*Journal of Anatomical Sciences*

*Email:anatomicaljournal@gmail.com*

*J Anat Sci 10 (2)*

# Histological and Biochemical Effects of Orally administered Lead acetate on the Liver of Adult Wistar Rats of both Sexes

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## ABSTRACT

Lead ($Pb^{2+}$) is a toxic heavy metal, unsafe even in small amounts. This study evaluated the histological and biochemical effects of lead acetate at different durations and dose on the liver of Wistar rats of both sexes. Thirty-Six (36) adult Wistar rats, were categorized into six groups, with each group containing 3 male and 3 female rats. Group 1 served as control for group 2 and 3. Group 2 and 3 received 30 and 60 mg/kg of lead acetate for 14 days respectively. Group 4 served as control for group 5 and 6. Group 5 and 6 received 30 and 60 mg/kg of lead acetate for 28 days respectively. At the end of the experiment, the rats were sacrificed and blood samples were collected in 5 ml plain bottle for liver enzymes evaluation. The liver tissue was excised for histological studies. A Significant ($p<0.05$) sex difference in serum levels of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) was observed in group 3 female Wistar rats when compared to group 3 male Wistar rats that were administered 60 mg/kg lead acetate for 14 days. Histological studies of the liver showed normal cytoarchitecture in the control group. However, in group 2, 3, 5 and 6 Wistar rats there were changes in the cytoarchitecture evidenced by vacuolation, ballooning degeneration and necrosis of hepatocytes**.** Exposure to lead acetate induces toxic effect on the liver of adult Wistar rats of both sexes and this is dependent on the dose and duration.

**Key words:** Lead Acetate, Liver, Wistar Rats, Histology, Liver Enzymes

## INTRODUCTION

Lead ($Pb^{2+}$) is a ubiquitous and toxic heavy metal which has caused extensive environmental contamination and significant public health problems in many parts of the world including Nigeria due to its widespread use in metal products, pipelines, ammunition, cables, as well as in paints and pesticides since prehistoric times[1,2]. Exposure of human populations to environmental lead was relatively low before the industrial revolution but has increased with industrialization and large-scale mining. Occupational and public health measures such as reduction in indiscriminate mining activities, the use of lead in petrol, paint and solder are being undertaken in order to control lead exposure[2]. However, cases of lead poisoning are still being reported. In Zamfara state, Nigeria, at least 400 people died between March and June 2010 from lead poisoning[3]. Also, between April and May 2015, 28 children in Kawo and Magiro villages in Niger state died owing to extremely high levels of lead in their bodies[4].

The liver is the first target organ of lead-induced injury because of its role in metabolism and its portal location within the circulation. Experimental animal studies have demonstrated alterations in hepatic cholesterol metabolism, vacuolar degeneration and necrosis of hepatocytes, increase in hepatic liver enzymes and decrease in antioxidant enzyme following exposure to lead[5,6,7]. However, few studies have related lead toxicity in the liver to the sex of the animal despite increasing evidence that health effects of toxic heavy metals differ in prevalence or are manifested differently in men and women[8]. Also, Significant gender differences in collagen content of the liver in rats have been found: 2.5 versus 1.9% in males and females, respectively[9] and it was shown that female rats have higher hepatocellularity with a larger proportion of binucleate hepatocytes[10]. These differences may have a possible implication in hepatic lead toxicity, therefore, this study is aimed at evaluating sex differences in biochemical and histological parameters following oral administration of lead acetate at different durations and dose on the liver of adult Wistar rats of both sexes.

## MATERIALS AND METHODS

**Experimental Animals:** Thirty-Six (36) apparently healthy adult Wistar rats, 18 males and 18 females were purchased from the Animal House of Human Anatomy Department, Faculty of Basic Medical Sciences, College of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. They were housed in clean plastic cages with soft wood shavings as their beddings

in the Animal House of the Department of Human Anatomy and allowed to acclimatize for two weeks prior to the commencement of the experiment. The animals were fed with pelletized vital feed manufactured by Grand Cereals and Oil Mills Ltd. Plateau State, Nigeria and allowed access to clean drinking water throughout the experimental period. All procedures were in accordance with the standard guideline for caring and using laboratory animals.

**Chemicals:** Analytical grade of Lead-acetate manufactured by Best of Chemical (BOC) Science was purchased from a reputable chemical store in Zaria, Kaduna, Nigeria.; Ketamine manufactured by Cayman Chemical was purchased from a reputable pharmacy in Zaria, Kaduna, Nigeria.

**Experimental Protocol:** Based on the oral $LD_{50}$ of lead acetate which was 600 mg/kg body weight for Wistar rats[11], 5% (30 mg/kg) and 10% of the $LD_{50}$ (60 mg/kg) were used in this study.

**Experimental Design:** A total number of 36 Wistar rats (18 male and 18 female) were distributed randomly into six groups with each group containing 3 male and 3 female rats that were kept separately.
Group 1 which served as control for group 2 and 3 were orally administered distilled water for 14 days. Group 2 and 3 were orally administered 30 and 60 mg/kg body weight of lead acetate daily for 14 days respectively. Group 4 which served as control for group 5 and 6 were orally administered distilled water for 28 days. Group 5 and 6 were orally administered 30 and 60 mg/kg body weight of lead acetate daily for 28 days respectively.

**Animal Sacrifice and Tissue Collection:** Twenty-four hours after the last administration, the animals were humanely sacrificed under anesthesia with ketamine at a dose of 75mg/kg IP[12]. through a midline incision on the anterior abdominal wall, the heart was accessed and blood samples were collected by cardiac puncture. The blood samples were stored in 5 ml plain bottle for biochemical evaluation of liver enzymes. The liver was quickly excised, rinsed in normal saline, gently blotted between the folds of a filter paper and was immediately fixed in Neutral Buffered Formalin (NBF) for subsequent histological studies.

**Biochemical assay of Liver Enzymes:** Blood in plain sample bottles were centrifuged at 3000 rpm using a centrifuge Machine (Made in England/ Serial No: 846307). The serum was collected and used to estimate the level of liver enzymes i.e. Aspartate Aminotransferase, Alanine Aminotransferase and Alkaline Phosphatase with the aid of Randox test kits according to the method described by Reitman and Frankel[13].

**Histological Studies:** The tissue preparation method used in the histological studies was as outlined by Bancroft and Stevens[14]. The tissues were stained using haematoxylin and eosin (H and E) technique for general liver histology at the Histopathology Department of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The stained sections were viewed under the light microscope at a magnification of ×250. Photomicrographs were taken using a digital microscope camera (AmScope Md900).

**Data Analysis:** All the data were analyzed using the Statistical Package for Social Sciences (IBM SPSS version 23, United States) and were expressed as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to compare the mean differences between and within the groups followed by Tukey's post-hoc test. Student t-test was used to compare mean differences between male and female Wistar rats. P-value less than to 0.05 was considered to be significant.

## RESULTS

**Biochemical Studies:** The result of the effect of lead acetate on liver enzymes is presented in Table 1 and 2. Serum levels of Aspartate Aminotransferase (AST) increased significantly ($p<0.05$) in group 3, 5 and 6 Wistar rats when compared to their respective control. This significant increase was found to be duration dependent when group 5 Male Wistar rats treated with 30 mg/kg lead acetate for 28 days was compared to groups 2 male Wistar rats treated with 30 mg/kg lead acetate for 14 days (Table 1). Serum levels of Alanine Aminotransferase (ALT) also increased significantly ($p<0.05$) in group 3 female, 5 and 6 Wistar rats when compared to their respective control. This significant increase was also found to be dose and duration dependent when group 2 and 3 male Wistar rats was compared with group 5 and 6 male Wistar rats respectively (Table 1). A significant increase ($p<0.05$) in Alkaline Phosphatase (ALP) was also observed in group 6 male and female Wistar rats (treated with 60 mg/kg lead acetate for 28 days respectively) when compared to the control (Table 1). Significant ($p<0.05$) sex difference in serum levels of AST and ALT was observed in group 3 female Wistar rats when compared to group 3 male Wistar rats that were administered 60 mg/kg lead acetate for 14 days as seen in table 2.

**Table 1:** Mean serum levels of liver enzymes of lead acetate treated Wistar rats.

| Parameter | Sex | Group 1 (Control 14 days) | Group 2 (30mg/kg Pb 14 days) | Group 3 (60mg/kg Pb 14 days) | Group 4 (Control 28 days) | Group 5 (30mg/kg Pb 28 days) | Group 6 (60mg/kg Pb 28 days) | P |
|---|---|---|---|---|---|---|---|---|
| AST (IU/L) | Male | 57.00±6.03$^{a}$ | 70.67±1.20$^{b}$ | 77.67±1.20$^{a}$ | 59.33±5.36$^{cd}$ | 90.67±3.84$^{bc}$ | 94.67±2.60$^{d}$ | 0.001 |
| | Female | 62.67±5.46$^{a}$ | 70.33±10.17 | 88.00±2.65$^{a}$ | 65.33±4.26$^{bc}$ | 92.00±2.08$^{b}$ | 96.00±2.08$^{c}$ | 0.026 |
| ALT (IU/L) | Male | 20.67±1.76 | 23.67±1.20$^{a}$ | 26.00±0.58$^{b}$ | 21.33±0.88$^{cd}$ | 42.33±2.91$^{ac}$ | 43.00±7.51$^{bd}$ | 0.047 |
| | Female | 20.67±0.67$^{a}$ | 35.67±6.69 | 39.33±0.67$^{a}$ | 25.00±1.73$^{b}$ | 34.00±3.06 | 43.33±2.40$^{b}$ | 0.046 |
| ALP (IU/L) | Male | 86.67±3.76 | 100.33±6.17 | 101.00±2.31 | 80.00±7.94$^{c}$ | 88.00±2.08 | 99.00±3.51$^{c}$ | 0.039 |
| | Female | 85.00±3.61 | 93.33±0.88 | 94.00±1.15 | 86.33±3.76$^{a}$ | 90.00±4.73 | 104.33±4.48$^{a}$ | 0.022 |

One-way ANOVA test followed by Tukey post hoc test. Results expressed as mean ± SEM. Cells carrying same superscripts on each row are significantly different (P<0.05). AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline Phosphatase

**Table 2:** Comparison of mean serum levels of liver injury biomarkers between male and female lead acetate treated Wistar rats.

| Group | Parameter | Male | Female | T | P |
|---|---|---|---|---|---|
| Group 1 (Control 14 days) | AST (IU/L) | 57.00±6.03 | 62.67±5.46 | -0.70 | 0.524 |
| | ALT (IU/L) | 20.67±1.76 | 20.67±0.67 | 0.00 | 1.000 |
| | ALP (IU/L) | 86.67±3.76 | 85.00±3.61 | 0.32 | 0.765 |
| Group 2 (30mg/kg Pb 14 days) | AST (IU/L) | 70.67±1.20 | 70.33±10.17 | 0.03 | 0.976 |
| | ALT (IU/L) | 23.67±1.20 | 35.67±6.69 | -1.77 | 0.212 |
| | ALP (IU/L) | 100.33±6.17 | 93.33±0.88 | 1.12 | 0.324 |
| Group 3 (60mg/kg Pb 14 days) | AST (IU/L) | 77.67±1.20 | 88.00±2.65 | -3.56 | 0.024* |
| | ALT (IU/L) | 26.00±0.58 | 39.33±0.67 | -15.12 | 0.000* |
| | ALP (IU/L) | 101.00±2.31 | 94.00±1.15 | 2.71 | 0.053 |
| Group 4 (Control 28 days) | AST (IU/L) | 59.33±5.36 | 65.33±4.26 | -0.88 | 0.430 |
| | ALT (IU/L) | 21.33±4.26 | 25.00±0.88 | -1.89 | 0.132 |
| | ALP (IU/L) | 80.00±1.73 | 86.33±7.94 | -0.72 | 0.511 |
| Group 5 (30mg/kg Pb 28 days) | AST (IU/L) | 90.67±3.84 | 92.00±3.84 | -0.31 | 0.776 |
| | ALT (IU/L) | 42.33±2.08 | 34.00±2.91 | 1.98 | 0.119 |
| | ALP (IU/L) | 88.00±2.08 | 90.00±4.73 | -0.39 | 0.718 |
| Group 6 (60mg/kg Pb 28 days) | AST (IU/L) | 94.67±2.60 | 96.00±2.08 | -0.70 | 0.524 |
| | ALT (IU/L) | 43.00±7.51 | 43.33±2.40 | 0.00 | 1.000 |
| | ALP (IU/L) | 99.00±2.40 | 104.33±4.48 | 0.32 | 0.765 |

AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; ALB: Albumin; GLB: Globulin

**Histological Studies**: Histological observations of liver sections from the control group of both sexes orally administered with distilled water showed normal cytoarchitecture of the liver parenchyma; the characteristic appearance of the hepatic cells (hepatocytes) having preserved cytoplasm, prominent nuclei and nucleoli, and hepatocytes radiating from the central vein. Fine vascular spaces separate the thin plate of hepatocytes, the sinusoids and Kupffer cells found within the sinusoid lining (Plate 1). Liver sections from group 2 Wistar rats orally administered with 30mg/kg lead acetate for 14 days showed vacuolation of hepatocytes in both sexes (Plate 2). Liver sections from group 3 Wistar rats treated with 60mg/kg lead acetate for 14 days showed some distortion in the cytoarchitecture of the liver parenchyma with vacuolation and ballooning degeneration of hepatocytes in both sexes (Plate 3). Liver sections from group 5 Wistar rats treated with 30mg/kg lead acetate for 28 days showed some distortion in the

cytoarchitecture of the liver parenchyma with vacuolation and ballooning degeneration of hepatocytes in both sexes (Plate 4). Liver sections from group 6 Wistar rats treated with 60mg/kg lead acetate for 28 days showed some distortion in the cytoarchitecture of the liver parenchyma with areas of focal necrosis of hepatocytes and cellular infiltration in both sexes (Plate 5).

**Histological Studies**

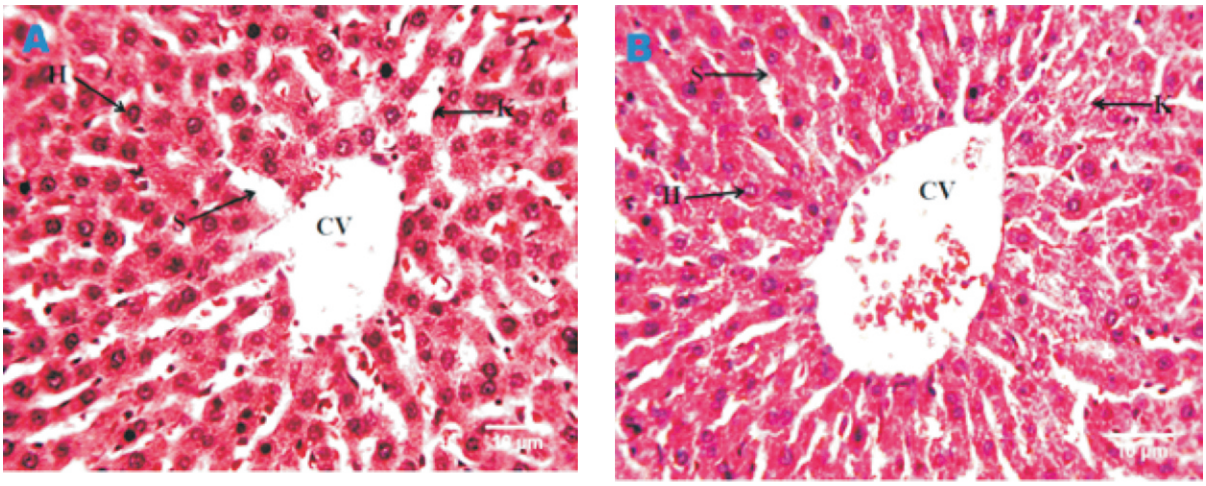


**Plate 1:** Photomicrograph of the liver of Male (A) and Female (B) Wistar rat of control group orally administered distilled water showing normal cytoarchitecture. Hepatocytes (H) Central Vein (CV), Sinusoid (S) and Kupffer Cells (K). (H & E stain; × 250).

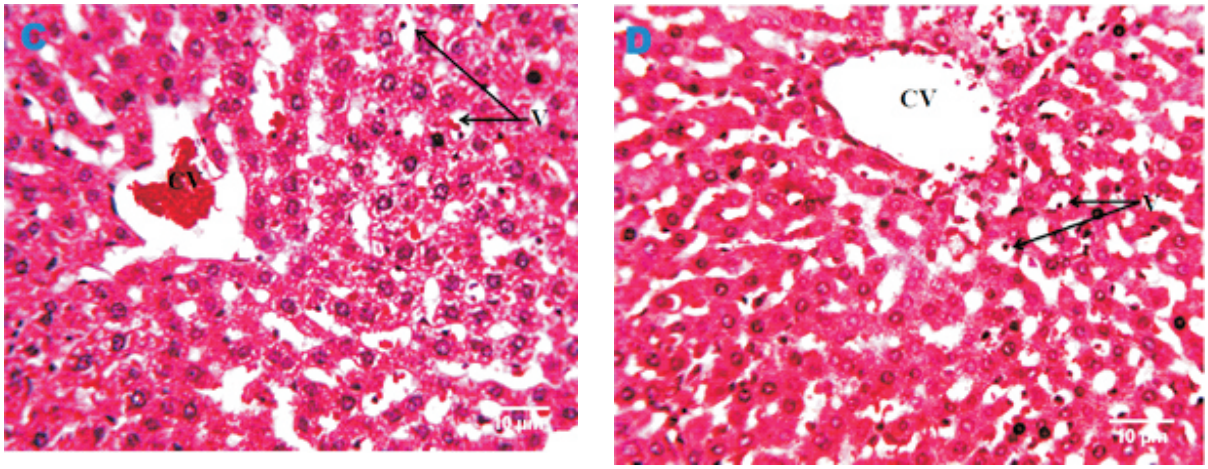


**Plate 2:** Photomicrograph of the liver of Male (C) and Female (D) Wistar rat of group 2 orally administered with 30 mg/kg of Lead Acetate for 14 days showing distortion in cytoarchitecture with Vacuolation of Hepatocytes (V). (Central Vein (CV); H & E stain; ×250)

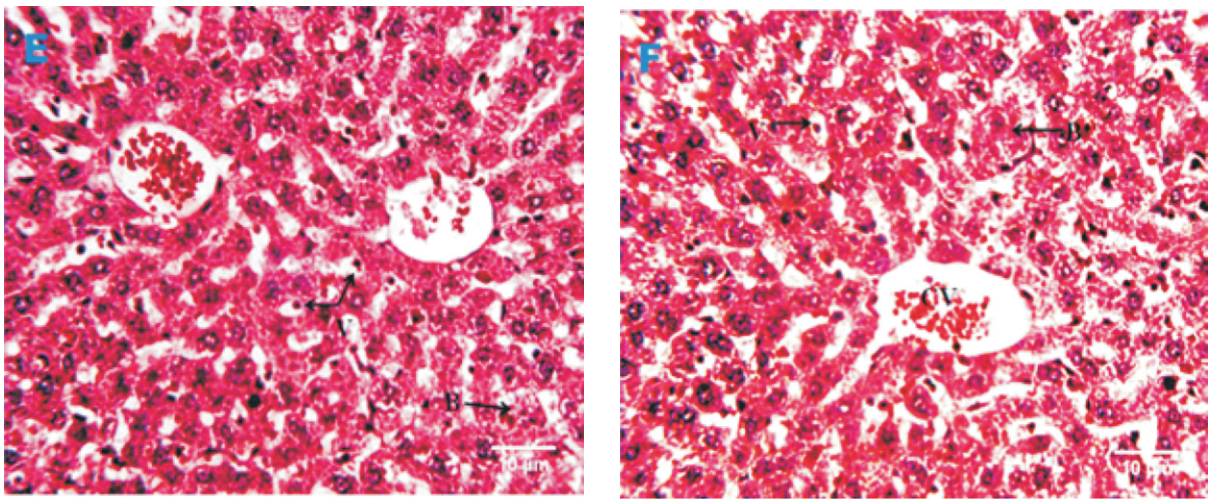


**Plate 3:** Photomicrograph of the liver of Male (E) and Female (F) Wistar rat of group 3 orally administered with 60 mg/kg of Lead Acetate for 14 days showing distortion in cytoarchitecture with Vacuolation of Hepatocytes (V) and Ballooning degeneration of Hepatocytes (B). (H & E stain; ×250)

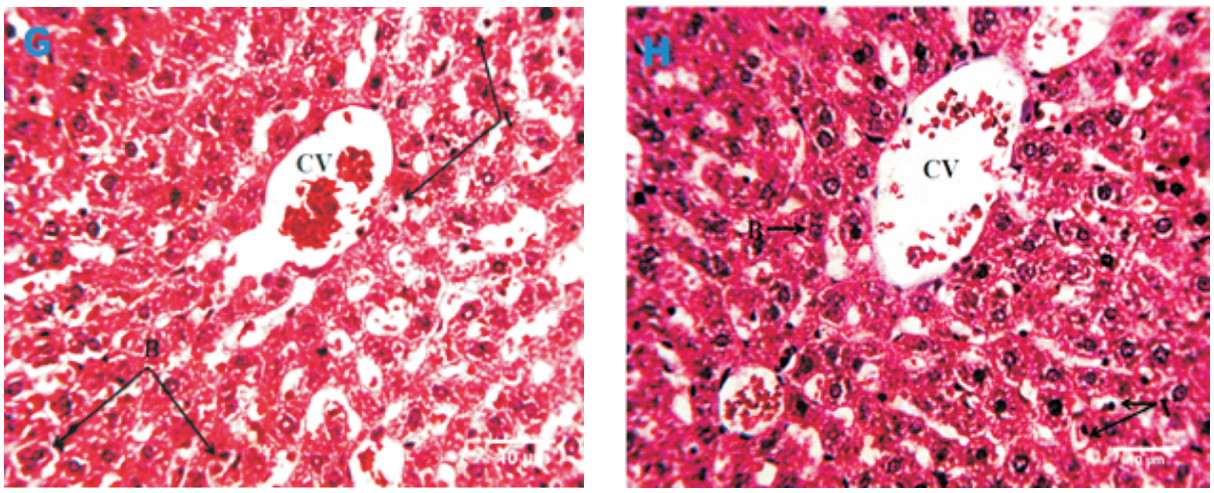


**Plate 4:** Photomicrograph of the liver of Male (G) and Female (H) Wistar rat of group 5 orally administered with 30 mg/kg of Lead Acetate for 28 days showing distortion in cytoarchitecture with Vacuolation of Hepatocytes (V) and Ballooning degeneration of Hepatocytes (B). (Central Vein (CV); H & E stain; ×250)

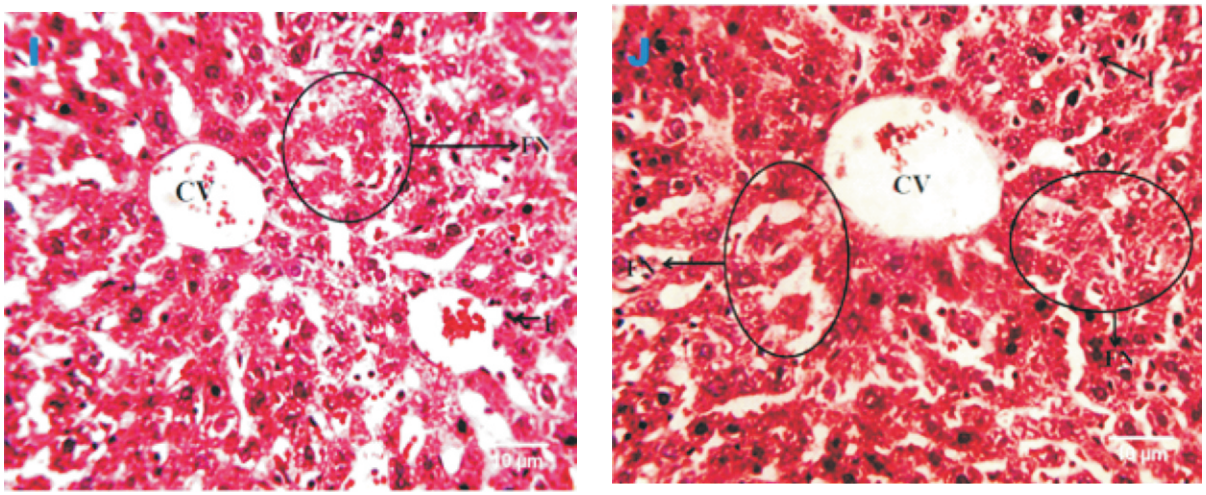


**Plate 5:** Photomicrograph of the liver of Male (I) and Female (J) Wistar rat of group 6 orally administered with 60 mg/kg of Lead Acetate for 28 days showing distortion in cytoarchitecture with areas of Focal Necrosis (FN) and Cellular Infiltration (I). (Central Vein (CV); H & E stain; ×250)

## DISCUSSION

The liver is the major target organ of toxicity due to its central role in xenobiotic metabolism and its portal location within the circulation[15]. Injury to the liver may affect the integrity of hepatocytes leading to the release of liver enzymes such as ALT, AST, and ALP since these enzymes are confined to hepatocytes and released into the blood following liver injury. Hence, these enzymes are commonly used as markers of hepatic injuries [16]. Lead absorbed into the liver can act by directly damaging the hepatocytes primarily by destroying the permeability of the cell membrane, with resultant release of cellular enzymes leading to increase their serum values[17]. In the present study, Wistar rats treated with lead acetate had an increased serum level of AST, ALT and ALP as compared with the control group in both sexes indicating hepatocellular damage, leakage of enzymes from cells and loss of functional integrity of cell. The level of AST and ALT was increased significantly in the groups that were treated with a higher dose and for a longer duration. This suggests that the effect of lead acetate on liver enzymes is dependent on its dose and hepatic tissue is more vulnerable to the toxic impact of lead acetate especially during continuous exposure for a longer time. The result of this study was consistent with previous findings[18,6,19] that reported that the activities of serum AST and ALT were significantly increased in lead exposed rats and [20] who reported a duration dependent increase in ALT, AST and ALP during exposure to lead acetate. However, the present result is contrary to previous findings [21] that reported a significant reduction in serum AST and non-significant decrease in serum ALT in lead exposed group. Also, significant difference in mean levels of AST and ALT was observed when both sexes were compared in group 3 Wistar rats administered lead acetate for 14 days with the female Wistar rats showing higher levels of liver enzymes in the serum compared to the male Wistar rats. This may be attributed to differential resistance to oxidative stress and cell damage due to a higher hepatocellularity in the liver of female Wistar rats [22,10].

Histopathological examination of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most sensitive end point for detecting organ toxicity[23]. It is also useful in providing information about acute or chronic effects of toxic substances that may not be detected by other biomarkers [24,25]. Light microscopic examination of the groups treated with lead acetate revealed various degrees of histological changes such as distortion in hepatic cytoarchitecture, vacuolation of hepatocytes, ballooning degeneration of hepatocytes and areas of focal necrosis of hepatocytes and cellular infiltration as compared to that of the control group. These changes were duration and dose dependent; the longer the duration and higher the dose, the more the damaging effects. Hepatic toxicity displays itself in the form of cell vacuolation which is a cellular defense mechanism against injurious substances[26]. The accumulation of fat droplets in the cytoplasm of the affected hepatocytes seen as vacuoles may suggest lead interference with lipid removal from these cells through impairment of ATP-dependent fatty acids [27]. The hepatocytes necrosis due to lead exposure might indicate oxidative stress on these cells due to antioxidant enzyme depletion. Several researchers also reported hepatocytes necrosis, vacuolization, and other signs due to lead toxicity in Wistar rats[26,27,728].

## CONCLUSION

Results from this study shows that female Wistar rats are more susceptible to the effect of lead acetate with respect to Aspartate Transaminase and Alanine Transaminase when compared to the male Wistar rats. Histologically no differences were observed in the liver sections of both sexes. However, further studies using stereological methods is recommended to determine if any sex differences actually exist.

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