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# Micro-Anatomical and Hematological Study of Aqueous and Ethanolic Extracts of *Ziziphus Mauritiana* Leaves on the Liver of Lead Acetate Treated Adult Male Wistar

* [1]Gebi, M.I., [1]Adebisi, S.S., [2]Mohammed, S.A. and [1,3]Usman, I.M.
[1]*Department of Human Anatomy, College of Medicine and Health Science, Ahmadu Bello University, Zaria, Nigeria*
[2]*Department of Human Physiology, College of Medicine and Health Science, Ahmadu Bello University, Zaria, Nigeria*
[3]*Department of Human Anatomy, Faculty of Biomedical Sciences, Kampala International University, Uganda*
**Corresponding Author :Gebi, M.I.*

**Abstract:** Lead poisoning is known to cause a number of adverse effects including neprophaty, infertility, liver, testis and heart damages in human and experimental animals. The present study was aimed at evaluating the effects of Aqueous and Ethanol extracts of *Ziziphus mauritiana* leaves (AZM & EZM respectivly) in lead acetate induced liver toxicity in adult male Wistar rats. Forty (40) Adult male Wistar rats were divided into 8 groups of containing 5 rats each. 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. Rats in Group 2 were sacrificed on the 22nd day of the administration. From the 22nd to 35th day; Groups 3 and 5 were treated with 100mg/kg bwt of AZM and EZM respectively, Groups 4 and 6 were treated with 400mg/kg bwt of AZM and EZM respectively, Group 7 was treated with 10mg/kg bwt of Succimer, Group 8 was administered with distilled water. At the end of the administrations the experimental rats were sacrificed humanely, blood samples and liver tissue were collected for haematological and histological studies respectively. The result of the present study showed alteration in body weight, organ body weight ratio, haematological indices and the liver histo-architechture of rats exposed to lead acetate, which were ameliorated following treatment with AZM and EZM. Thus, the present study has concluded that AZM and EZM at doses of 100mg/kg bwt and 400mg/kg bwt were 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.

**Keyword**: Lead acetate, Liver, Wistar rat, *Ziziphus mauritiana*

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## I. INTRODUCTION

Lead is a poisonous metal, which exist in both organic and inorganic forms in the environment (Shalan *et al*., 2005). In many developing and industrializing countries, occupational and environmental exposures to this metal remain a serious problem (Tong *et al*., 2000). Lead is primarily found in leaded gasoline (Tong *et al*., 2000). Among urban residents, particularly in areas with congested traffic, automobile emissions have been an important source of lead exposure (Rahbar *et al*, 2002). 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, colica pictonum, saturnism, Devon colic, or painter's colic, which is a medical condition caused by increased levels of lead in the body (Rossi, 2008). The manifestations of lead poisoning in humans are nonspecific, and may include weight loss, anemia, nephropathy, infertility, liver, testis and heart damages (Kim *et al.*, 2015). Symptoms of lead toxicity include abdominal pain, confusion, headache, anaemia, irritability, and in severe cases seizures, coma and death (Barbosa *et al*., 2005).

The use of medicinal and aromatic plants for the treatment of diseases is as old as mankind (Sofowora *et al*., 2013). 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 (Karthikeyan *et al.*, 2009), secondary metabolites are grouped as alkaloids, glycosides, flavonoids, saponins, tannins and essential oils. 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). *Ziziphus mauritiana* 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). The present study aim to evaluate the changes in body weight, organ body ratio, haematological parameter and micro-anatomy of the liver following treatment with aqueous and ethanolic extracts of *Ziziphus mauritiana* leaves in lead acetate exposure in adult male Wistar.

## II. MATERIALS AND METHODS

**Materials**

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

**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 fed with rat chow and water was allowed *ad libitum.* Animal handling was carried out in accordance with animal use and care policy guideline Ahmadu Bello University.

**Obtaining 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 authentication.

**Obtaining and identification of *Ziziphus mauritiana* leaves**

Fresh leaves of *Ziziphus mauritiana* were obtained from a private farmland in GRA Zaria, Zaria local Government, Kaduna state of Nigeria. The leaves were identified and authenticated with a voucher number of 3253 by Mallam Namadi Sanusi at Department of Biological Science Herbarium, Ahmadu Bello University, Zaria.

**Preparation of extract**

Extraction of *Ziziphus mauritiana* was done in the Department of Pharmacognosy and drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The fresh leaves of *Ziziphus mauritiana* collected were washed thoroughly, shade-dried and pulverized using a mechanical grinder for the purpose of aqueous and ethanolic extraction.

**Aqueous extraction of *Ziziphus mauritiana* leaves:** Aqueous extraction of *Ziziphus mauritiana* leaf was carried out as outlined by Ofodile *et al*., (2013). The powder obtained was macerated with distilled water for 72 hours at 30±4°C. The mixture was filtered, followed by concentration of the filtrate in water bath at 50°C. Finally, the concentrated crude aqueous extract was subjected to drying.

**Ethanolic extraction of *Ziziphus mauritiana* leaves:** The powder obtained was macerated by putting 1kg put in 4 liters of 70% ethanol for 48 hours. The mixture was 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).

**Dosage determination**

After establishing the LD50 of the leaves extract of aqeous and ethanolic *Ziziphus mauritiana* to be above 5000mg/kg bw, a doses of 100mg/kg bwt and 400mg/kg bwt (low and high dose respectively) were used in this study for both aqeous and ethanolic extracts.

Based on the reported oral LD50 of Lead acetate which was 600 mg/kg bwt for Wistar rats (Sujatha et al., 2011), 20% of the LD50 (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).

**Experimental design**

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

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

**Group 2** was administered 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** was administered 120 mg/kg bwt of lead acetate for 21 days and then administered with 100mg/kg bwt of aqeous extract of *Ziziphus mauritiana* leaves from day 22 to day 35 of the administration.

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

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

**Group 6** was administered 120 mg/kg bwt of lead acetate for 21 days and then treated with 400 mg/kg bwt of ethanolic extract of *Ziziphus mauritiana* leaves from day 22 to day 35 of the administration.

**Group 7** was administered 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.

**Group 8** was administered 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.

All the administrations were carried out orally once per day.

**Wistar rats sacrifice**

After the last administration, the rats were fasted for 24 hours before they were humanely anaesthesized by using ketamine. Blood samples were collected via heart puncture from each rat and stored in tripotassium salt of ethylenediamine tetra acetic acid (K3 EDTA) coated sample bottles for haematological estimation.

Incision was made through the abdomen to allow access to the liver, which were removed, weighed with an electronic analytical and precision balance (BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany). The liver tissue were fixed in 10% neutral buffered formalin solution, and then taken to the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria for subsequent tissue processing.

**Determination of haematological parameters**

The blood samples collected into anticoagulated (EDTA) specimen bottle were analyzed within 6 hours of collection using systemex XE-2100 Automated Haematology Blood Analyser. Each blood sample was well mixed at room temperature using a blood rotor machine for five minutes. The automated analyzer was put on about 30 minutes for the system to warm. A 10 µl of blood sample was aspirated through tube of the machine. Hematological parameters; differential leucocytes count (DLC), white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV), hemoglobin concentration (Hb) and Platelets count were analyzed.

**Tissue preparation and microscopy**

The tissue processing method for the hist-pathological studies was carried out as outlined by Jali *et al.*, (2015). The stages of the technique include; fixation, dehydration, clearing, infiltration, embedding and sectioning. Haematoxylin and Eosin (H and E) staining technique was employed to demonstrate the general tissue architecture. Photomicrographs were snapped using MD900 Amscope digital camera and a light microscope**.**

**Data analysis**

All the results were analyzed using the Statistical Package for Social Sciences (SPSS version 16). 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 post-hoc test. Values were considered statistically significant at p-value less than to 0.05.

## III. RESULTS

**Changes in weight and organ body weight ratio**

Fig.1 Showed that weight was affected by the Lead Acetate treatment as the weight gain was significantly lower ($p \leq 0.05$) when compared to control group. However, treatment with extract at low and high doses therapeutically resulted in significant weight gain higher than lead acetate treated group ($p \leq 0.05$) and no significant change ($p \leq 0.05$) was observed between aqueous and ethanolic extract at both high and low dose, but there was a significant difference between the high and low dose in both extract. A significant difference was observed ($p \leq$

0.05) between the standard drugs treatment group and the high dose of both Extract (Aqueous and ethanol extraction). Likewise a significant ($p \leq 0.05$) difference was not observed between the natural recovery group and the lead acetate treated group.

Fig. 2 shows the result of organ body weight ratio. Treatment with lead acetate was associated with significant increased ($p \leq 0.05$) in organ body weight ratio when compared to the control group and the entire treatment groups. A significant difference was not ($p \leq 0.05$) observed between each of the treatment group compared with the control group. However, the natural recovery group had an insignificant increase ($p \leq 0.05$) in organ body weight ratio compared with the entire treatment group and the control group

**Changes in Hematological indices**

Table 1 showed a significant decreased ($p \leq 0.05$) in RBC, Hb, HCT, MCV, MCH and MCHC in the lead treated group when compared with the control group and the entire treatment group while a significant increased ($p \leq 0.05$) in WBC in the lead treated group compared with the control group and the entire treatment group. There was therapeutic increased in RBC, Hb, HCT, MCV, MCH and MCHC in the extract (Aqueous and Ethanolic) treated group compared with the lead treated and natural recovery group. The natural recovery group shows no significant change in RBC, Hb, HCT, MCV, MCH and MCHC compare with the lead treatmen group. The high dose of the extract proves to be therapeutically effective than the low dose of the extract and the extract treated group is more therapeutic effective than the standard drugs (Succimer) treatment groups.

**Histopathology of the Liver**

The histo-pathological findings obtained in this study showed relatively normal liver tissue from the control group (Plate 1). Treatment with lead acetate was hepatotoxic as seen below in the liver section characterized by lose of cellular membrane, due to fatty degeneration necrotic changes (Plate 2). Extract when administered to rats attenuated the changes associated with lead acetate toxicity by reducing vacuolation of the hepatocytes and necrotic changes (Plate 3, 4, 5 and 6), thereby restoring almost normal architecture of the liver. Treatment with the standard drug (Plate 7) also attenuated the effect of lead acetate exposure. There was no significant therapeutic effectiveness in the liver tissue micro-anatomy in the natural recovery group (Plate 8) compared with the lead acetate group.

## IV. DISCUSSION

**Changes in body weight and organ body weight ratio**

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 agreement with the report of previous studies where lead exposure in experimental animals was associated with reduction in growth rate (Barker, 2002). 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 Ethanolic) resulted in therapeutic weight gain though not significant. On the other hand low dose of the Ethanolic *Ziziphus mauritiana* leaf extract resulted in a therapeutic 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) 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. This result is in accordance with the work of Ibrahim *et al*., (2012), according to them the detected elevation in the organs weight was thought to be due to necrosis which could be attributed to the accumulation of the lipids in the organ. 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 *Ziziphus mauritiana* 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 ethanolic extracts of *Ziziphus mauritiana* leaves.

**Haematological indices**

These are simply normal values of blood in which deviations indicate anormaly and/or disease state (Ganong, 2005). The present study showed a significant reduction in Red blood cell (RBC), Hb, HCT, MVC, MCH and MCHC counts 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 increased 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 increased in RBC values and reduction in the WBC values in the *Ziziphus mauritiana* 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 ethanolic extracts of *Ziziphus mauritiana* 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 *Ziziphus mauritiana*.

**Histopathology of the liver**

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 in line 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 ethanolic) 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.

## V. CONCLUSION

From the present studies it can be concluded that treatment with aqueous and ethanolic extracts of *Ziziphus mauritiana* leaves may be of therapeutic value in the management of adverse effects associated with lead exposure, especially on the liver in adult Wistar rats, based on the results of this study.

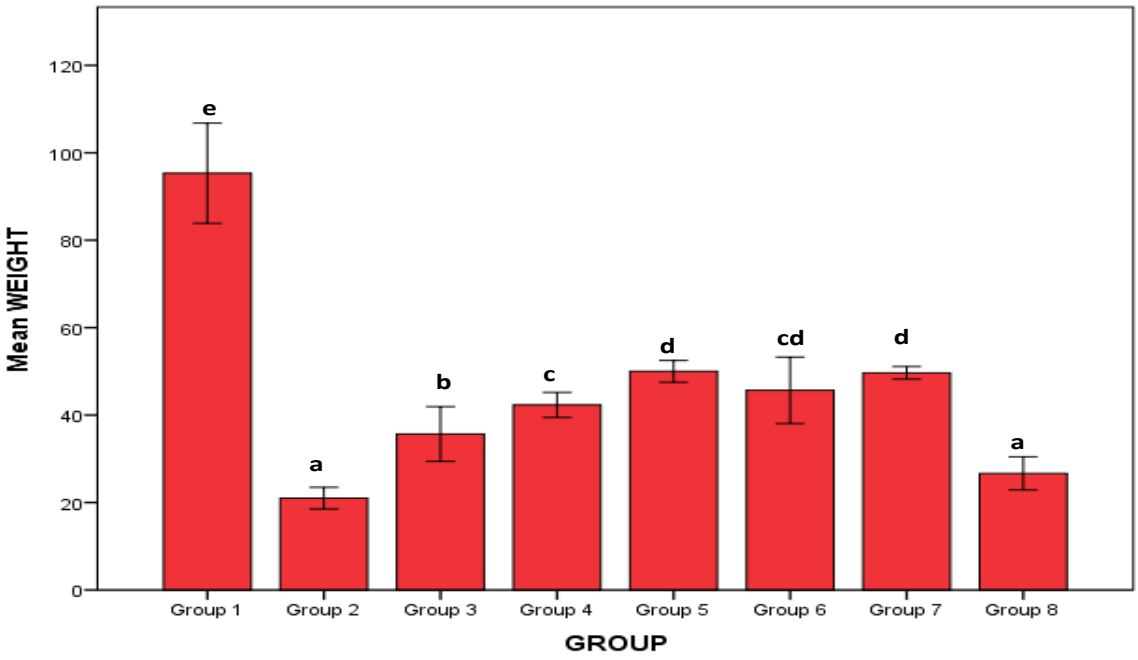



**Fig. 1: Change in Body Weight in Wistar rats following the administration of lead acetate and Ziziphus mauritiana extracts.**

**Legend:** n=5; Data are presented as Mean±SEM; Values along the same columns with different superscripts [a, b, c] and [d] are significantly different ($p \leq 0.05$).

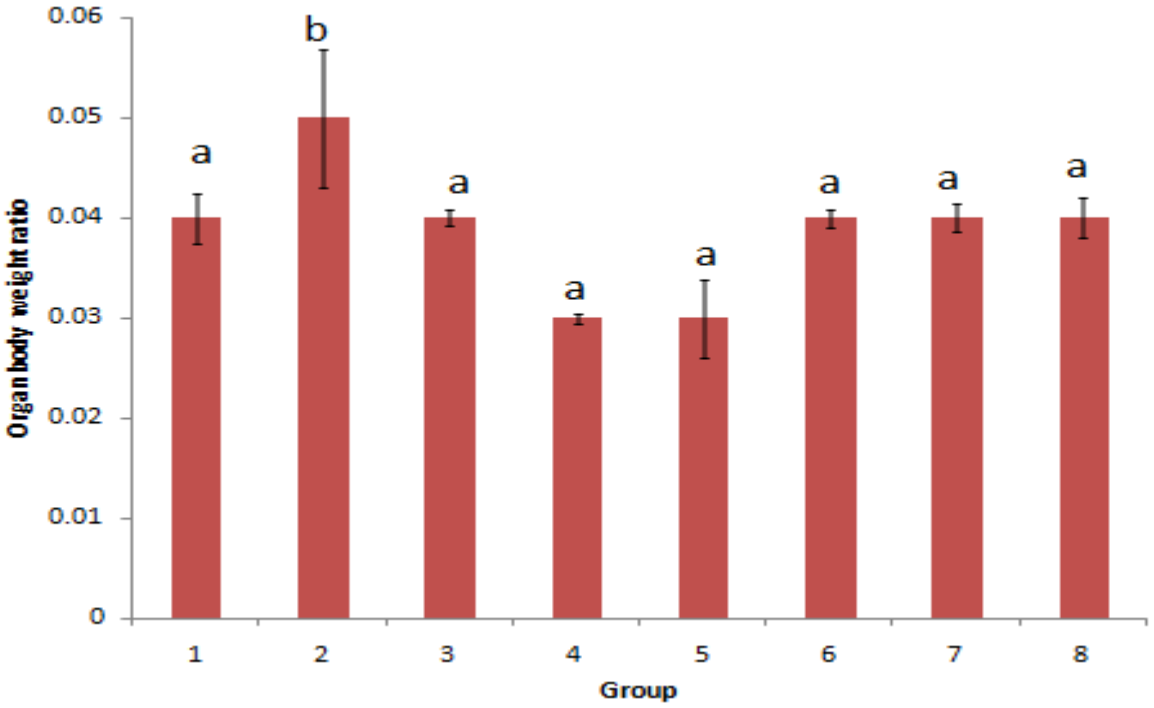



**Fig. 2: Change in Organ Body Weight Ratio in Wistar rats following the administration of lead acetate and *Ziziphusmauritiana* extracts.**

**Legend:** n=5; Data are presented as Mean±SEM; Values along the same columns with different superscripts [a] and [b] are significantly different ($p \leq 0.05$).

**Table 1: Change in Haematological indices in Wistar rats following the administration of lead acetate and *Ziziphusmauritiana* extracts**

| Group | Treatment | WBC | RBC | Hb | HCT | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|---|
| 1. | Control | 4.07 ± 0.47$^{a}$ | 8.07 ± 0.32$^{c}$ | 155.00 ± 1.15$^{f}$ | 0.45 ± 0.00$^{d}$ | 56.97 ± 0.52$^{e}$ | 18.43 ± 0.71$^{c}$ | 362.62 ± 9.39$^{d}$ |
| 2. | 120mg/kg Pb | 12.86 ± 1.14$^{b}$ | 3.65 ± 0.39$^{a}$ | 100.00 ± 2.89$^{a}$ | 0.23 ± 0.01$^{a}$ | 39.17 ± 2.75$^{a}$ | 8.77 ± 1.11$^{a}$ | 229.00 ± 5.29$^{a}$ |
| 3. | Pb + LW. AQ. ZM | 6.40 ± 1.00$^{a}$ | 6.38 ± 0.39$^{bc}$ | 117.67 ± 1.20$^{bc}$ | 0.32 ± 0.01$^{abc}$ | 52.23 ± 1.24$^{cde}$ | 15.27 ± 0.32$^{bc}$ | 310.67 ± 2.33$^{bc}$ |
| 4. | Pb + HG.AQ.ZM | 5.00 ± 0.75$^{a}$ | 7.19 ± 0.08$^{bc}$ | 132.67 ± 1.45$^{de}$ | 0.42 ± 0.01$^{cd}$ | 53.03 ± 0.68$^{cde}$ | 17.27 ± 0.09$^{bc}$ | 335.33 ± 0.88$^{c}$ |
| 5. | Pb + LW.ETH.ZM | 5.33 ± 1.14$^{a}$ | 5.81 ± 0.35$^{b}$ | 120.00 ± 0.58$^{c}$ | 0.35 ± 0.02$^{bcd}$ | 48.56 ± 1.68$^{bcd}$ | 14.83 ± 0.45$^{b}$ | 311.67 ± 1.20$^{bc}$ |
| 6 | Pb + HG.ETH.ZM | 4.07 ± 0.31$^{a}$ | 7.93 ± 0.50$^{c}$ | 134.00 ± 1.00$^{e}$ | 0.44 ± 0.01$^{d}$ | 55.23 ± 0.73$^{de}$ | 17.63 ±0.32$^{bc}$ | 337.00 ± 6.43$^{cd}$ |
| 7 | Pb + Succimer | 7.17 ± 1.04$^{a}$ | 7.92 ± 0.27$^{c}$ | 122.67 ± 1.45$^{c}$ | 0.38 ± 0.05$^{bcd}$ | 46.83 ± 0.95$^{abc}$ | 16.37 ± 0.13$^{bc}$ | 306.00 ± 2.65$^{b}$ |
| 8 | Pb + Recovery | 7.58 ± 1.40$^{a}$ | 3.75 ±0.33$^{a}$ | 108.33 ± 4.41$^{a}$ | 0.27 ± 0.16$^{ab}$ | 41.07 ± 2.46$^{ab}$ | 9.44 ± 1.22$^{b}$ | 231.00 ± 8.50$^{a}$ |

n=5; Data are presented as mean±SEM; Pb=Lead acetate; LW=Low, AQ= Aqueous, ZM=*Zizipus Mauritania*, HG= High, ETH= Ethanol, RBC=Red blood cells, WBC= White blood cells, Hb= Hemoglobin, HCT= Hematocrit, MCV=Mean corpuscular volume and MCHC= Mean corpuscular hemoglobin concentration. Values along the same columns with different superscripts $^{a,b,c}$ and $^{d}$ are significantly different ($p \leq 0.05$).

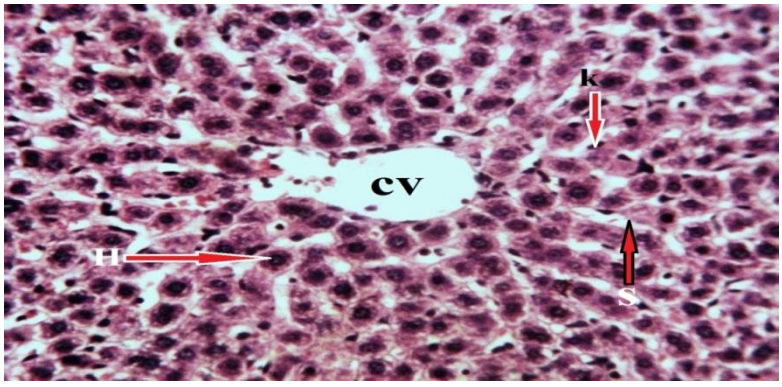
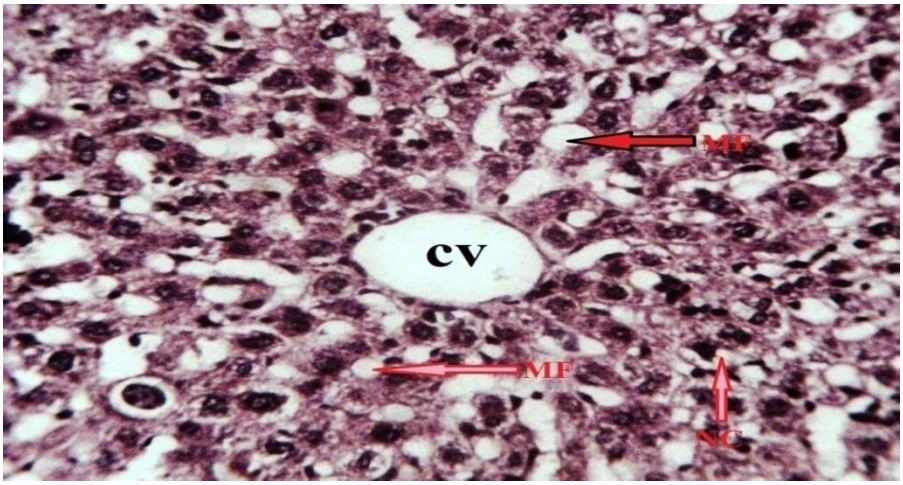



**Plate 1: Section of Liver tissue from Control group showing normal Hepatocytes (H) Central Vein (CV), Sinusoid (S) and Kupffer Cells (K) (H and E Stain x250)**




**Plates 2: Section of the Liver Tissue treated 120 mg/kg Lead Acetate showing both Macro and Microvascular fatty droplet (MF), loss of cellular boundaries including necrotic hepatocyte (NC)(H and E Stain)**

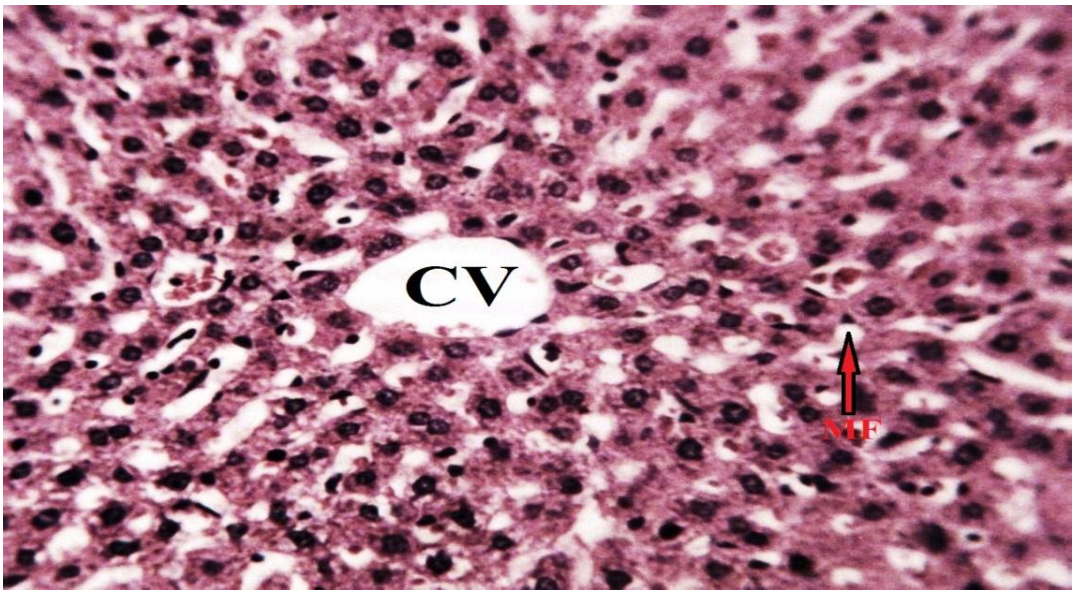



**Plate 3: Section of the Liver treated 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Aqueous Extract) showing slightly restored liver cytoarchitecture of the Central Vein (CV), with scanty microvascular Fatty droplet (MF) (H and E stain X250)**

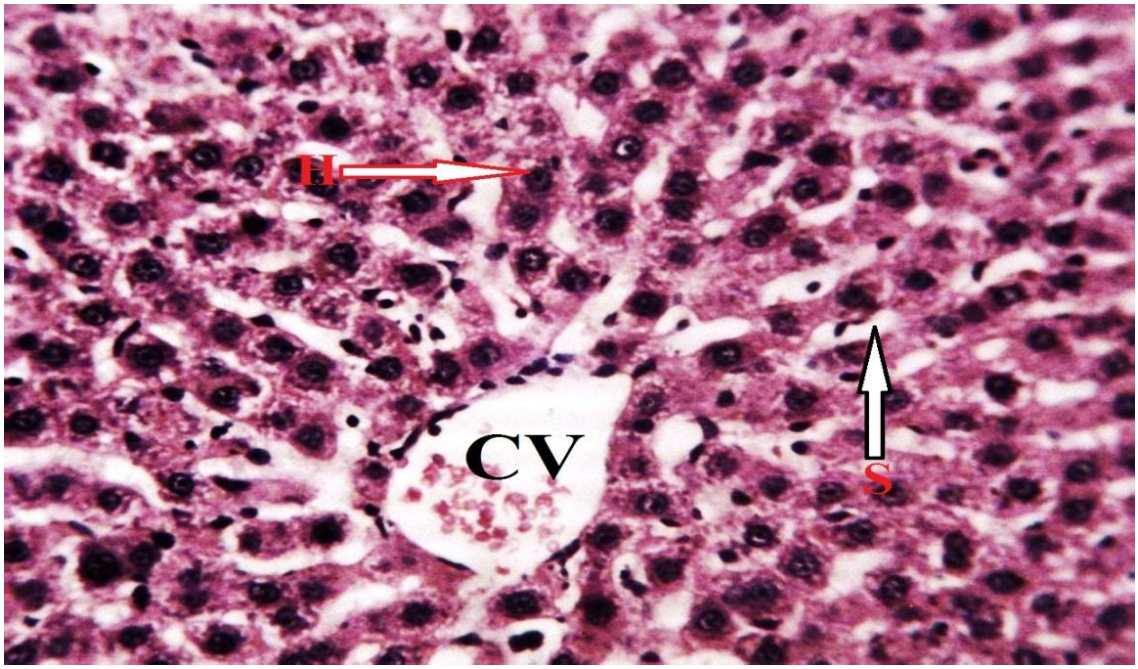



**Plate 4: Section of the Liver tissue treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Aqueous Extract) showing restored liver cytoarchitecture with scanty dilated Sinusoid (S) (H and E Stain, X250).**

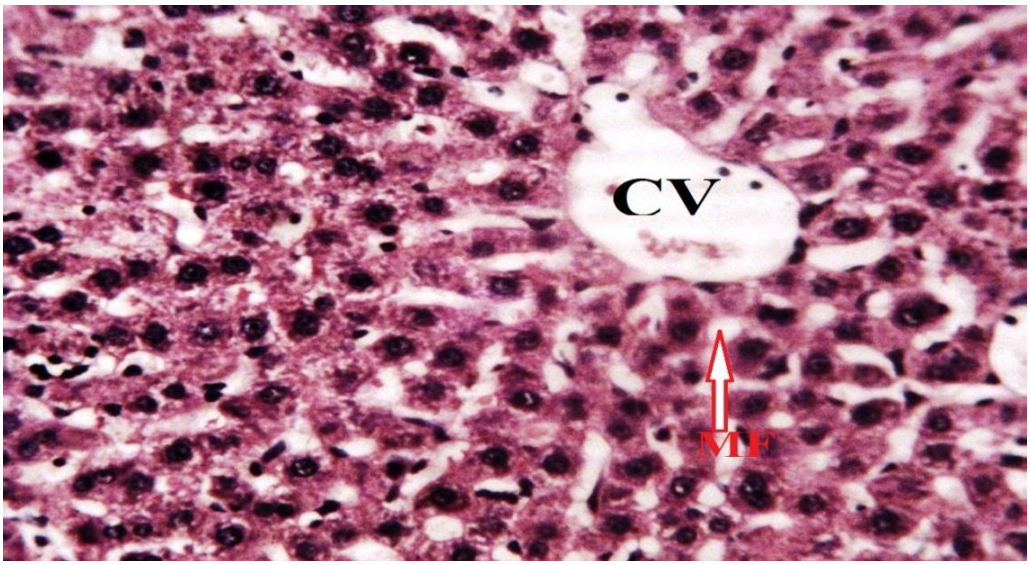



**Plate 5: Section of the Liver Treated with 120 mg/kgLead Acetate + Low Dose of *Z. mauritiana* (Ethanolic Extract) Restored Liver Cytoarchitecture with small amount of Fatty droplet (MF) (H and E Stain, X 250).**

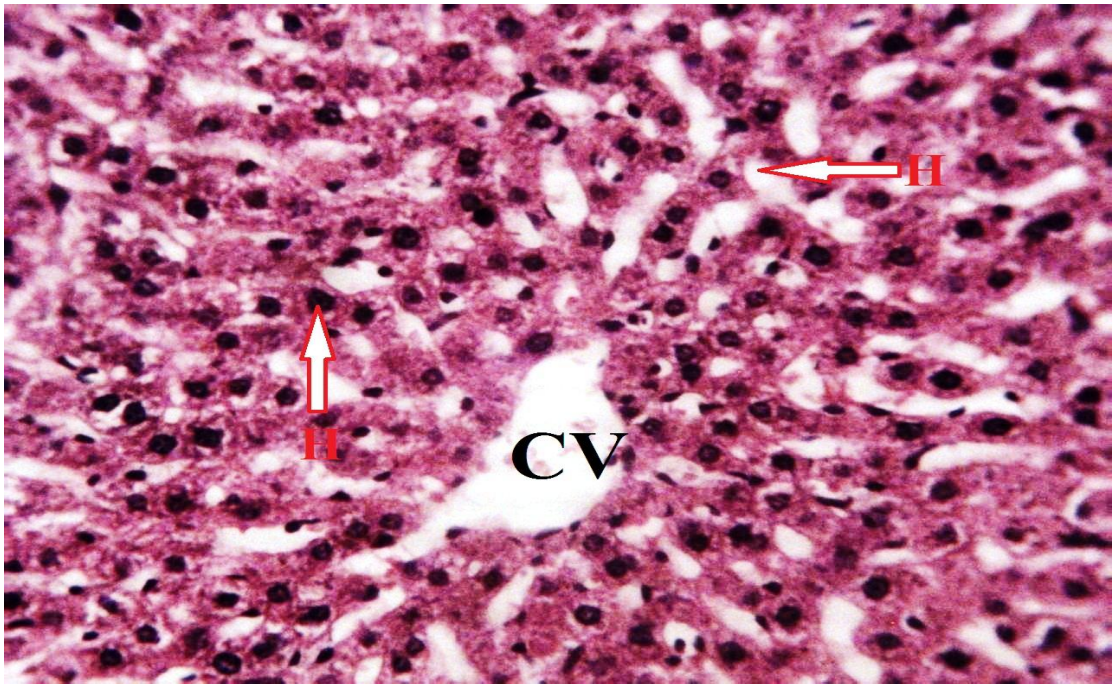



**Plate 6: Section of the Liver tissue treated with 120 mg/kgLead Acetate + High Dose of *Z. mauritiana* (Ethanolic Extract) showing restored liver cytoarchitecture with scanty dilated Hepatocyte (H) (H and E Stain, X250).**

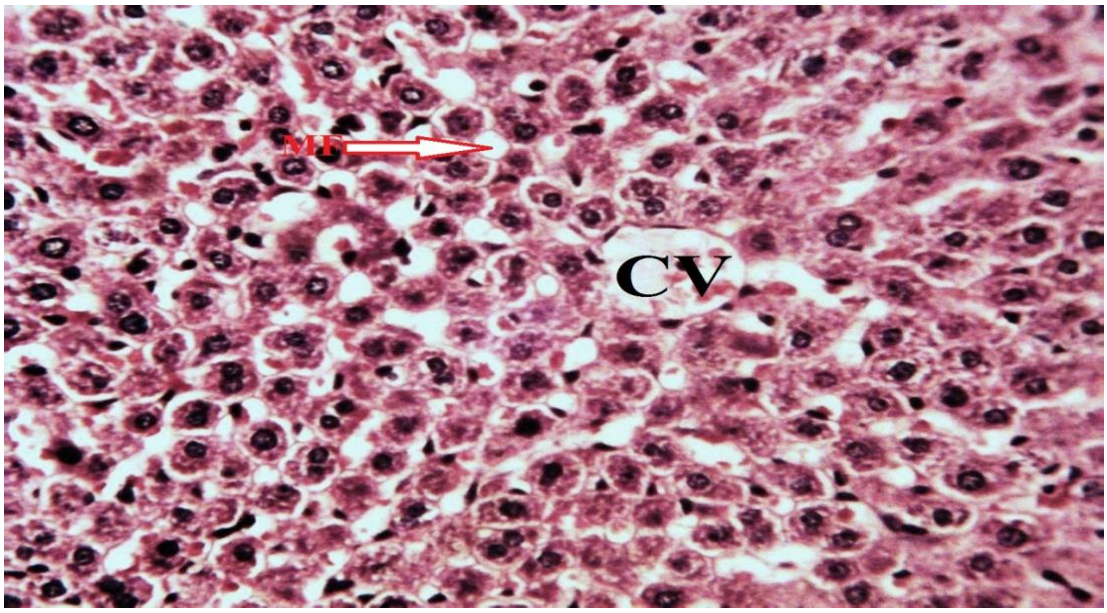
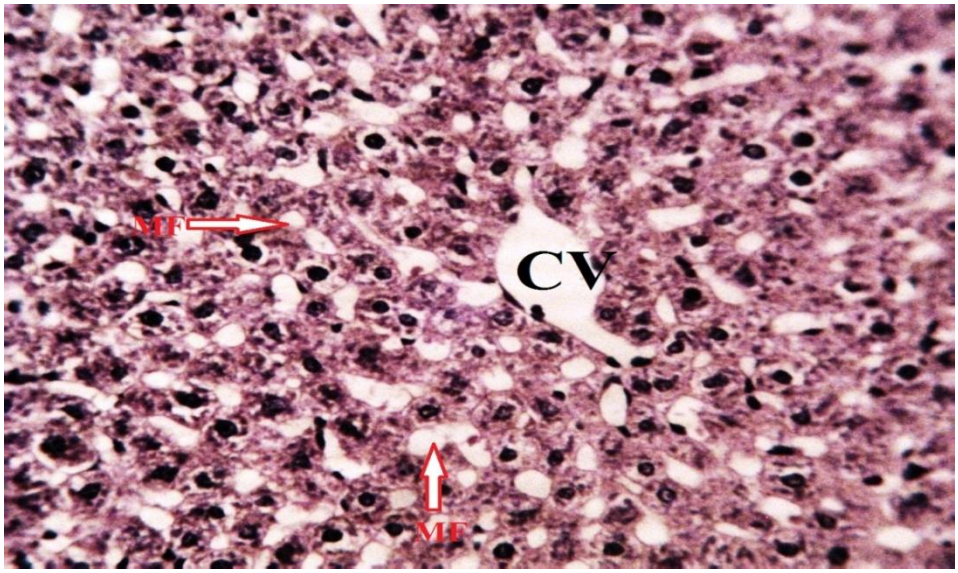



**Plate 7: Section of the Liver tissue treated with 120 mg/kgLead Acetate + 10 mg/kg showing restored liver cytoarchitecture with and Fatty Droplet (MF) (H and E Stain, X250).**




**Plates 8: Section of the Liver Tissue treated 120 mg/kgLead Acetate + Distill Water showing macro and microvascular fatty droplet (MF), loss of cellular boundaries including necrotic hepatocyte (S)(H and E Stain)**

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