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Evaluation of Gender-Specific Variation in Lead-Induced Hepatotoxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The heavy metal lead is toxic and triggers oxidative stress mediated toxicity in many organs, including the liver. This study aimed to evaluate the gender-specific variation in lead-induced hepatotoxicity in wistar rats. 10 male and 10 female Wistar rats (180-220g) were each divided into 2 groups (n=5 each): Control (M), Lead alone (M), Control (F), Lead alone (F). Male and female rats of the experimental groups administered a daily dose of 100 mg/kg/BW of lead acetate dispersed in distilled water for 21 days. All rats were anaesthesized and sacrificed 24 hours after the last administration. Blood was collected through cardiac puncture for biochemical analysis. Liver tissue was also collected, homogenized and analyzed for antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), lipid peroxidation (malondialdehyde). Results

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showed that the weight gain difference in lead alone (F) group is decreased significantly compared to the lead alone (M) group ($p<0.01$). Lead acetate induced oxidative damage, demonstrated by a significantly decreased antioxidant enzymes, significantly increased lipid peroxidation in both male and female experimental groups but these was more significant ($p<0.05$) in female than male. The findings also revealed that lead exposure induced hepatotoxicity with a significant increase in liver function markers. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) levels in the lead alone (M) and lead alone (F) group were also increased when compared to their respective control groups ($p<0.05$). Furthermore, the albumin and total protein level were significantly decreased in this study ($p<0.05$) when compared with their control groups. The study concludes that liver exposure induced hepatotoxicity in both male and female rat but more significantly advanced in female than the male. This advancement might have been mediated by the more lead-induced oxidative stress exhibited in the female than male rats.

Keywords: Lead acetate; oxidative stress; antioxidant enzymes; lipid peroxidation; liver function.

1. INTRODUCTION

Lead is a heavy metal, one of the leading causes of environmental toxicity. Several adverse effects are caused by its primary impacts on the hematological, hepatic, renal, and central neurological systems [1]. It is a hazardous environmental pollutant with no nutritional value, and has numerous detrimental impacts on the body [2]. Lead acetate is a white crystalline compound of lead with a sweetish taste [3]. Sources of heavy metals exposure including lead in particular are in mining, agriculture, coal production and burning [4]. One of the inappropriate characteristics of heavy metals is their easy access into the food as well as accumulation in the body of the organism [5]. Bioavailability of lead is either through inhalation of air or dust, food and water contaminated with this element [6]. The gastrointestinal system is the main route of absorption for lead acetate in Wistar rats, while the chemical may also be absorbed through the skin and inhalation [7]. After being absorbed, lead acetate spreads throughout the body and builds up in a number of organs, including the brain, liver, kidneys, and bones [8].

"The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a center for metabolism of nutrients and excretion of waste metabolite" [9]. The liver controls absorption and distribution of substances, but lead can cause liver damage, causing elevated blood enzyme levels and decreased protein synthesis, as confirmed by histopathological findings [10]. "Lead is absorbed, conjugated in the liver, and passed to the kidney, where a small amount is excreted, while the rest accumulates and interferes with body organ function" [11].

Among the numerous organs affected by lead exposure, the liver stand out due to their crucial roles in metabolism and excretion of xenobiotics [12]. However, prolonged exposure to lead can overwhelm the detoxification capacity of these organs, leading to hepatotoxicity characterized by hepatic damage and liver impaired function. Lead has effects on and many biological molecules through it influence on regulatory proteins and enzymes by changing the molecular signaling for these molecules [5]. Studies on rats exposed to lead acetate have shown changes in liver enzyme levels, histological abnormalities suggestive of liver damage, and oxidative stress in the liver tissue [13].

"The mechanism by which lead causes damage to the cell is principally through oxidative stress. It is an enlargement that can be easily accumulated in the cells, and can induce oxidative stress by lowering the action of endogeneous anti-oxidant and also by inducing the production of reactive oxygen species (ROS)" [14]. "Documentation has shown that chronic ingestion of lead leads to a significant decrease in liver enzymatic and non-enzymatic anti-oxidant including reduced glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) with concomitant increase in reactive oxygen species (ROS), Malondialdehyde (MDA) content, generation of superoxide anion ($O2$ •−), hydrogen peroxide ($H2O2$) content" [15-17]. "Similarly, ROS have been reported to play a critical role in both physiological and pathological conditions with resultant increase in DNA damage and apoptosis. The levels of hepatic and renal markers such as alanine aminotransferase, aspartate aminotransferase, triglycerides, cholesterol, urea, and uric acid have been

reported to increase significantly following administration of lead acetate" [18].

Gender-specific differences in response to toxic insults have been well-documented across a range of environmental pollutants and pharmaceutical agents [19]. Factors such as hormonal fluctuations, metabolic differences, and genetic predispositions contribute to variability in susceptibility and response between males and females [20]. In the context of lead toxicity, emerging evidence suggests that gender-specific variations exist in the absorption, distribution, metabolism, and excretion of lead, as well as in the molecular pathways involved in its toxicity [21].

Furthermore, evidence suggests that gender differences may influence the toxicokinetics and toxicodynamics of lead exposure [22]. For example, some studies have reported that female rats exhibit greater accumulation of lead in the liver and kidneys compared to males, potentially due to differences in hormone-mediated alterations in lead metabolism and excretion [23]. Therefore, lead toxicity poses significant health risk, particularly affecting the liver with evidence suggesting gender-specific variations in susceptibility. Despite existing research on general lead toxicity, there is a critical gap in understanding the precise hepatic gender-specific variances induced by lead exposure in Wistar rats. Gender disparities in hepatic detoxification pathways, enzymatic activities, and hormonal interaction may underlie this variations [24]. Furthermore, lead has been shown to exacerbate oxidative stress and inflammatory responses in the liver, impacting both genders [25].

This study evaluate and compare the hepatic effect of lead toxicity in male and female wistar rats, focusing on elucidating gender-specific differences in biochemical markers and oxidative stress responses. Understanding gender-specific susceptibility to lead-induced organ damage is of paramount importance due to its profound implications for public health [26]. It is substantiated that males and females indeed respond differently to lead exposure, this findings can inform targeted interventions and preventive measures to alleviate lead toxicity in at-risk populations [27]. And insights into the mechanisms underpinning gender-specific responses can significantly enhance the development of more efficacious medical interventions [28].

2. MATERIALS AND METHODS

2.1 Chemical and Compounds

Lead acetate (100g) $(CH_3CO_2)_2$ $Pb.3H_2O$ were acquired from Kermel, China. Normal saline, distilled water was purchased from Department of Pure and Applied Chemistry, LAUTECH, Oyo, Nigeria, Buffered formalin was purchased from Department of Anatomy, FBMS, LAUTECH, Oyo, Nigeria and Phosphate buffer saline was purchased from Department of Science Laboratory Technology, LAUTECH, Oyo, Nigeria).

2.2 Experimental Planning and Animals

The standard Farm of Laboratory Animals provided 10 male and 10 female adult Wistar rats, which were kept in a typical laboratory environment (12/12 h light/dark cycle, 25 ± 1 °C). Prior to the experimental activity, the rats were allowed two weeks of acclimation and unrestricted access to clean water and animal feed.

The period of the experiment istwenty-one (21) days. The groups and their treatments are described below.

Group 1: Control (M) in which the animals were given the usual water and food pellets throughout the period of the experiment.

Group 2: Lead acetate (M): Male rats were treated with daily dose of 100 mg/kg/BW of lead acetate through an oral means of administration for 21 days

Group 3: Control (M) in which the animals were given the usual water and food pellets throughout the period of the experiment.

Group 4: Lead acetate (F): Female rats were treated with daily dose of 100 mg/kg/BW of lead acetate through an oral means of administration for 21 days

2.3 Collection and Processing of Samples

Twenty-four (24) hours after the last oral administration of lead acetate, the animals were each per time placed inside a dessicator containing a chloroform soaked cotton wool for anaesthesia. Blood was collected through cardiac puncture with the use of 2ml syringe and needle. The blood collected into the plain bottles

were centrifuged at 2500 revolutions per minutes for 10 minutes to obtain serum. The serum was collected into Eppendorf bottles with Pasteur pipettes and refrigerated for biochemical analysis. The liver tissues from each animal was then swiftly removed, weighed, and homogenized in ice-cold Phosphate-buffer saline after being rinsed in ice-cold saline buffer (20 mM Tris-HCl, 0.14 M NaCl buffer, pH 7.4). In order to further examine the oxidative stress marker and antioxidant properties in the liver, the homogenate was centrifuged. The supernatants were carefully collected and stored at −20°C [29].

2.4 Biochemical Tests

2.4.1 Evaluation of liver markers

Serum liver transaminase enzyme (ALT and AST) activities were measured using kits from Human (Magdeburg, Germany). Spinreact (Barcelona, Spain) kits were used to assess alkaline phosphatase (ALP). Albumin, and total protein were measured using commercial kits obtained from Human (Magdeburg, Germany). All were determined using a spectrophotometer (BM, Magdeburg, Germany, model 5010) in accordance with the methods recommended by the manufacturer.

2.4.2 Evaluation of hepatic antioxidant parameters

Using commercial kits purchased from Bio-diagnostic (Cairo, Egypt), glutathione perioxide (GPx), catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) activities in liver tissues were evaluated spectrophotometrically in accordance with the enclosed pamphlets.

2.5 Analysis of Statistics

The study's numerical data were expressed as mean ± standard error of mean (Mean ± SEM). A one-way Analysis of variance (ANOVA) with Graph Pad Prism version 7.0 (Graph Pad statistical software, Inc., USA) was used to compare within groups and Tukey's Post-hoc test was used for multiple comparison. $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Result

Results showed that lead acetate has effect on animal weight gain in both male and female Wistar rat when compared to their control groups ($p<0.05$). The weight gain in lead alone (F) group is decreased significantly compared to the lead alone (M) group ($p<0.01$). This study induced oxidative damage, demonstrated by a significantly decreased antioxidant enzymes, significantly increased lipid peroxidation in both male and female animal but the significance is more prone in female induced group. In the lead alone (F) group, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were significantly decreased, while malondialdehyde (MDA) increased significantly compared to the lead alone (M) group ($p<0.05$). The findings also revealed that lead exposure induced hepatotoxicity with a significant increase in liver function markers. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in the lead alone (M) and lead alone (F) group were also increased when compared to the control groups ($p<0.05$). Furthermore, the Albumin and Total Protein (TP) level were significantly decreased in this study ($p<0.05$) when Lead (M) is compared to Lead (F).

3.2 Discussion

Oxidative stress is thought to be involved in lead-induced toxicity [17] and proposed to be a principal mechanism involved in lead toxicity. "One of the most important effects of lead poisioning is the induction of oxidative stress via the production of free radicals and lowering of antioxidant system. Free radicals are produced from endogenous (mitochondria, cytP450 mechanism, peroxisomes) as well as exogenous sources (xenobiotics, chemical reactions) as described" by Valko et al. [30], Patrick [31]. Oxidative stress induced by lead exposure can disrupt cellular homeostasis, leading to oxidative damage and alterations in organ weight. Increased levels of reactive oxygen species may contribute to enlarged liver [32]. Studies have shown that lead exposure can induce apoptosis and cell death in liver tissues, leading to structural changes and alterations in organ weight. Increased cell turnover may contribute to enlarged liver [33].

"The effects of the lead acetate on body weight gain (100mg/kg BW) of the male and female Wistar revealed that the body weight of the lead alone groups was significantly different from that of the control groups after 21 days of exposure. In this study, the weight gain of the male and female rats first increased with exposure time;

however, the rate of increase gradually decreased, and the weights gain slightly decreased at the end of study" [29]. A decrease in the rate of body weight gain was also observed in the study of Jadhav et al. (2007), "where it was explained as a progressively severe systematic toxemia and an aversion to drinking water containing a heavy metal mixture" [34,35].

The present study demonstrates that lead exposure significantly affects the organ weights of both male and female Wistar rats when compared to the control groups.

Table 1. Effect of lead acetate administration on the liver weight and weight gain in male and female Wistar rats

Weight (g)	Control (M)	Lead alone (M)	Control (F)	Lead alone (F)
Liver weight	6.92 ± 0.66	5.44 ± 0.22	6.98 ± 0.65	5.58 ±1.42
Body (weight gain)	11.60 ± 0.68	6.40 ± 0.51	13.80 ± 1.02	3.60 ± 0.60@

Mean body weight gain difference (+) (Experimental change compared with mean control values)
Data were represented as mean ± SEM., n=5. (P<0.05) was considered as statistically significant. @ represent a statistical significance in lead alone (F) groups when compared to lead alone (M) group.
Table 1 has a statistically significant decrease (P< 0.01)

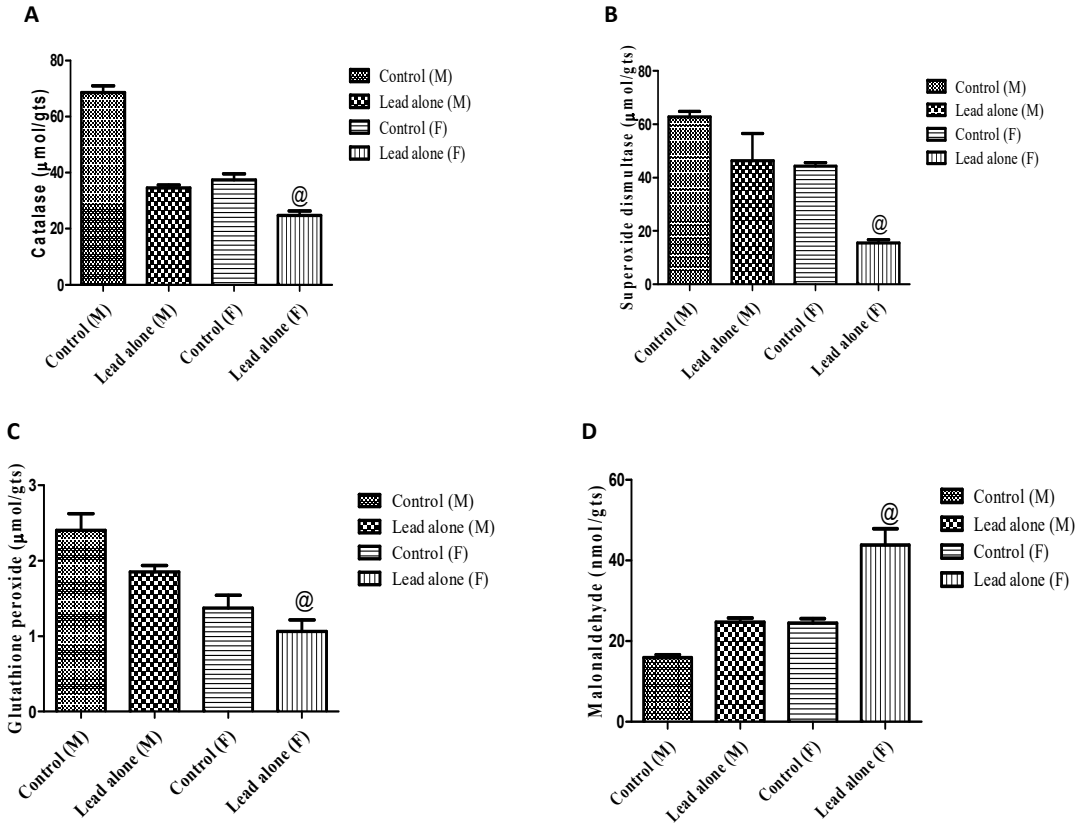

Fig. 1A-D. Effects of lead acetate on Hepatic Antioxidant System in male and female Wistar rats in both control and experimental groups

Data were represented as mean ± SEM., n=5. (P<0.05) was considered as statistically significant. @ represent a statistical significance in lead alone (F) groups when compared to lead alone (M) group
Graph A-C has a statistically significant decrease (P< 0.01)
Graph D has a statistically significant increase (P< 0.001)

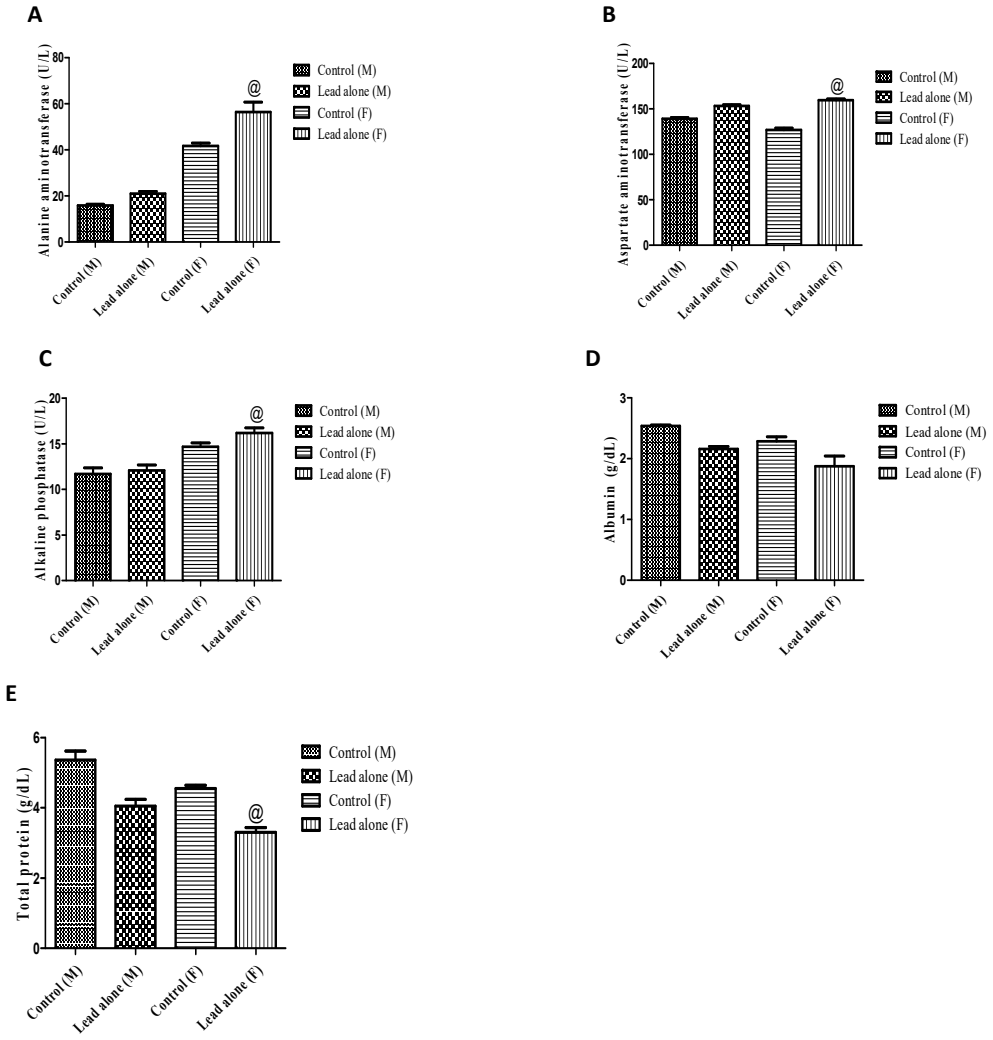

Fig. 2A-E. Effects of lead acetate on Liver Function Markers in male and female Wistar rats in both control and experimental groups

Data were represented as mean ± SEM., n=5. (P<0.05) was considered as statistically significant. @ represent a statistical significance in lead alone (F) groups when compared to lead alone (M) group
Graph A & C has a statistically significant increase (P< 0.001)
Graph B has a statistically significant increase (P< 0.01)
Graph E has a statistically significant decrease (P< 0.01)

Notably, the extent of these effects varies between genders, with females exhibiting more pronounced changes than males. This variation in females may be attributed to higher estrogen levels,which influence lead absorption, retention, and metabolic factors such as higher fat-to-body mass ratios. Male rats have higher levels of testosterone, which might confer some protective effects against lead toxicity. Testosterone has been associated with the upregulation of antioxidant enzymes, which can mitigate oxidative stress induced by lead exposure. Research indicates that females are more prone to lead-induced bone demineralization and oxidative stress due to lower antioxidant defenses.

Antioxidants investigated include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In this study, the observed decrease in SOD, GPx and catalase activities in the male and female lead alone groups is an indication of oxidative stress caused as a result of increase depletion of these

antioxidants by free radicals generated during the period of lead acetate induction. The observed statistically significant decrease in hepatic CAT levels in both male and female groups indicates a response to elevated oxidative stress. The pronounced reduction in females can be attributed to inherent baseline differences in antioxidant enzyme levels, with females typically having lower antioxidant activity than males which renders them more susceptible to oxidative damage under stress conditions [36].

Decreased SOD activity in the female lead alone group suggest a response to excessive superoxide radicals generated leading to increased lipid peroxidation and decreased activity of antioxidant enzymes such as SOD. Ramesh et al. (2006) found that female rats exhibited a more substantial reduction in SOD activity upon exposure to oxidative stressors compared to male rats, aligning with these findings [37]. The significant reduction in GPx activity in both genders compare to controls, with a more significant decrease observed in female aligns with previous research showing that estrogen, which has antioxidant properties, may enhance GPx expression under normal conditions but becomes overwhelmed during oxidative stress, leading to a substantial reduction in GPx activity [38].

Previous studies have documented that females generally exhibit lower baseline levels of antioxidant enzymes, when compared to male [39]. The baseline differences means that females may start with a lower capacity to neutralize ROS which makes them more susceptible to oxidative damage when exposed to lead [21]. Hormonal influences play a greater role in this differential response. Estrogen also known as estradiol (E2), which is prevalent in higher concentrations in female acts as a greater factor that impacts oxidative stress response genes but may not sufficiently upregulate the antioxidant activity to counteract lead-induced oxidative stress [40]. Conversely, testosterone, which is more abundant in males, is associated with elevated levels of catalase, Superoxide dismutase and other antioxidant enzymes [41]. This androgenic upregulation of antioxidant enzyme provides males with a greater protective buffer against the oxidative damage.

Moreover, the liver role in detoxification and metabolic process makes it particularly vulnerable to oxidative stress leading to increased cellular damage and inflammation [42]. Lead's interaction with estrogen signaling and metabolism may further disrupt antioxidant defenses in females, intensifying the reduction in CAT, SOD and GPx activity [43]. This hormonal disruption, combined with the already lower baseline antioxidant capacity in females may have resulted in increased vulnerability to lead-induced oxidative damage.

Malondialdehyde is a byproduct of lipid peroxidation, which occurs when oxidative stress damages cell membranes [44]. High levels of MDA in the blood or tissues can indicate increased oxidative stress and potential damage to cells, often associated with various diseases and conditions [45]. In this study, administration of lead acetate for 21 days resulted in significant increases ($p<0.001$) in MDA of Lead (M) group when compared with Lead (F) group. This clearly indicates an induction of oxidative stress during the period of lead exposure. Elevated MDA levels point to enhanced lipid peroxidation probably due to the production of superoxide, peroxyl, and hydroxyl radicals [46]. Generation of peroxyl radicals following intoxication by lead stimulates lipid peroxidation by production of endoperoxides through cyclization reactions [18].

However, the greater increase in MDA levels in females compared to males suggests that females might be experiencing a higher degree of oxidative damage under similar exposure conditions. The observed disparity in MDA levels between genders may be influenced by hormonal differences. As stated earlier, previous studies have demonstrated that estrogen can modulate the expression of antioxidant enzymes, thereby enhancing the cellular defense against oxidative stress [47].

“Increased liver enzymes are indicators of liver damage since they are released into the blood as a result of the loss of hepatocyte membrane integrity through lead exposure, which also result in lipid peroxidation” [48,49]. “The activity of ALT and AST are sensitive indicators of acute hepatic necrosis” [50]. Lombardi et al. [51] “reported that elevated activities of AST and ALT in plasma are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver. Hence, when elevated activity of ALT and AST is observed; liver function is already compromised which ultimately results in liver damage”.

“This study shows that lead acetate induced hepatotoxicity led to a significant increase

($p<0.05$) in the serum levels of liver enzymes (Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)) compared with the control rats of both male and female groups. These alterations following lead exposure could be the result of cell membrane modifications triggered by oxidative stress that cause the extended release of liver enzymes into the blood" [52]. "ALT and AST are often used to reflect the degree of hepatocyte damage, and ALT is generally a better indicator of this damage than AST. ALT is often released into circulation following hepatic injury" [53]. "These results demonstrate that the hepatocytes were damaged to some degree in both male and female rats, consistent with the results" of Nehad et al. (2013).

"The elevated Alkaline Phosphatase (ALP) levels in the lead-only groups also suggested that hepatobiliary tract cell death from subchronic lead exposure may have led to bile duct obstruction and infiltrative liver disease" [54]. "ALP has a role in several metabolic activities, including the synthesis of proteins, phospholipids, and nucleic acids; thus, the change in ALP activity may also have an impact on these processes" [55].

Furthermore, the findings from this studyare consistent with those of El-Boshy et al. [56], in which rats were exposed to lead acetate for 6 weeks at a dose of 500 mg/L. In addition, the study further revealed that lead acetate induced rats demonstrated a marked decline in their albumin and total protein. "Plasma proteins, particularly albumin (Alb), are predominantly synthesized in the liver therefore, the significant drop in serum total protein also indicated liver disease" [57]. "Lead disrupts a significant number of hepatocyte enzymes by attaching to plasma proteins, which prevents hepatocytes from producing proteins" [58]

Additionally, it decreases in the quantity of free amino acid used protein and interfere with intracellular Ca+2 signaling, which damages the endoplasmic reticulum [59,60]. Therefore, elevated Alkaline Phosphatase resulted from the impairment of bilirubin excretion caused by liver injury and/or hemolysis [61]. Increased hemolysis and liver damage might have contributed to the increased Alanine Aminotransferase (ALT) levels revealed in the study. Similar results were noted when rats were treated orally with lead acetate at a dose of 60 mg/kg for 28 days [62].

4. CONCLUSION

Lead toxicity poses a potential toxic effect as far as its concern. In this study, Liver damage was seen along with a considerable increase in free radical production and a severe inhibition of the antioxidant machinery. In all, lead toxicity is a major threat globally because the environment is heavily contaminated by it and female are more pronounced to it effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

The Animal Research Ethical Committee of the Faculty of Basic Medical sciences at Ladoke Akintola University of Technology, Oyo, Nigeria developed guidelines for all animal studies, and these regulations were adhered to throughout the research process (ERC Approval number: ERCFBMSLAUTECH:055/08/2024).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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