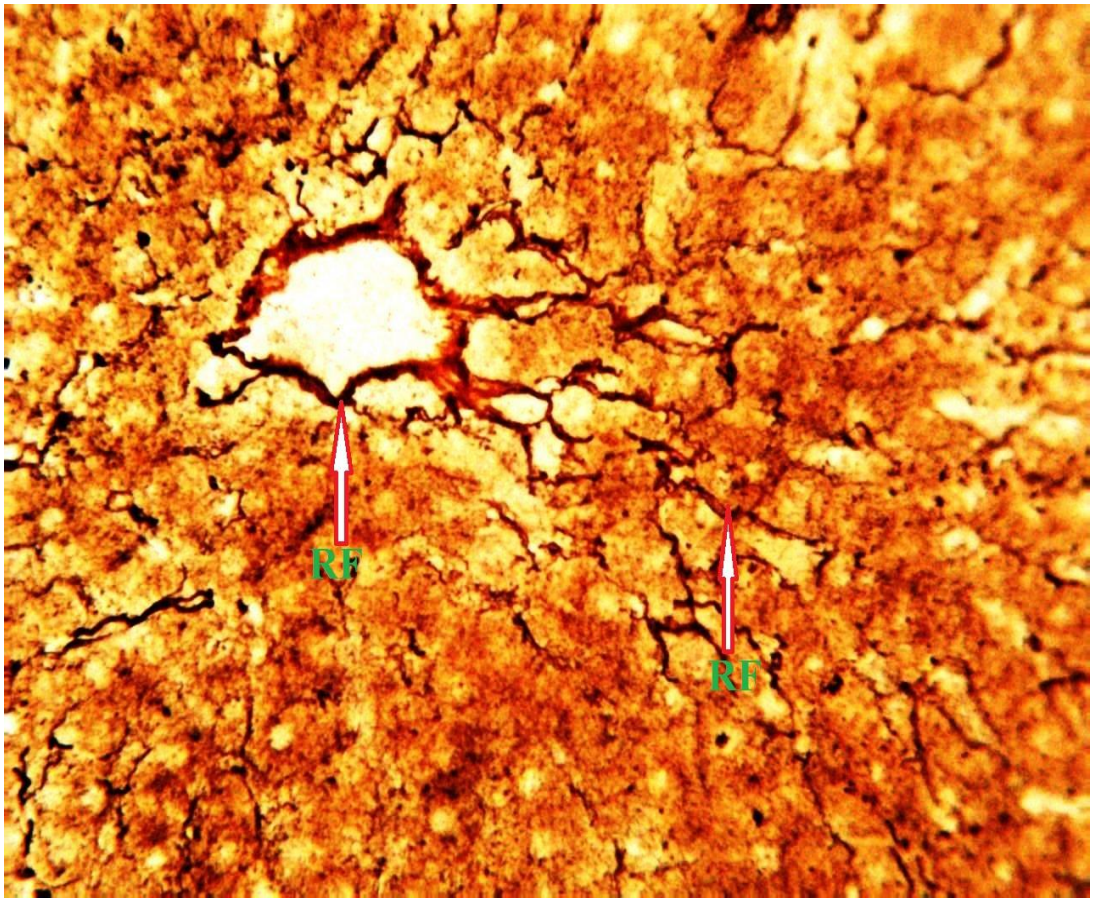


Plate XV: Microscopic section of the liver treated with 120 mg/kgLead Acetate + 10 mg/kg Succimer. RF:. Reticular fibres (Gordon Stain ×250)

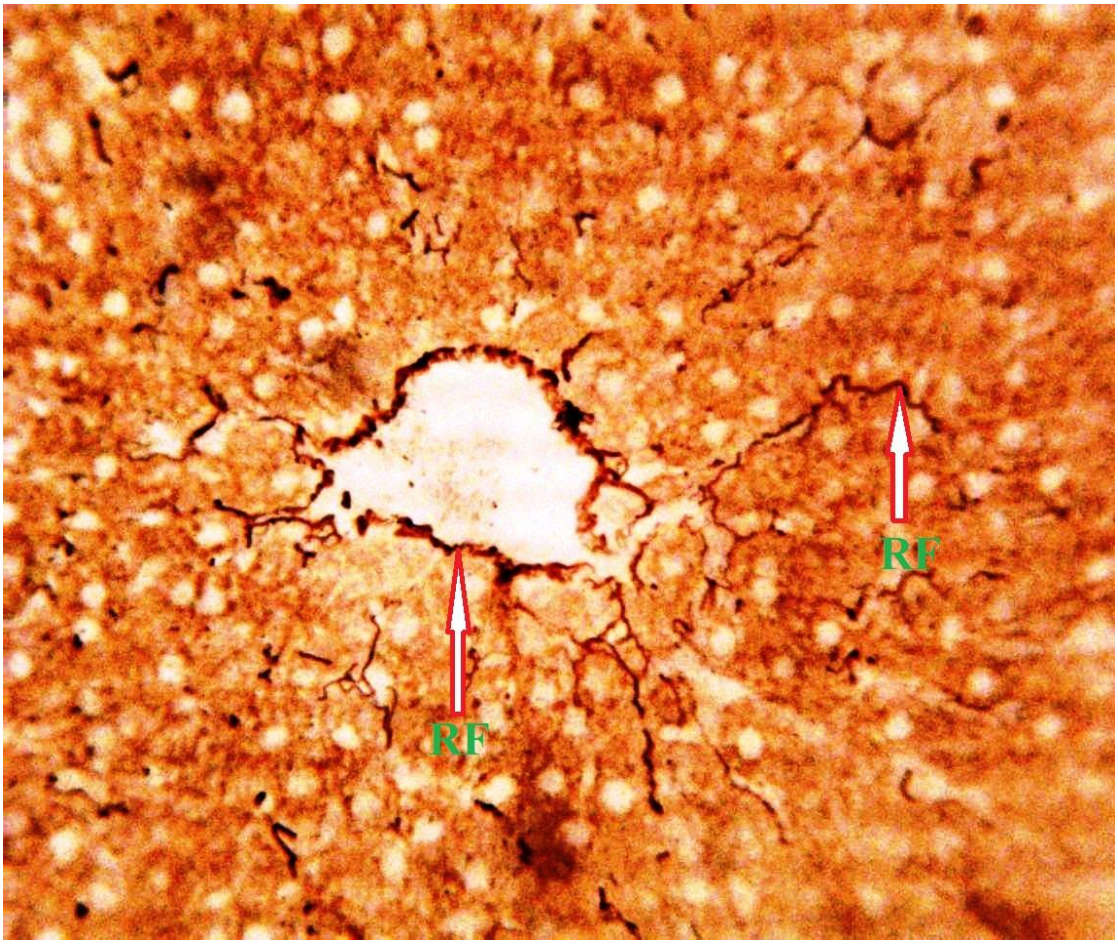


Plate XVI: Microscopic section of the liver of 120 mg/kg of Lead Acetate + Distill Water group.RF:Reticular fibres (Gordon, ×250).

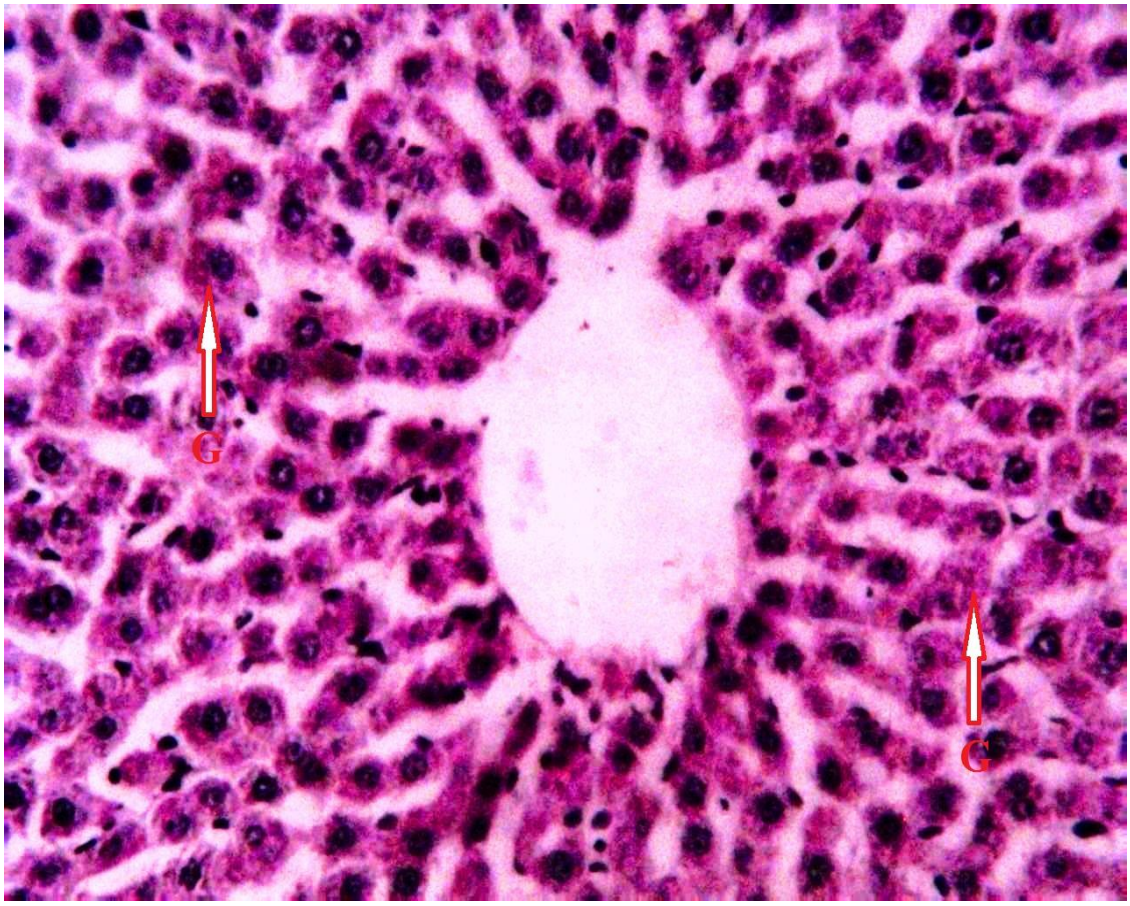


Plate XVII: Microscopic section of the liver of control group. (PAS; ×250).With normal pattern of glycogen storage in cytoplasm of the liver hepatocytes.Glycogen (G).

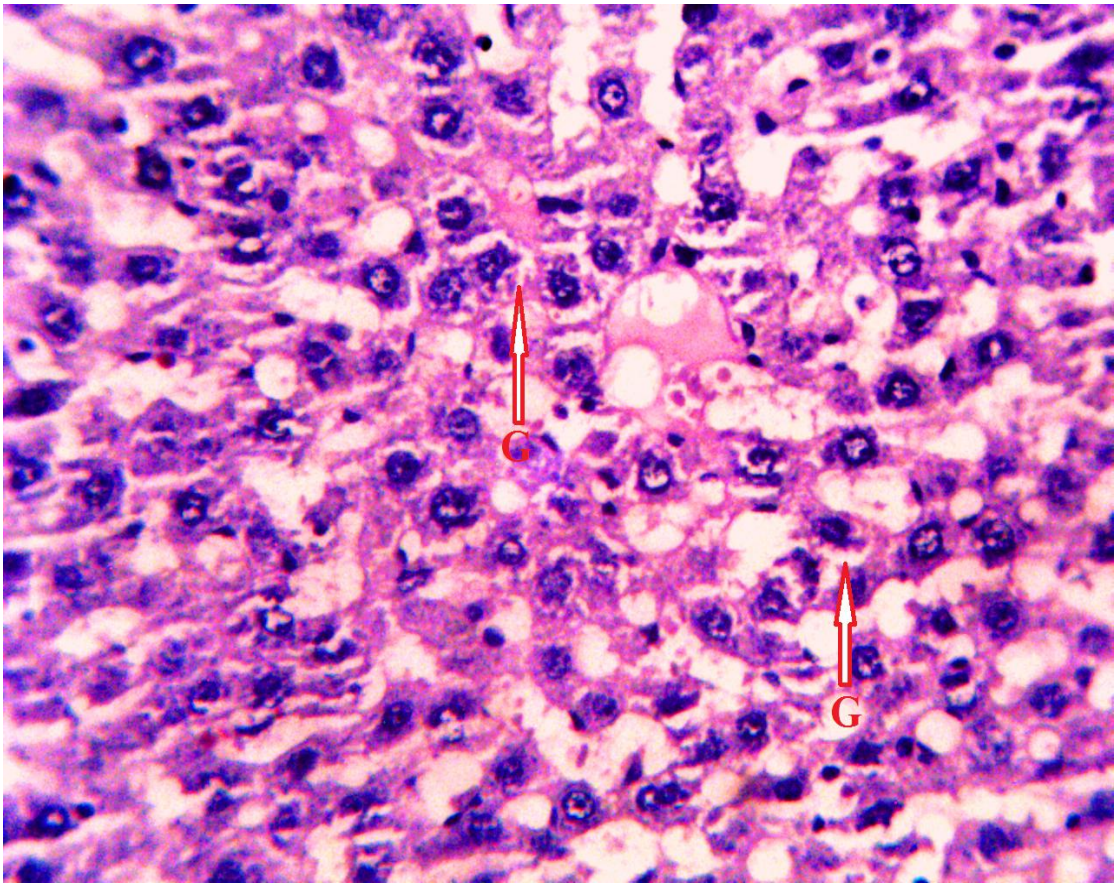


Plate XVIII: Microscopic section of the liver with treated 120 mg/kgLead Acetate. (PAS; ×250). With depletion in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

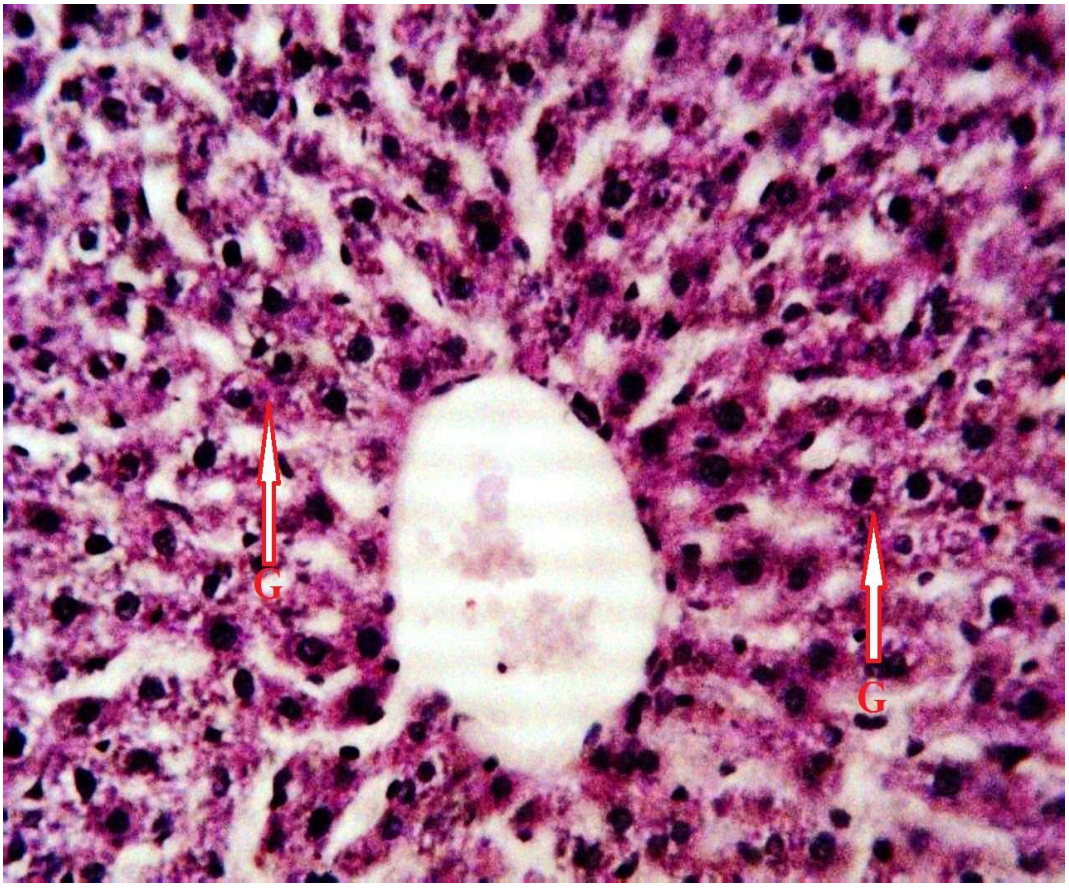


Plate XIX: Micoscopic section of the liver treated 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Aqueous Extract). (PAS, ×250).With a slight improvement in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

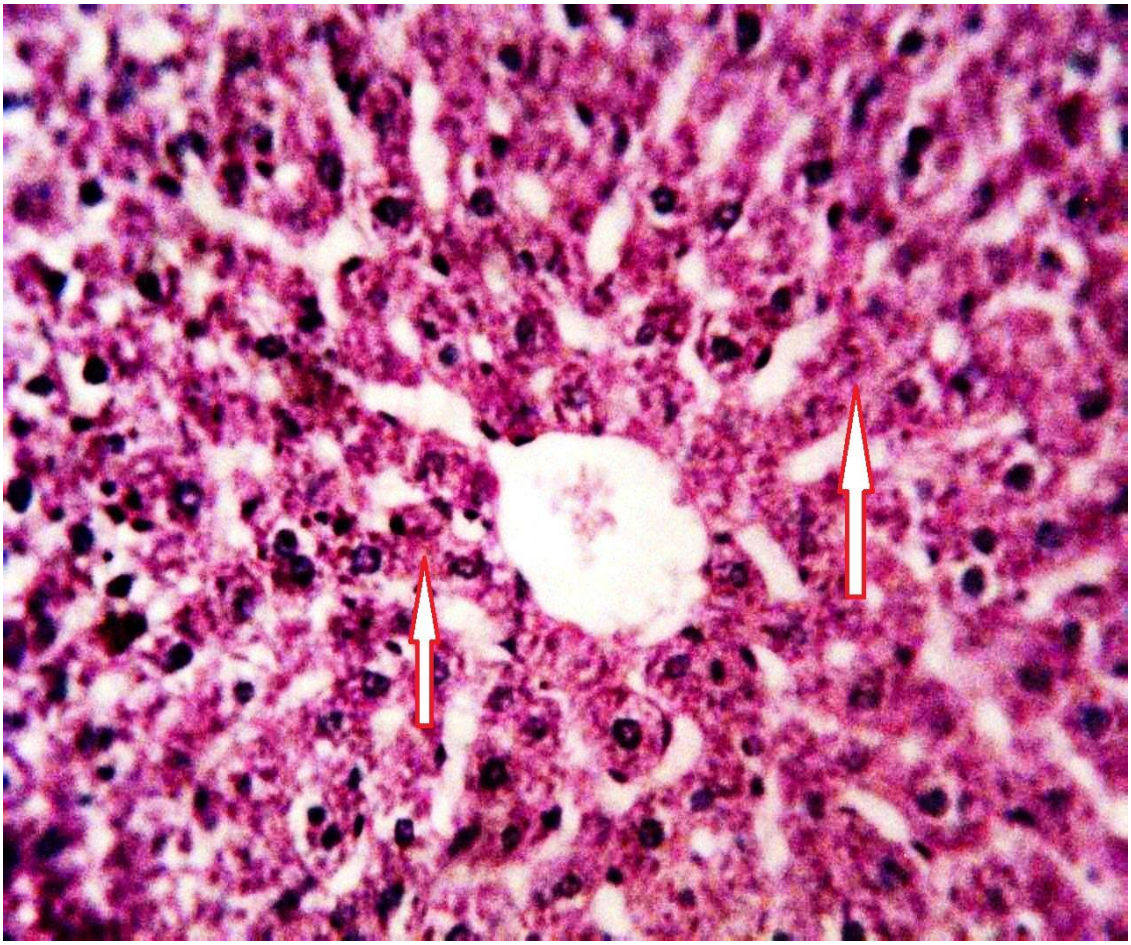


Plate XX: Microscopic section of the liver 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Aqueous Extract). (PAS, ×250). With significant improvement in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G)

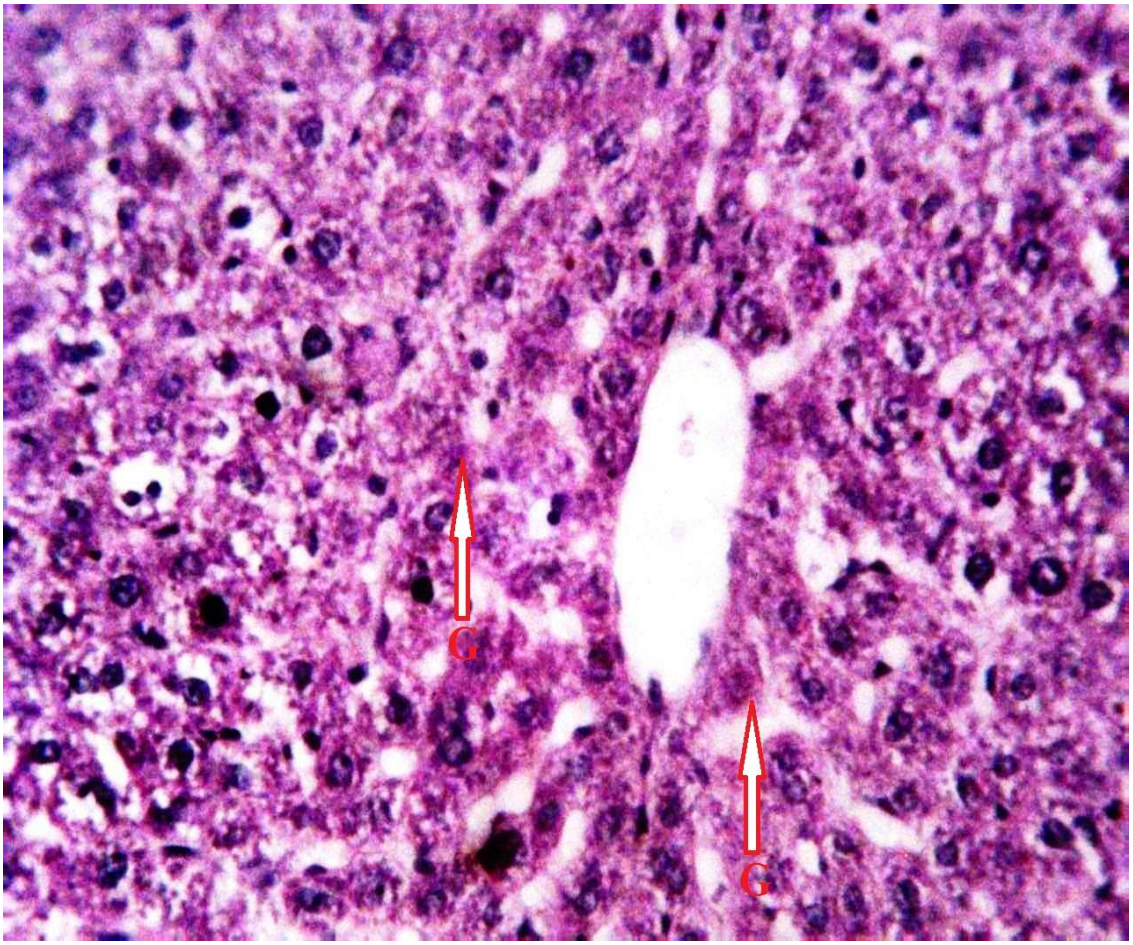


Plate XX1: Microscopic section of the liver treated with 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Ethanolic Extract). (PAS, ×250).With no significant change in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

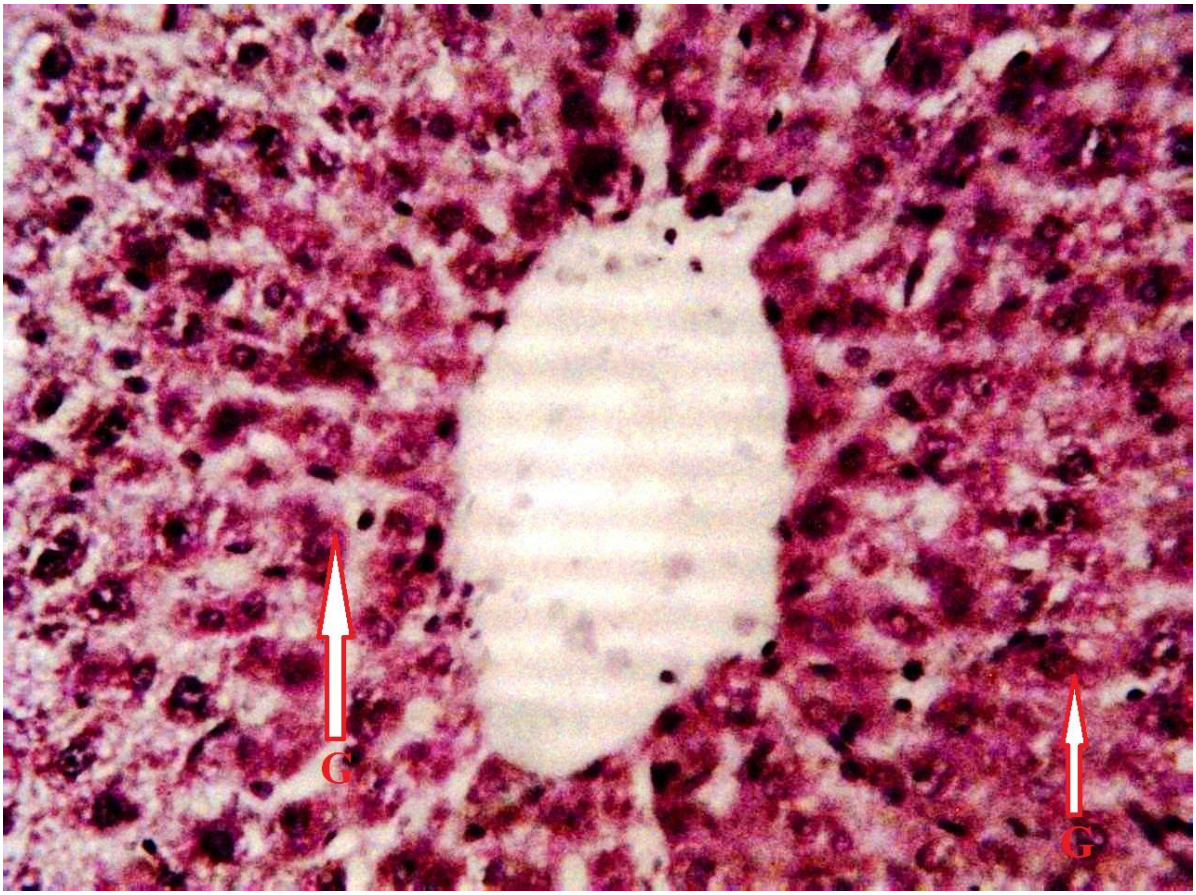


Plate XXII: Microscopic section of the liver treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Ethanolic Extract). (PAS, ×250). With significant increase in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

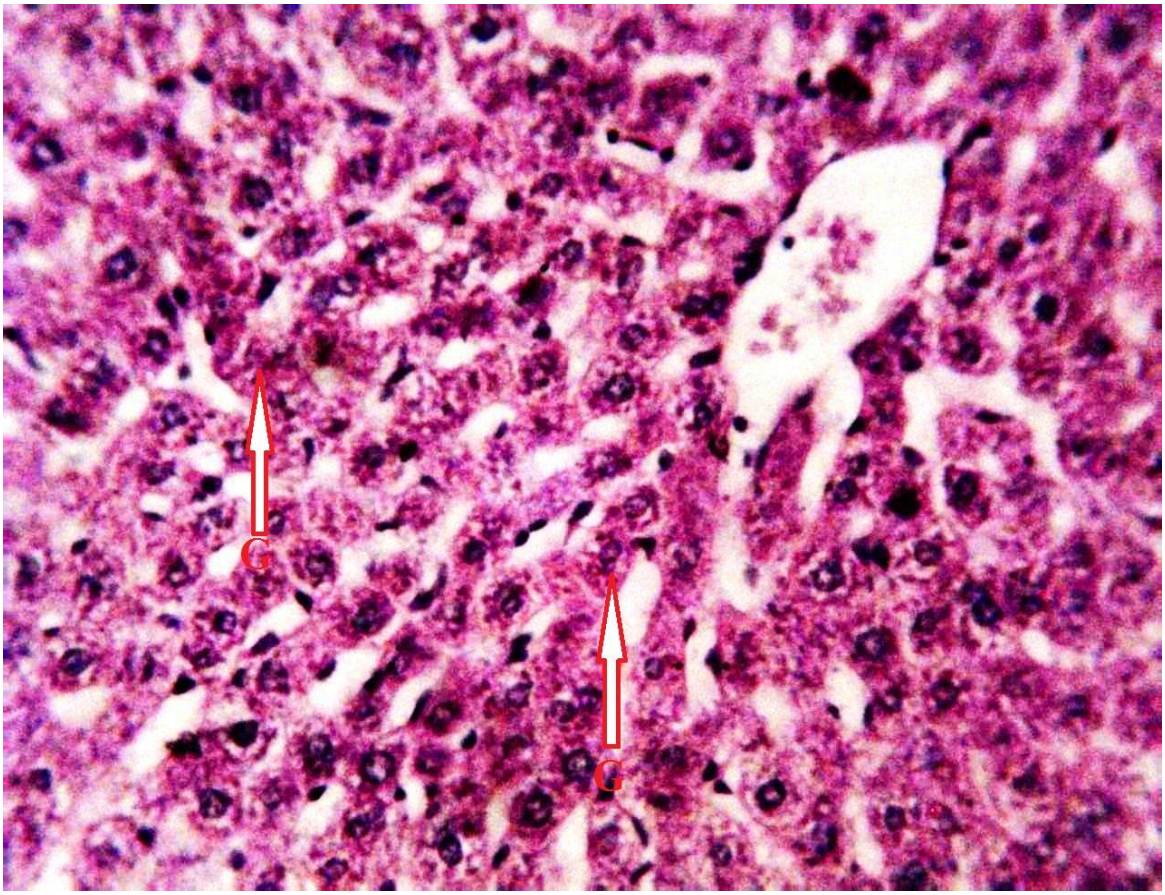


Plate XXIII: Microscopic section of the liver treated with 120 mg/kgLead Acetate + 10 mg/kg Succimer. (PAS, ×250).With significant change in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

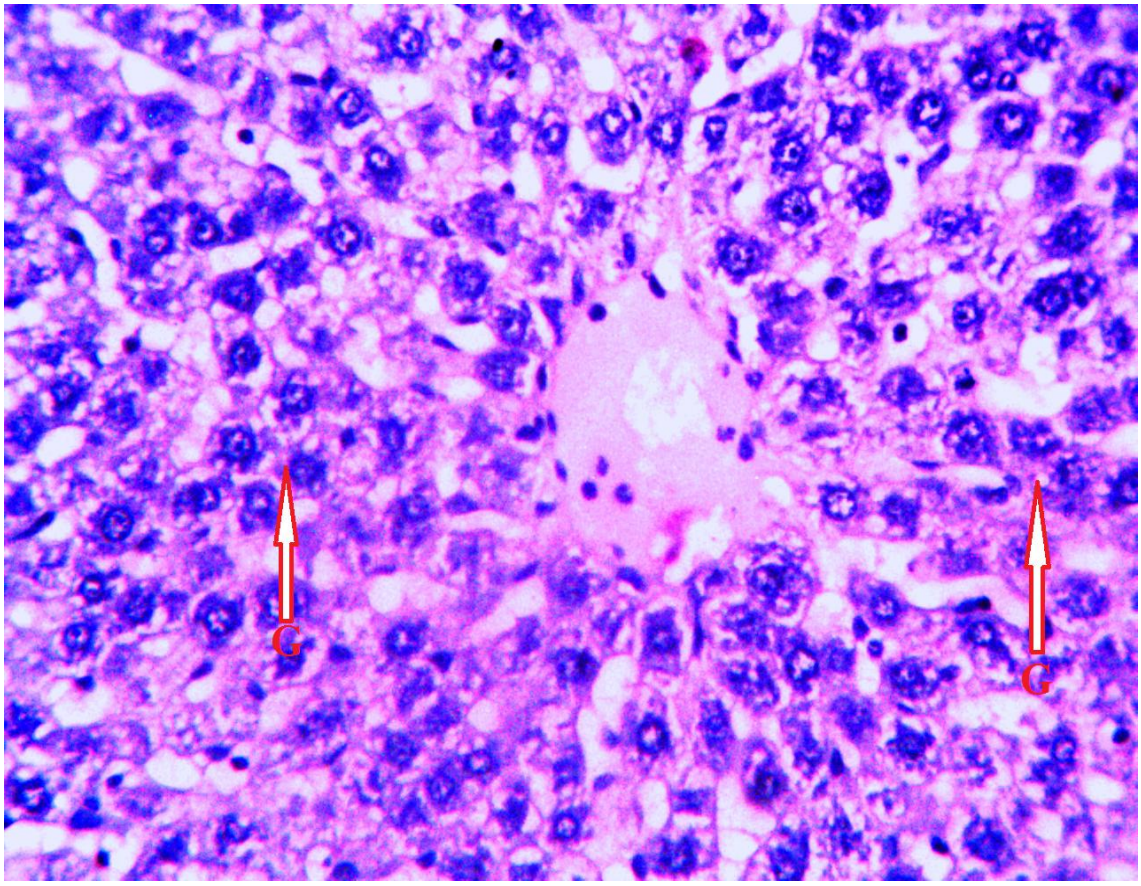


Plate XXIV: Microscopic section of the liver with treated 120 mg/kg Lead Acetate + Distill Water. (PAS; ×250). With depletion in the pattern of glycogen storage in the cytoplasm of liver hepatocytes, Glycogen (G).

CHAPTER FIVE

5.0 DISCUSSION

In the present study, there was a significant decrease in weight gain in the treated animals when compared to the control group (Group one). These observations are in similar with the report of previous studies where lead exposure in experimental animals was associated with reduction in growth rate (Barker, 2002; El-Sayed*et al*., 2015). Aseth*et al*., (1995) and Teijon*et al*., (2006) also reported reduction in body weight caused by lead induces toxicity in Wistar rats. The decreased in body weight gain in the lead treated groups may be possibly attributed to reduction in food intake from the toxic effects of lead acetate (Sakata *et al*., 2007). However, treatment with extracts (Aqeous and Ethanol) resulted in weight gain though not significant. On the other hand low dose of the Ethanol*Ziziphusmauritiana* leaf extract resulted in a weight gain similar to that observed in the Succimer (standard drug) treated group; although the high dose of our extracts proves to be more effective than the low dose. The efficacy of our extracts could possibly be owed to its antioxidant activities which could be hinged on the presence of tannins (Adzu*et al*., 2001), carotenes (Guil-Guerrero *et al*., 2004) and flavonoids (Pawlowska*et al*., 2009) in some *Ziziphus* species.

The present study showed a significant increase in the organ body weight ratio in the rats administered with lead acetate, this is likely caused by the increase in weight of the liver in animals treated with lead acetate. Upasani and Balaraman, (2001) studied the effects of lead and found that lead treatment produced a significant accumulation of lipids in Liver and Kidney cells of rats. Valko*et al*., (2006) attributed the increased weight of Liver to tumorigenicity of lead salts in general. This study further established that treatment with *Ziziphusmauritiana* leaves extract could lead to a recovery from lipid accumulation caused by lead acetate as weight of the liver insignificantly decreased in the rats administered with lead acetate and then treated with aqeous or ethanol extracts of *Ziziphusmauritiana* leaves.

Serum liver enzyme parameters (AST, ALP and ALT) are sensitive markers of hepatocellular injury, injury to the liver cells results in the leakage of these enzymes into circulation, therefore resulting in increased serum concentration of these enzymes. The present study showed that treatment with lead acetate has effect on liver enzyme markers as treatment with lead acetate resulted in an increase AST, ALP and ALT concentrations. This is simlilar with most researches done on lead toxicity on hepatic enzymes, where significant increase in serum liver enzymes such as AST, ALP, ALT, creatinine and bilirubin and various serum lipids were reported (Kumar *et al*., 2010; Sujatha*et al*., 2011). Lead induced hepatotoxicity was reported to be associated with the impairments of liver structural and functional integrity (Aziz *et al*., 2012).

However, in rats treated with lead acetate followed by extracts (Aqeous or ethanol) there was a decreased in serum liver enzymes. In an earlier experiment, Dahiru*et al*., (2005) investigated the hepatoprotective activity of ethanol extracts of *Ziziphusmauritiana* leaf in carbon tetrachloride-induced liver damage. They reported pretreatments of rats with 200mg and 300mg/kg bw of *Ziziphusmauritiana* leaf extract protected rats against carbon tetrachloride liver injury by significantly lowering AST, ALT, ALP and total bilirubin compared to control, according to the result we can say that *Ziziphusmauritiana* has good hepatoprotective property and can prevent liver damage. Hepatoprotective property of both aqeous and ethanolic extracts of *Ziziphusmauritiana* leaf can also not be separated from antioxidant activity of phytochemicals such as tannins. Tannins are known to exert antihepatotoxic action (Hikino*et al*., 1985). The basis for the hepatoprotective action of tannins has been attributed to the formation of an impervious poly-phenol protein and/or polysaccharide layer under which the natural healing process can occur.

Oxidative stress is caused by an imbalance in the production of free radicals and compromise in the system's ability to readily detoxify the body of reactive oxygen species resulting in

cellular damage (Flora, 2011). Reduction in glutathione (GSH) concentrations, as well as modifications in CAT and SOD activities are the most frequently used markers of oxidative stress in tissues (Khaki *et al*., 2010). The present study provided evidence that lead acetate can induce oxidative stress in Wistar rats by decreasing CAT, SOD and GSH, and increasing MDA. Lead induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande and Flora, 2002). The decrease in the activity of antioxidative enzymes such as SOD concentration and the increase in the concentration of MDA indicate formation of free radicals and the participation of free radical induced oxidative cell injury in mediating the toxic effect of lead (Jomova*et al*., 2010). The increase in the concentration MDA might be due to peroxidation of unsaturated fatty acids within the cells, with resultant increase in membrane permeability and rigidity, and disruption of structural and functional integrity of cell organelles (Gemma *et al*., 2007). Lead promotes oxidative stress directly through excess production of reactive oxygen species (ROS), originating from interference with the heme synthetic pathway (Becharа*et al*., 1996; Jangid*et al*., 2012), but also indirectly by tampering with cellular antioxidant pool (Ercal*et al*., 2001). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasani*et al*., 2001). However, this study further established that treatment with *Ziziphusmauritiana* leaves extract could lead to recovery from oxidative stress caused by lead acetate as there was a therapeutic increase in oxidative stress indicator SOD, GSH and CAT in the different extracts (Aqeous and Ethanol) treated groups compared to the positive control group and Succimer treated group. This observation is similar with the report of Dahiru and Obidoa (2007) that aqeous extract of *Ziziphusmauritiana* leaves was protective against chronic ethanol induced hepatoxicity in Wistar Rats. In that earlier study, treatment with aqeous extract of *Ziziphusmauritiana* significantly restored the level of oxidative stress markers, and therefore

was concluded that aqeous extract of *Ziziphusmauritiana* leaf is effective against oxidative liver damage induced by chronic alcohol administration. Flavonoids are naturally occurring polyphenolic compounds found present in both ethanol and aqeous extracts of *Ziziphusmauritiana* leaves. Polyphenolic compounds have been shown to protect various cell types from oxidative stress mediated cell injury (Sasaki *et al*., 2002). These compounds, like other anti-oxidants, can cure or prevent oxidative stress by chelating redox active metal ions and also by terminating the free radical chain reaction (Rice-Evans, 2001; Terao, 2009). Therefore, it could be pertinent to speculate that the ability of the leaf extract of *Ziziphusmauritiana* to lower the MDA concentrations in the treatment groups may be attributed to similar antioxidant properties.

The present study showed a significant reduction in White Blood cell (WBC), Red blood cell (RBC) and Hemoglobin (HGB) in rats administered with lead acetate. Studies have shown that lead intoxication has destructive effects on blood (Elias *et al*., 2014). Helmy*et al*. (2000) reported a significant decreased in Hb and PCV following exposure of rats to lead acetate. Suradkar*et al*., (2009) who observed that the reduction could be as a result of the effect of lead acetate on the activity of aminolevulinic Acid Dehydratase (ALAD), a key enzyme in haeme synthesis. Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is quite sensitive to lead. It is also reported that, lead also inhibit the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in haemoglobin production and shortened life-span of erythrocytes (Klassen, 2001). Progressive destruction of RBCs due to binding of lead with RBCs, leading to increased fragility and destruction could be the main reason for decrease haematological values (Rous, 2000). The present study also showed a significant increase in White blood cell (WBC), this result is in line with the work of Nwokocha*et al*., (2011), who established a significant reduction in haematological parameters i.e. RBC, Hb, HCT and an increased in platelet count and WBC was induced by

lead intoxication. He speculated the increased could have been as a result of marrow infiltration by toxic substances and with a reaction increase in some parameters.

However, there was a significant increase in RBC values and reduction in the WBC values in the *Ziziphusmauritiana* extracts and succimer treated groups which caused amelioration when compared to lead only administered group. This observation supports the findings of El-Desouky*et al*., (2014), that the ethanol extracts of *Ziziphusmauritiana* leaves was protective against irradiation and an improvement in haematological values was also noticed following the treatments of experimental rats with gamma irradiation. This was attributed to the haemoprotective and antioxidant property of *Ziziphusmauritiana*. Montilla*et al*., (1998) investigated the high power of *Ziziphus* leaves ethanol extract as an antioxidant which neutralized the damaging effect of the both free radicals (hydroxyl and peroxyl) and regulated the antioxidative defense enzyme system in the kidney tissues.

In the present study, there was a significant increase in the level of lead accumulation in the liver of lead only treated animals when compared to the control group. This observation is similar with the work of Nwokocha *et al*., 2012. These increased in concentration of lead is associated with increased susceptibility to liver damage. Injury to the liver cells results in leakage of liver function enzymes into the circulation and serum levels of these enzymes increases. The high plasma ALT and AST activities are accompanied by high liver membrane fluidity, free radical generation and alteration in the liver histology. These study further esterblished that treatment with *Ziziphusmauritian* leaves extract could lead to a recovery from lead accumulation caused by lead acetate.

The results of the histological studies of the liver showed congested sinusoids, loss of cellular boundaries including necrotic hepatocytes, and also fatty droplets. These showed that lead acetate was hepatotoxic to the liver. Lead induced hepatotoxicity was reported to be

associated with the impairments of liver structure and function (Aziz *et al*., 2012). This observation was similar with the findings of El- Nahal (2010) who reported that liver of rats treated with lead acetate shows congestion of hepatoportal blood vessels and edema in the portal tract, forcal hepatic hemorrhage and vacuolar degeneration of hepatocytes. This is also similar with the findings of Omotoso*et al*., (2015), who administered 50mg/kg bw of lead nitrate for 14 days and observed disruption of the normal structural organization of the hepatic lobules and loss of the characteristic cord-like arrangement of the normal cells. Hepatocytes appear mostly hyperchromatic with occasional vacoulations and congestion of sinusoids. Treatment with both extracts (aqeous and ethanol) of *Ziziphusmauritiana* leaves were both observed to show lesser degenerative changes, this could be attributed to the protective potential of *Ziziphusmauritiana* leaf extract. Dahiru and Obidoa (2007) reported that pretreatment with aqeous extract of *Ziziphusmauritiana* leaf reduced the morphological changes that were associated with chronic alcohol administration. The presence of tannins, saponins and phenolic compounds observed in our extracts could be responsible for the observed effects.

Reticular fibres provide most of the supporting connective tissue of the liver (Cotran*et al*., 2005). They line the sinusoids, surrounding the individual sheets of liver cells (hepatocytes) forming the only fibrous connective tissue components supporting the cells (Ross *et al*., 2006). Reticular fibres also facilitate the interchange of substances between the hepatocytes and the blood, which flows in irregularly shaped blood vessels (sinusoids) between the hepatocytes. The present study shows that lead exposure causes a decreased in the distribution of reticular fibres in liver parenchyma. This observation is similar with the findings of Omotoso*et al.*, (2015) who administered 50mg/kg bw of lead nitrate for 14 days and observed a degeneration of reticular fibres especially around and in the wall of the central vein. However treatments with both aqeous and ethanolic*Ziziphusmauritiana* restored the

distribution of the reticular fibres to a near normal state. This could be owned to the protective potential of *Ziziphusmauritiana* leaves extracts.

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

From the present studies it can be concluded that treatment with aqueous and ethanol extracts of *Ziziphus mauritiana* leaves may be ameliorative against the adverse effects associated with lead exposure on the liver in adult Wistar rats, observed from the Liver function test, oxidative stress studies, haematological parameters, lead accumulation and histological studies. Thus, aqueous and ethanol extracts of *Ziziphus mauritiana* leaves maybe useful therapy in lead exposed patients, especially in lead poisoning endemic area.

6.2 Recommendations

From the present study, we recommended that;

1. The use of *Ziziphus mauritiana* leaves should be encouraged especially in the population who may be exposed to increased risk of lead poisoning.
2. Further work should be carried out on the protective role of *Ziziphus mauritiana*, since it contains flavonoid which is a known plant antioxidant.
3. Further study should be carried out using immunohistochemical methods to elucidate the deposits of lead in respective tissues of the liver of exposed animals treated with *Ziziphus mauritiana* leaves extracts.

6.3 Contribution to Knowledge

Oral Administration of *Ziziphus mauritiana* at high dose following lead exposure significantly increased SOD acttivities (12.24±0.17 and 11.68±0.40) of Aqueous and Ethanol extracts respectively compared with natural recovery (9.11±0.25).

Oral Administration of *Ziziphus mauritiana* at high dose following lead exposure significantly increased CAT activities (22.89±1.64 and 23.53±1.38) of Aqueous and Ethanol extracts respectively compared with natural recovery (11.36±0.29).

Oral Administration of *Ziziphus mauritiana* at high dose following lead exposure significantly reduced ALT activities (22.80±2.65 and 20.83±0.74) of Aqueous and Ethanol extracts respectively compared with natural recovery (38.83±0.88).

Both aqueous and ethanol extracts of *Ziziphus mauritiana* were seen to pocess hepatoprotective potentials from the study, however ethanol *Ziziphus mauritiana* extract was more effective.

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Appendix I

Lorke method of determination of LD_{50} of Animals

Preparation of doses

The ethanol extract of *Ziziphus mauritiana* leaves was weighed and the appropriate dose dissolved in distilled water to make a solution. The dose was contained in mg per kg body weight of the rats and prepared shortly before administration.

Administration of extract

The extract was administered orally, for the first phase, 9 rats were used and were divided into group A, B and C, with three rats in each group.

For group A, 1000mg/kg body weight was administered

For group B, 100mg/kg body weight was administered

For group C, 10mg/kg body weight was administered

Observation in first phase

There was no weakness, behavioral changes or death recorded after the administration of the extract. Hence the LD_{50} lies above 1000mg/kg. For the 2nd phase, the remaining 3 rats were used. Each of the animals was given different dose.

The 1st rat administered 5000mg/kg body weight

The 2nd rat was administered 2900mg/kg body weight

The 3rd rat was administered 1600mg/kg body weight

Observation in second phase

There was no behavioral changes or death recorded after the administration of the extract. Hence, the final oral LD_{50} of the rats is above 5000mg/kg.

Appendix II

Phytochemical screening

Test for Glycosides:

Keller-Killani Test: Glacial acetic acid was added into 2 ml. extract and one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown color appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides (Kokate et al., 2001).

Molisch's Test: 2 drops of Molisch's regent was added into 1 ml of extract, and 2 ml of concentrate H_2SO_4 was added carefully into above solution. Formation of violet ring at the junction indicates the presence of glycosides (Kokate et al., 2001).

Test for saponins

Foam Test: The extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicates the presence of saponins (Kokate et al., 2001).

Test for Sterols

Liebermann-Burchard Test: chloroform was mixed into 2ml. extract. 1-2 ml. acetic anhydride and 2 drops of concentrated H_2SO_4 were dropped into the test tube. First red, then blue and finally green colour indicates the presence of sterols (Kokate et al., 2001).

Test for Flavonoids

Shinoda Tests:- 2-3 ml. extract and few fragments of magnesium metal were added into a test tube, followed by dropwise addition of concentrated HCl. Formation of magenta colour indicates the presence of flavonoids (Kokate et al., 2001).

Test for Alkaloids

Dragendorff's Tests: Few drops Dragendorff's reagent was added into 2 to 3 ml extract. Formation of orange brown precipitate indicates the presence of alkaloids (Kokate et al,. 2001).

Appendix III

Group 1= Control (distilled water 2ml/kg body weight)

Group 2= 120 mg/kg Lead Acetate

Group 3= 120 mg/kg Lead Acetate + Low Dose of *Z. mauritiana* (Aqueous Extract)

Group 4= 120 mg/kg Lead Acetate + High Dose of *Z. mauritiana* (Aqueous Extract)

Group 5= 120 mg/kg Lead Acetate + Low Dose of *Z. mauritiana* (Ethanol Extract)

Group 6 = 120 mg/kg Lead Acetate + High Dose of *Z. mauritiana* (Ethanol Extract)

Group 7 = 120 mg/kg Lead Acetate + 10 mg/kg of Succimer

Group 8 = 120 mg/kg Lead Acetate + Distill Water (Natural Recovery).