Original Research Article

Evaluation of Gender-Specific Variation in Lead-Induced Nephrotoxicity in Wistar rats.

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ABSTRACT

The kidney is sensitive to heavy metals because of its intensive metabolic activity and multiple functions namely those of excretion and pollutants concentration. The study aimed to evaluate the gender-specific variation in lead-induced nephrotoxicity 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 were administered a daily dose of 100 mg/kg/bw of lead acetate dispersed in distilled water for 21 days. All rats were anesthetized and sacrificed 24 hours after the last administration. Blood samples were collected via cardiac puncture for biochemical analysis, kidney tissues were harvested, homogenized, and analyzed for antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and lipid peroxidation (measured by malondialdehyde levels). Results indicated a significantly ($p<0.01$) lower weight gain in the lead-only female (F) group compared to the lead-only male (M) group. Lead acetate exposure caused oxidative damage, evidenced by significantly reduced antioxidant enzyme levels and increased lipid peroxidation in both male and female groups, with the effects being more pronounced in females ($p<0.05$). Serum creatinine and Urea levels in the lead alone (M) and lead alone (F) group were increased when compared to their respective control groups ($p<0.05$) however serum creatinine was significantly ($p<0.001$) higher in lead alone (F) than lead alone (M). The electrolyte function increased significantly in this study ($p<0.05$) when compared with their control groups. Sodium ion in lead alone (F) increased significantly ($p<0.01$) when compared to lead alone (M). The study concludes that lead exposure induced nephrotoxicity in both male and female rats, but more significantly pronounced in females than the males. This increased severity may have been mediated by the higher lead-induced oxidative stress observed in the female rats compared to males.

KEYWORDS: Kidney; Lead acetate; Oxidative stress; Antioxidant enzymes; Lipid peroxidation

1. INTRODUCTION

Lead is a common environmental toxicant which results in several adverse effects caused by its primary impacts on the hematological, renal and central neurological system (Collin *et al.,* 2022). Sources of heavy metals exposure including lead in particular are in mining, agriculture, coal production and burning (Liang *et al.,* 2017). 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 (Munir *et al.,* 2021). Bioavailability of lead is either through inhalation of air or dust, food and water contaminated with this element (Behrooz *et al.,* 2021).

Lead damages cellular material and alters cellular genetics and produces oxidative damage (Olujimi and Rodríguez, 2022). It causes increased production of free radicals and decreased availability of antioxidant reserves to respond to the resultant damage (Lushchak, 2014). It also interrupts enzyme activation and competitively inhibits trace mineral absorption. Lead binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis and lowers the levels of available sulfhydryl antioxidant reserves in the body (Abdulqader *et al.,* 2022).

Lead excretion from the body is mostly carried out by the kidneys, and lead levels in renal tissue have been found to be higher than in the liver and brain of lead- intoxicated animals (Amin *et al.,* 2021). Lead exposure is particularly damaging to the kidneys, as they are one of the primary sites for lead accumulation and processing. Toxic effects on kidneys are represented through the structure damage of kidneys and changes in the excretory function (Kellum *et al.,* 2021). However, information available on changes in kidney function in lead toxicity in animals is meager. Impaired Kidney functions have been reported as one of the most silent feature of lead toxicity (Tejchman *et al.,* 2021). Lead nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Mishra *et al.,* 2022).

Decreased glomerular filtration rate, low and high-molecular weight proteinuria, reduced transport of organic anions and glucose, and enzymuria are among the functional deficiencies in humans linked to high lead exposure. (Kim *et al.,* 2020). A few studies have revealed histopathological features of renal injury in humans, including intranuclear inclusion bodies and cellular necrosis in the proximal tubule and interstitial fibrosis (Sirac, *et al.,* 2021). Therefore, the purpose of the present study is to investigate the effect of biochemical alterations of the kidneys following experimental lead poisoning of Wistar rats by chronic exposure to lead acetate as one of the initial events responsible for impairment of renal function.

It is generally known that there are gender-specific variations in how the body reacts to toxic assaults, including a variety of pharmaceuticals and environmental contaminants (Halappanavar *et al.,* 2021). Factors such as hormonal fluctuations, metabolic differences, and genetic predispositions contribute to variability in susceptibility and response between males and females (Tramunt *et al.,* 2020). Regarding lead poisoning, previous study indicates that there are gender differences in lead absorption, distribution, metabolism, and excretion, as well as in the molecular mechanisms that contribute to lead toxicity. (Mitra *et al.,* 2017).

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Furthermore, Research indicates that variations in gender might impact the toxicokinetics and toxicodynamics of exposure to lead. (Gochfeld, 2017). For example, some studies have reported that compared to male rats, female rats had higher levels of lead buildup in their liver and kidneys, possibly as a result of variations in hormone-mediated changes in lead metabolism and excretion. (Jasim and Mosa, 2023). Therefore, there is substantial health risk associated with lead poisoning, especially to the kidney, where there may be gender-specific differences in susceptibility. While general lead toxicity is well-studied, there is still a significant gap in knowledge regarding the gender-specific impacts of lead poisoning on kidney function in Wistar rats.

This study aimed to assess and compare the impact of lead poisoning on the kidney of male and female Wistar rats, emphasizing the identification of gender-specific variations in biochemical markers and oxidative stress responses. Comprehending the gender-specific vulnerability to lead-induced organ damage is crucial because of the significant consequences for public health. (Cuomo *et al.,* 2022). It is substantiated that males and females do react to lead exposure differently, as has been demonstrated. These findings can help develop targeted treatments and preventative strategies to reduce lead poisoning in groups that are at its detrimental effects (Jaffee, 2019). And understanding the processes underlying gender-specific reactions can greatly advance the creation of medical interventions that are more effective (Nindl *et al.,* 2018).

2. Material and methods

2.1. Chemical and Compounds

Lead acetate $(CH_3CO_2)_2$ $Pb.3H_2O$ 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. Maintenance of animals

Male and female Wistar rats weighing approximately 180–220 g were procured. The animals were acclimatized for 14 days and unrestricted access to clean water and animal feed prior to this experiment. The animals procured were kept in a typical laboratory environment and a 12/12 h light/dark schedule was maintained. 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).

2.3 Experimental protocol

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10 male and 10 female adult Wistar rats were used randomly in Group-I (water and food pellet alone); Control (M). Group-II (Lead acetate (100 mg/kg/BW) given orally and daily); Lead alone (M). Group-III (Water and food pellet alone); Control (F). Group IV (Lead acetate (100 mg/kg/BW) given orally and daily); Lead alone (F). Each group consists of five rats and oral administration took place by oral beaded canula. The experimental duration was 21 days. Dose was selected based on the previously standardized doses for lead.

2.4 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 samples were obtained by cardiac puncture and left for 30 minutes to coagulate then centrifuged at 2500 revolutions per minutes for 10 minutes. Serum samples were separated and stored at -80℃ till when analysis was conducted on them. After blood collection, kidney organs were harvested for biochemical studies. Kidneys were carefully removed, washed in ice-cold (20 mM Tris-HCl, 0.14 M NaCl buffer, pH 7.4) and homogenized immediately. The homogenates were centrifuged at 2500 revolutions per minutes for 10 minutes. The supernatants were used for the various biochemical determinations.

2.5 Biochemical Tests

2.5.1 Evaluation of kidney function parameters

Blood urea nitrogen, creatinine and electrolyte were assessed in serum using a commercially available kit (Roche Diagnostics GmbH, Mannheim, Germany) and analyzed by auto analyzer (Roche Diagnostics Cobas Integra 800).

2.5.2 Oxidative stress markers/enzymatic antioxidant status

For enzymatic antioxidant status, kidney homogenates were used for the determination of malondialdehyde (MDA), superoxide dismutase activity (SOD), catalase (CAT) activity, and glutathione peroxidase (GPx) activity

2.6 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.

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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 ($p<0.05$) (Table 1). The weight gain in lead alone (F) group declined significantly when compared to the lead alone (M) group ($p<0.01$). This study indicated a lead-induced oxidative damage, demonstrated by the 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, there is no significance in catalase (CAT) when compared to the lead alone (M) group ($p<0.05$). However, the SOD and GPx activities were significantly decreased, while the MDA levels increased significantly ($p<0.05$) when compared to the lead alone (M) group (Figure 1A-D). The findings further revealed that lead exposure induced nephrotoxicity with a significant increase in kidney function parameters. Serum creatinine and Urea level in the lead alone (M) and lead alone (F) group were increased when compared to the control groups ($P<0.05$). However, the serum creatinine level showed a statistical significant increase ($P<0.01$) in lead alone (F) when compared with lead alone (M). Furthermore, the electrolytes (K^+, Na^+, Cl^- and Hco_3^-) levels in the lead alone (M) and lead alone (F) group were increased when compared to the control groups ($P<0.05$). The sodium ion level was however significantly ($p<0.01$) increased in the Lead (F) group when compared to Lead (M) group (Table 2).

3.2 Discussion

Humans are exposed to heavy metals through the environment and some of them can lead to physiological, biochemical and histological disorders. These metals are found in numerous places, including contaminated air, water, soil and food. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances. Previous studies indicated that the degree of toxic manifestation of different metals depends on dose, duration, route of administration and other physiological factors, especially nutrition. The kidney's high metabolic activity and several roles, including those of excretion and pollution concentration, make it vulnerable to the effects of poison (Ungureanu and Mustatea, 2022). Previous studies sought to demonstrate that exposure to hydrocarbons, pesticides, and various heavy metals might cause tubular dysfunction or tubulo-interstitial nephritis, which can result from a renal insufficiency (Rees et al., 2022). Hence, interstitial tubular nephrosis, a major cause of chronic renal insufficiency, may be caused by lead poisoning. Numerous researchers have considered various biomarkers such as urinary hippuric acid, the rate of metabolic clearance of the creatinine (Lara-Prado et al., 2021).

Kidneys are particularly susceptible to the effect of toxic agents that can cause renal damage and even renal failure (Rosner et al., 2021). Several studies shows strong association between lead exposure and renal effects (Wang et al., 2018). However, continued or repetitive exposures can cause a toxic stress on the kidney that, if unrelieved, may develop into chronic and often irreversible lead nephropathy (that is

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interstitial nephritis), confirming the view of a deleterious role for lead (Al-Attar, 2022). Developmental studies without concomitant under nutrition are still lacking in the literature (Thurstan *et al.,* 2022). The aim of this study was to present the effect of chronic lead intoxication on the body weight, oxidative stress and biochemical parameters of male and female Wistar rats.

In table 1, the study showed that adult male Wistar rats treated with lead acetate for 21 days cause decrease in body weights gain of lead alone (F) group when compared to lead alone (M). 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. The weight growth of both the male and female rats in this study grew initially with exposure time, but the rate of rise progressively dropped, and at the end of the study, the weight gain marginally decreased. 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 (Sidhu *et al.,* 2005; Jadhav *et al.,* 2007).

This study further demonstrated the difference in the effect of lead induction on the organ weight of both male and female Wistar rats when compared to their respective control group (Table1). The degree of these impacts differs based on the gender, with females showing more noticeable modifications than males. The rate at which lead is absorbed and retained may be affected by high levels of estrogen, and metabolic parameters such a greater fat-to-body mass ratio may also be responsible for these alterations (Sheng et al., 2021). Furthermore, higher testosterone levels in male rats may have some protective benefits against lead damage. (Behairy et al., 2022). Research indicates that females are more prone to lead-induced bone demineralization and oxidative stress due to lower antioxidant defenses (Sheng *et al.,* 2020).

Oxidative stress is believed to play a role in lead-induced toxicity and is proposed as a primary mechanism behind lead toxicity (Dzugkoev *et al.,* 2022). One of the critical impacts of lead poisoning is the induction of oxidative stress through free radical production and a decrease in antioxidant defenses (Unsal et al., 2021). Free radicals are generated from both endogenous sources (such as mitochondria, the cytP450 pathway, and peroxisomes) and exogenous sources (like xenobiotics and chemical reactions), as explained by Valko et al. (2006) and Patrick (2006). Lead exposure disrupts cellular balance by inducing oxidative stress in the body. This can result in damage to the cells and changes in their weight (Kidney) (Guo et al., 2021). Increased levels of reactive oxygen species may contribute to inflammatory kidney (Gwozdzinsk *et al.,* 2021). Studies have shown that exposure to lead can cause kidney tissues to undergo apoptosis and cell death, which can alter organ weight and cause structural abnormalities (Tang, 2020; Mishra et al., 2022).

Renal antioxidants investigated in (Fig. 1A-D) of this study includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The study observed decrease SOD, GPx and catalase activities in the male and female lead alone groups. This finding is signaling to an indication of oxidative

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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 renal CAT, SOD and GPx levels in both male and female lead groups when compared to their control groups indicates a response to elevated oxidative stress. It is further observed that there is statistically significant decrease in lead alone (F) when compared to lead alone (M). The significant reduction in females can be linked to natural baseline differences in antioxidant enzyme levels, with females typically having lower antioxidant activity than males which renders them more vulnerable to oxidative damage under stress conditions (Botero, 2017).

Lower SOD and GPx activity in the female lead alone group suggest a reaction towards excessive superoxide radicals generated leading to elevated lipid peroxidation and lower 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 (Ramesh and Knuckle, 2006). Previous research also shows 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 (Hussein *et al.,* 2021).

Malondialdehyde is a byproduct of lipid peroxidation, resulting from oxidative stress that damages cell membranes (Mohideen *et al.,* 2023). 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 (Jelic *et al.,* 2021). In fig. 1D of this study, induction 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. Increased lipid peroxidation is likely caused by the generation of superoxide, peroxyl, and hydroxyl radicals, as indicated by elevated MDA levels (Niki et al., 2009). Following lead poisoning, the generation of peroxyl radicals promotes lipid peroxidation by cyclization processes producing endoperoxides (Valgimigli *et al.,* 2023).

However, the larger rise in MDA levels in females than in males implies that, given identical circumstances, females may be more oxidatively damaged. 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 (Chainy and Sahoo, 2020).

The kidney is sensitive to the action of poison because of its intensive metabolic activity and multiple functions namely those of excretion and of pollutants concentration (Vervaet et al., 2017). Previous research attempted to show that intoxication by several heavy metals, hydrocarbons, pesticides, induces a tubular disfunctioning or a tubulo-interstitial nephropathy which can be due to a renal deficiency (Rees *et al*., 2022). Hence, lead poisoning may be a common cause of chronic renal deficiency by interstitial tubular nephrosis. Authors have shown that lead toxicity acts upon various systems, mainly the nervous system (Boskabady *et al*., 2018). Epidemiological studies suggest that chronic exposure to this metal

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increases the accumulation of lead in the blood and contributes to the increase of the chronic renal absence (Orr and Bridge, 2017).

According to Innih and Ubhenin, (2021) an increase in the serum creatinine rate indicates a decrease in the glomerulary filtration probably due to a decrease in functional nephrons number since the blood creatinine rate rises only if 50% of nephrons are destroyed. Serum creatinine and Urea can to some extent reflect renal function (Wani and Pashs, 2021). In table 2, creatinine levels reflect the degree of damage to the glomerular filtration function more accurately than urea, because urea can be affected by many factors besides renal function, such as a high protein diet, gastrointestinal bleeding, dehydration and hypermetabolism, whereas creatinine levels mainly depend on glomerular filtration (Jonsson *et al*., 2020; Molina *et al*., 2022). In this study the results showed that exposure to lead led to significant increases in both male and female rats, whereas serum creatinine increased significantly only in the female rats exposed to lead acetate. Similar results were also found in studies of Moneim *et al*. (2011). Together, these results indicate that exposure to heavy metals affect renal function to some extent in terms of the increase in serum creatinine and urea (Lentini et al., 2017).

Furthermore, the study showed that administration of lead acetate cause increase ($P<0.05$) in the electrolyte functions (K^+, Na^+, Cl^- and HCO_3^-) of the kidney when compared with their respective groups (Table 2). The result shows a statistically significance increased ($P<0.001$) in sodium ion (Na^+) in lead alone (F) when compared to lead alone male. This increase indicate the high level of susceptibility of lead exposure in female rat. The significance increase seen in the electrolyte function in the lead exposed group of Wistar rat could be the trace of nephrotoxic effect of lead acetate which implies the impairment of the glomerular function and tubular damage of the kidney (Kucukler et al., 2021). This aligned with the findings Innih and Ubhenin, (2021). The degeneration and destruction observed in the renal tissue can be linked to the generation of reactive oxygen species initiated as result of lead exposure (Makhdoumi et al., 2020).

4. CONCLUSION

Lead acetate administration in both male and female Wistar rats led to a reduction in body weight gain, reduced antioxidant levels and increased creatinine, urea and electrolytes in their serum. This is indicating the nephrotoxic effect of lead. These observations were however more pronounced in female than male indicating that females are more susceptible to lead toxicity. The susceptible of female rats to this lead toxicity may be attributed to certain specific female hormones.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was conducted following the guidelines of the Animal Ethical Committee of the Faculty of Basic Medical sciences, 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).

INFORMED CONSENT STATEMENT

Not applicable

Disclaimer (Artificial intelligence)

Option 1:

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.

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3.

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

Weight (g)	Control (M)	Lead alone (M)	Control (F)	Lead alone (F)
Kidney weight	0.54 ± 0.02	0.64 ± 0.04	0.54 ± 0.02	0.60 ± 0.03
Mean body weight gain difference (+) (Experimental change compared with mean control values				
Body (weight gain)	11.60 ± 0.68	6.40 ± 0.51	13.80 ± 1.02	3.60 ± 0.60@

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).

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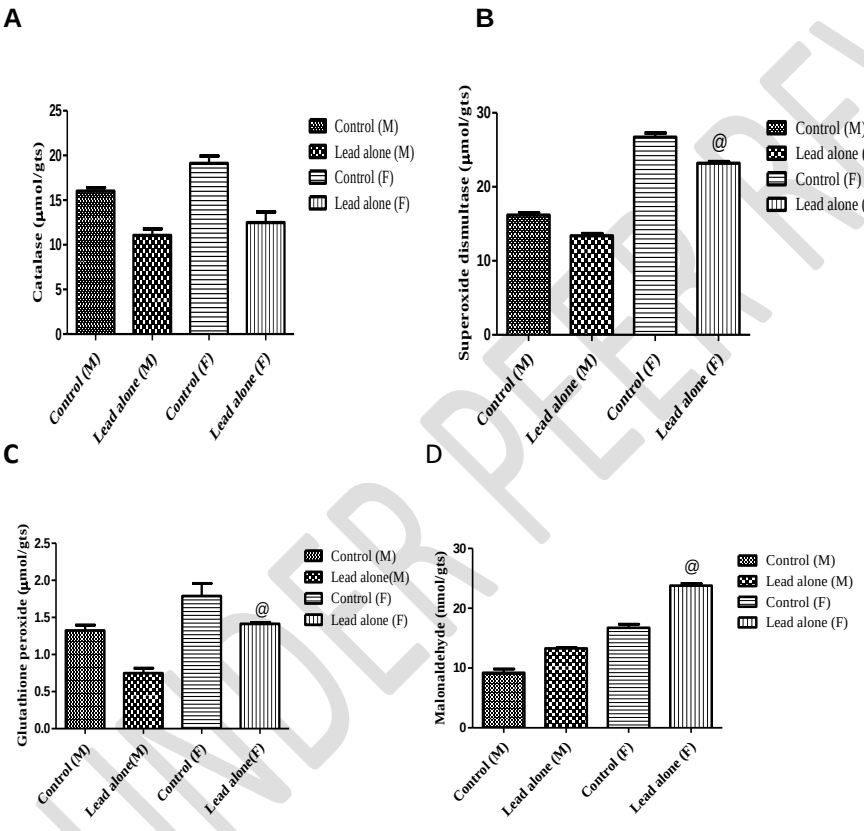

Fig. 1A-D: Effects of lead acetate on Renal Antioxidant System in male and female Wistar rats in both control and experimental groups.

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 B&D has a statistically significant decrease (P< 0.001).

Graph C has a statistically significant increase (P< 0.01).

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Table 2: Effects of lead acetate on kidney function parameters level in male and female Wistar rats in both control and experimental groups.

Functions	Control (M)	lead alone (M)	Control (F)	Lead alone (F)
SC (mg/dL)	0.66 ± 0.03	0.92 ± 0.03	0.93 ± 0.012	1.23 ± 0.07@
Urea (mmol/L)	6.64 ± 0.08	8.86 ± 0.05	6.01 ± 0.06	8.45 ± 0.22
HCO_3 (mml/L)	16.83 ± 0.66	27.94 ± 1.01	17.63 ± 0.65	25.82 ± 1.42
K^+ (mml/L)	1.80 ± 0.04	4.51 ± 0.33	2.54 ± 0.14	4.34 ± 0.07
Na^+ (mml/L)	65.96 ± 6.08	84.58 ± 10.77	80.85 ± 3.16	101.12 ± 0.93@
Cl- (mml/L)	70.57 ± 3.64	78.38 ± 4.72	62.69 ± 2.24	80.91 ± 0.68

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.

Na^+ *has a statistically significant increase (P< 0.01).*

SC *has a statistically significant increase (P< 0.001).*

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