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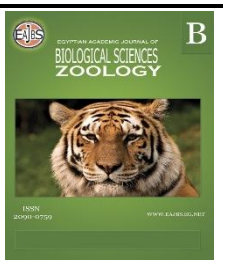

Impact of Silver Nanoparticles on the Effectiveness of *Moringa oleifera* Leaf Extract Against Hepatorenal Toxicity of Lead in Male Rats

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ABSTRACT

This study aimed to enhance drug delivery using silver nanoparticles (Ag NPs) combined with *Moringa Oleifera* (*M. Oleifera*) extract to mitigate the adverse effects of conventional therapies and reduce lead acetate toxicity with minimal side effects. Fifty adult male albino rats were divided into five groups: **Group I** (negative control group); **Group II** (positive control group exposed to lead acetate); **Group III** (lead acetate + Ag NPs); **Group VI** (lead acetate + *M. Oleifera*); **Group V** (Lead acetate + Ag NPs + *M. Oleifera*). Biochemical parameters, including liver enzymes (ALT, AST, ALP), lipid profile (CHOL, HDL-c, LDL-c, TG), kidney function markers (BUN, CREA, UA), and oxidative stress indicators (GSH, SOD, MDA) were measured. Lead exposure significantly increased ALT, AST, ALP, TBIL, LDL-c, BUN, UA, CREA, IL-6, TNF-α, and LDH while decreasing ALB, HDL-c, GSH, SOD, and MDA. However, treatment with Ag NPs and *M. Oleifera* mitigated these alterations, restoring biochemical balance. Histological examination revealed severe liver and kidney damage in lead-treated rats, including hepatocyte degeneration, blood sinusoid dilation, and renal tubular necrosis. Treatment with Ag NPs and *M. Oleifera* significantly reduced these pathological changes, suggesting their protective effects against lead-induced oxidative stress and inflammation. The findings support the potential of Ag NPs combined with *M. Oleifera* as a novel therapeutic approach to reduce lead toxicity, demonstrating promising hepatorenal protective properties by regulating oxidative stress and inflammatory response.

INTRODUCTION

Lead is a toxic heavy metal widely used in industrial applications such as batteries, paints, cosmetics, and X-ray shielding (Nag & Cummins, 2022; USFDA, 2015). However, Pb contamination poses serious environmental and health risks, including soil degradation, water pollution, and air contamination due to industrialization and urbanization (Masindi & Muedi, 2018). Pb exposure is linked to hepatotoxic effects, as it damages cell membranes, elevates liver enzymes (ALT, AST, ALP), and impairs hepatic function (Sharma *et al.*, 2015).

Silver nanoparticles have been identified as highly promising biomedical agents due to their antibacterial, anticancer, and therapeutic properties (Almatroudi *et al.*, 2020). Studies indicate that AgNPs provide hepatoprotection by restoring liver enzyme levels and mitigating oxidative stress in cases of acetaminophen-induced toxicity (Reshi *et al.*, 2017) and cadmium (Cd)-induced liver damage (Laib *et al.*, 2024).

The *M. Oleifera* extract has also demonstrated hepatoprotective effects against lead toxicity. *M. Oleifera* supplementation reduced lipid profile alterations, oxidative stress

markers, and liver enzyme disruptions in lead-exposed animals, aiding in tissue recovery (Melebary & Elnaggar, 2023). Furthermore, *M. Oleifera* administration counteracted lead-induced histological liver damage and restored antioxidant enzyme activity, underscoring its significance as a natural hepatoprotective agent (Omotoso *et al.*, 2015).

This study explores the hepatoprotective potential of AgNPs and M. oleifera against lead-induced liver toxicity, emphasizing their biochemical and histological benefits.

MATERIALS AND METHODS

Materials:

Silver Nanoparticles Preparation:

All chemicals and reagents must be of analytical quality. Aqueous sodium borohydride was introduced into a solution of silver nitrate at a concentration of 0.05M. A precipitate was formed by gradually adding drops of the solution while stirring at room temperature. Once the sodium borohydride addition is finished, the stirring process will continue for 10 minutes. Monitoring the reduction of Ag+ ions by sodium borohydride in the solution will involve taking samples of the aqueous component (Diantoro *et al*., 2014).

Transmission Electron Microscope (TEM) Examination:

Silver nanoparticles were characterized using a high-resolution transmission electron microscopy (HR-TEM) apparatus, namely the JEM-2100F model manufactured by Jeol in Tokyo, Japan. The device operated at an accelerating voltage of 200 KV (Ramkumar *et al*., 2017).The resulting suspension was dispersed in a solution of 90% Ethyl alcohol using a Sonicator (BRANSON 1510). Afterward, the suspended Ag NPs were attached to a carbon-coated copper grid. Their particle size and surface form were then examined using high-resolution transmission electron microscopy.

Methods:

Experimental Groups:

Fifty adult male Wister albino rats weighing 180-200 g were used in the present study. The rats were obtained from the Animals Section of the King Fahad Medical Research Center at King Abdulaziz University and were classified into five groups (n=10), as follows:

- **Group I:** Negative control group received no treatment.
- **Group II:** Positive control group administered with 0.1 ml lead acetate (60 mg/kg b. wt.) by stomach gavage for 3 weeks, then in the next 3 weeks the dose increased to 0.2 ml.
- **Group III:** $AgNO_3$ + Lead group received 0.2 ml $AgNO_3$ (10 mg/kg b. wt.) then administered 0.1 ml lead acetate (60 mg/kg b. wt.) for 3 weeks, then in the next 3 weeks, the dose of lead acetate increased to 0.2 ml.
- **Group IV:** *Moringa oleifera* leaf extract + Lead group administered 0.2 ml of *Moringa oleifera* leaf extract (200 mg/kg b. wt.) followed by 0.1 ml lead acetate (60 mg/kg b. wt.) for 3 weeks, then in the next 3 weeks the dose of lead acetate increased to 0.2 ml.
- **Group V:** $AgNO_3$ + *Moringa oleifera* leaf extract + Lead group treated with 0.2 ml of *Moringa oleifera* leaf extract (200 mg/kg b. wt.) followed by 0.2 ml $AgNO_3$ (10 mg/kg bwt) then administered 0.1 ml lead acetate (60 mg/kg b. wt.) for 3 weeks, then in the next 3 weeks the dose of lead acetate increased to 0.2 ml.

Blood Serum Analysis:

During the next 6 weeks, the experimental rats underwent a 12-hour fasting period, with free access to water before being anesthetized using diethyl ether. Blood samples were drawn from the retro-orbital venous plexus into non-heparinized tubes, then centrifuged at 2500 rpm for 15 minutes. The separated serum was collected and stored at -80°C until analysis. Serum samples were later used to assess liver enzyme activity and various biochemical parameters.

Biochemical Assays:

Liver Function Tests: Enzymatic activities for ALT and AST was estimated using the method of Reitman and Frankel (1957). ALP enzyme activity was determined by using McComb and Bowers (1972) method. TP level was measured using Peters (1968) method. Doumas *et al*. (1973) method was used to determine TBIL level. Albumin (ALB) concentration was determined by using the Busher (1990) method.

Lipid Profile Tests: Cholesterol (CHOL) concentration was determined by using the method of Richmond (1973). Triglycerides (TG) concentration was determined by using the method

of Fossati and Prencipe (1982). High-density lipoprotein-cholesterol (HDL-c) was determined by using the method of Warnick *et al*. (1983). Low-density lipoprotein-cholesterol (LDL-c) concentration was determined using Friedewald *et al*. (1972) method.

Oxidative Stress Markers: Glutathione (GSH) level was estimated using the Moron *et al*. (1979) method. The activity of the enzyme Superoxide Dismutase (SOD) was estimated using Misra and Fridovich (1972) method. Malondialdehyde (MDA) was determined by using the method of Zeb and Ullah (2016).

Renal Function Tests: Blood urea nitrogen level was estimated according to the method of Patton and Crouch (1977). Uric Acid (UA) level was estimated using the Burtis and Ashwood (1994) method. CREA level was estimated according to the method of Moore and Sharer (2017). Interleukin-6 (IL-6) was determined using the Boulanger *et al.* (2003) method.

Inflammatory Biomarker Tests: Tumor Necrosis Factor-α (TNF-α) was determined using the method of Petrovas *et al*. (1999). Lactate dehydrogenase (LDH) was measured using the Stevens *et al*. (1983) method.

Histological Examinations:

At the end of the experimental period, all animals were sacrificed, and their organs were examined for gross lesions. The livers and kidneys were then collected and immersion-fixed in Karnovsky's fixative before being processed for paraffin embedding. Tissue sections, 7 µm thick, were stained with hematoxylin and eosin (H&E) and analyzed using an Olympus trinocular microscope. Photomicrographs were primarily captured at high magnification (Sulimani *et al*., 2024).

Statistical Analysis:

Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc multiple comparisons test in GraphPad Prism software (version 5, San Diego, CA, USA). A p-value of < 0.05 was considered statistically significant.

RESULTS

Transmission Electron Microscopy for Ag NPs:

Figure 1, confirms the successful synthesis of Ag NPs. (A) The low-magnification TEM image reveals uniformly dispersed nanoparticles with a spherical morphology. (B) The high-resolution TEM image shows well-defined lattice fringes, indicating the crystalline nature of the Ag NPs. (C) The line profile analysis highlights distinct interplanar spacings, consistent with the characteristic crystallographic planes of silver nanoparticles. These results validate the structural integrity and crystalline properties of the synthesized Ag NPs.

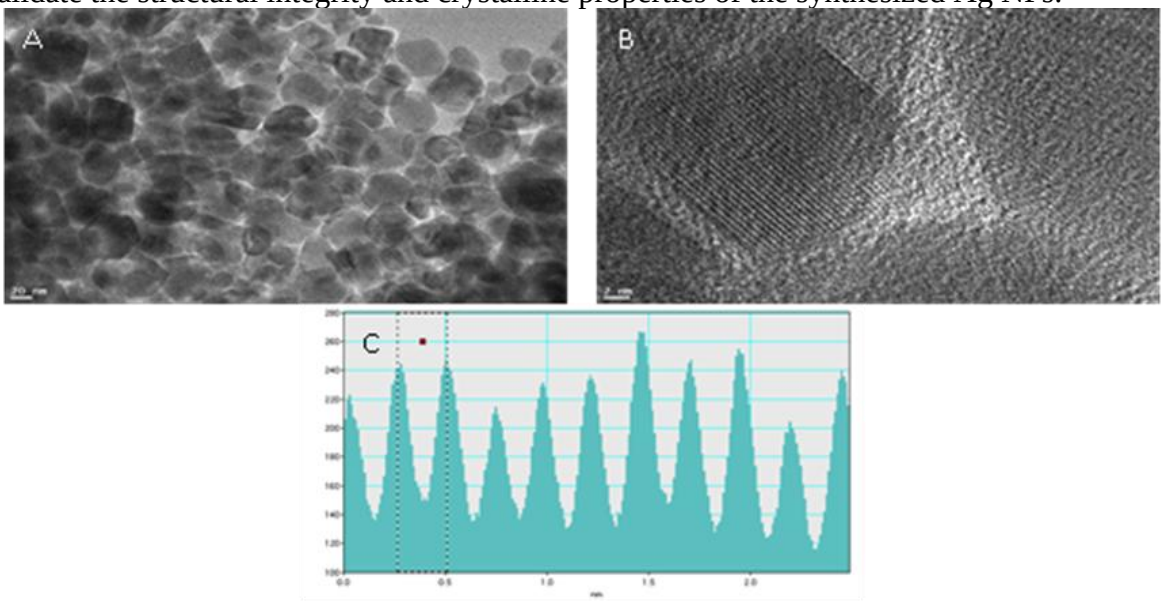

Fig. 1. Transmission Electron Microscopy (TEM) characterization of Ag NPs. (A) TEM image showing the morphology and uniform distribution of Ag NPs. (B) High-resolution TEM image revealing lattice fringes, confirming the crystalline structure of Ag NPs. (C) Line profile analysis illustrating the interplanar spacing characteristics of Ag NPs.

The present study results revealed that, in lead-treated groups, serum levels of AST, ALT, ALP, TP, and TBIL were significantly increased compared with negative control groups. Meanwhile, serum levels of albumin were significantly decreased in lead versus negative control. The treatment of the animals by *M. Oleifera* + lead caused a significant decrease in serum levels of AST, ALT, ALP, TP, and TBIL and a significant increase in ALB versus the positive control group but still showed significant changes versus the negative control group. On the other hand, treatment of the animals, in the present work, by AgNPs + *M. oleifera* revealed a significant reduction in serum levels of AST, ALT, ALP, TP, and TBIL and significant elevation in ALB and TP versus AgNPs + lead groups but still showed significant changes versus negative control group.

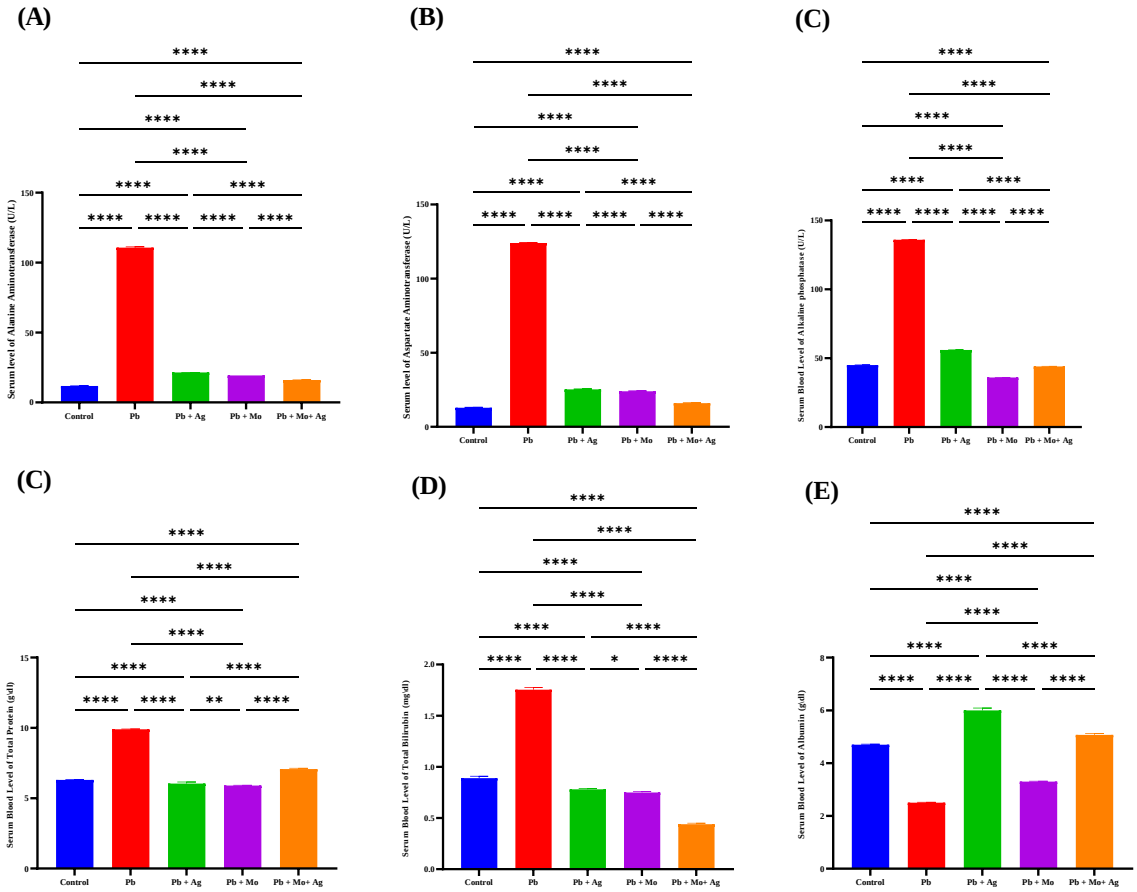

Fig. 1. Serum levels of (A) ALT, (B) AST, (C) ALP, (D) TP and (E) TBIL in different groups being studied. Data is presented as mean ± SD. Using One-way ANOVA (Tukey) test at $P < 0.05$.

Figure (2A-D), showed the levels of serum lipid profile: CHOL (cholesterol), TG (triglycerides), HDL-c (high density lipoprotein cholesterol), and LDL-c (Low density lipoprotein cholesterol) measured across different studied groups. The results revealed the oral administration of Lead-induced abnormal lipid profile in treated rats. The levels of serum CHOL, TG, and LDL-c were significantly elevated, while HDL-c decreased in lead-induced toxicity in comparison with negative control groups. Treatment of *M. oleifera* extract + Lead significantly decreased the levels of serum CHOL, TG, and LDL-c in comparison with Lead treated positive control group. The restoration of HDL-c to approximately its normal range was at the dose level when compared with the lead-treated group.

Figures (3A-C), show the impact of Lead administration on antioxidant markers, lead caused significant decrease in levels of antioxidant markers, including GSH, SOD and MDA versus the untreated group. Whereas treatment of AgNPs + Lead caused significant elevation in GSH and SOD compared to Lead group but still lower than negative control. On the other hand, the treatment of *M. Oleifera* + Lead caused a significant reduction in antioxidants versus Lead and *M. Oleifera* + Lead groups. The level of MDA was elevated versus the

control group, then after administration of AgNPs, its level was significantly decreased versus the Lead group. Administration of Mo led to significantly decreased levels of MDA versus Lead and *M. oleifera* + Lead groups, but the level was still higher than the control.

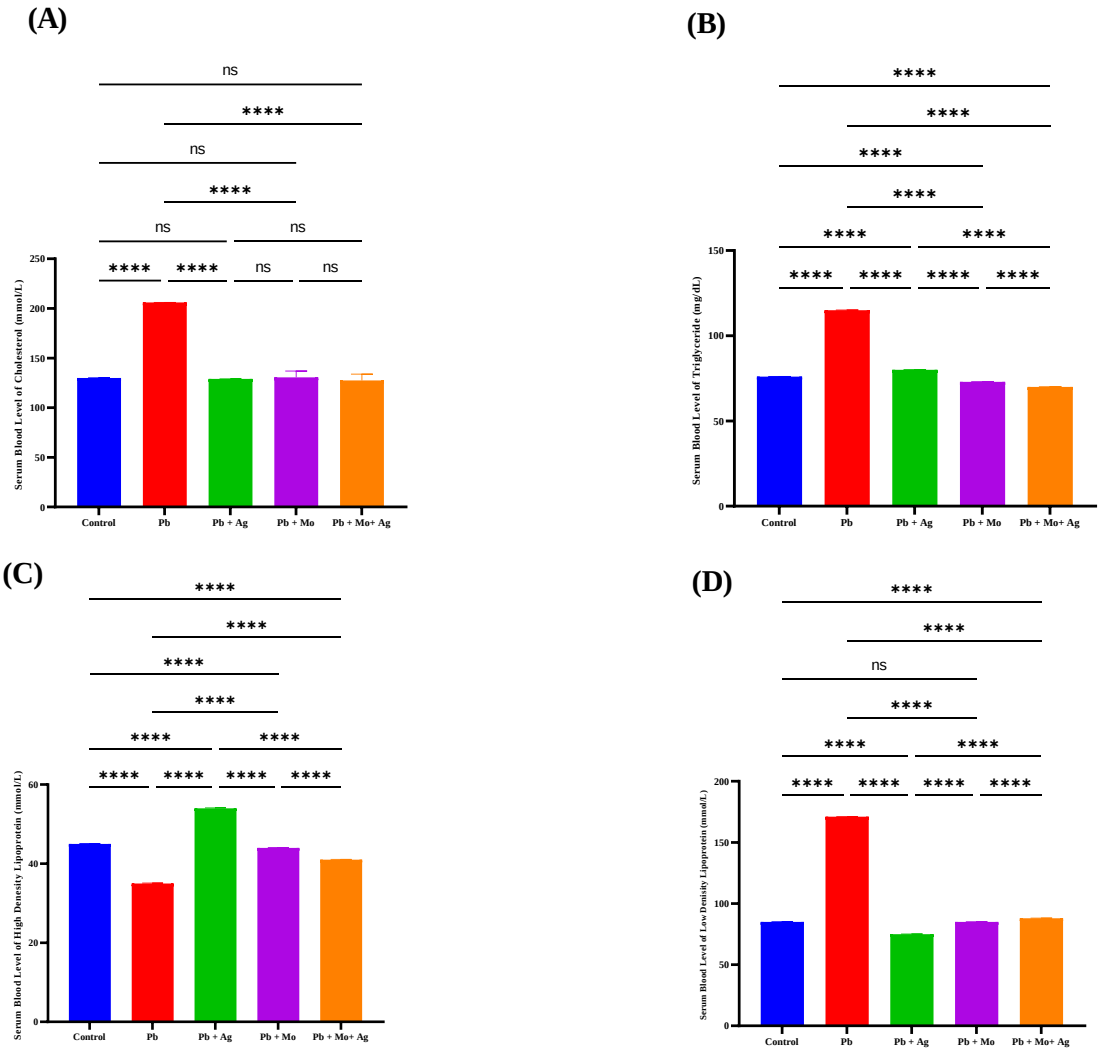

Fig. 2. Serum levels of (A) CHOL, (B) TG, (C) HDL-c, (D) LDL-c in different groups being studied. Data is presented as mean ± SD. Using One-way ANOVA (Tukey) test at $P < 0.05$.

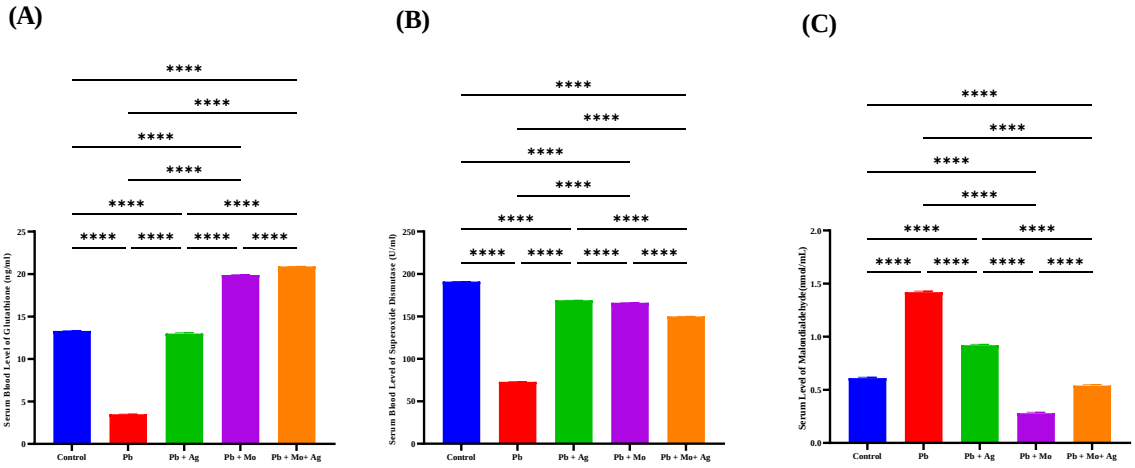

Fig. 3. Serum levels of (A) GSH, (B) SOD and (C) MDA in different groups are being studied. Data is presented as mean ± SD. Using One-way ANOVA (Tukey) test at $P < 0.05$.

Figures (4A-C), show the levels of serum BUN (Blood Urea Nitrogen), UA, and CREA measured across different studied groups. The findings indicated that oral exposure to lead caused impaired kidney function in the treated rats, as evidenced by a significant increase in BUN, UA, and CREA levels compared to the negative control group. However, co-administration of M. oleifera with lead significantly reduced serum BUN, UA, and CREA levels compared to rats exposed to lead alone.

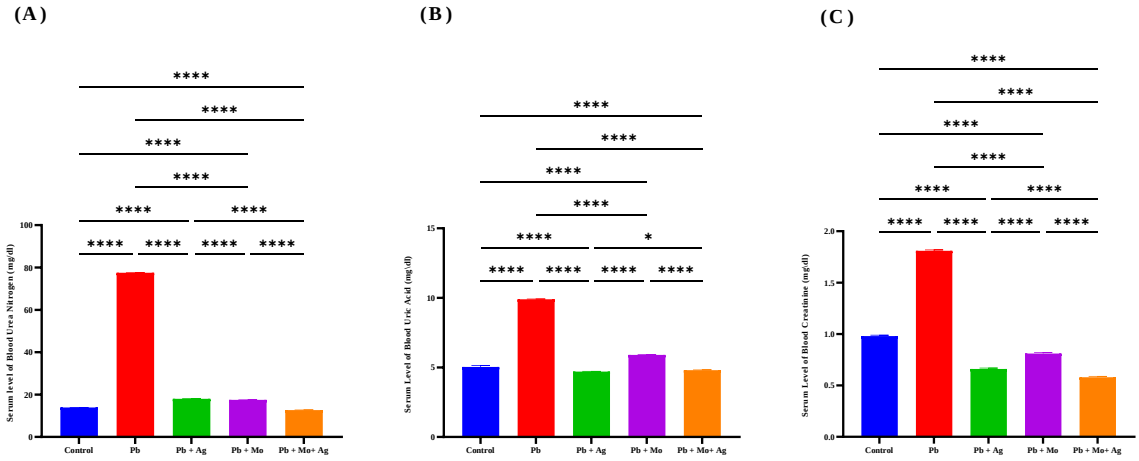

Fig. 4. Serum levels of (A) BUN, (B) UA, and (C) CREA in different groups are being studied. Data is presented as mean ± SD. Using One-way ANOVA (Tukey) test at $P < 0.05$.

Figures (5A-C), shows the serum levels of Inflammatory Biomarkers: Interleukin (IL-6), Tumor Necrosis Factor-α (TNF-α) and Lactate Dehydrogenase (LDH) measured across different studied groups. The present results showed that the levels of serum IL-6, TNF-α and LDH were significantly increased, in Lead-treated rats when compared with negative control group. Administration of *M. oleifera* with Lead significantly decreased the levels of serum IL-6, TNF-α and LDH decreased compared to Lead-treated rats. Restoration of inflammatory biomarkers to the normal range was at the dose level when compared with Lead-treated rats.

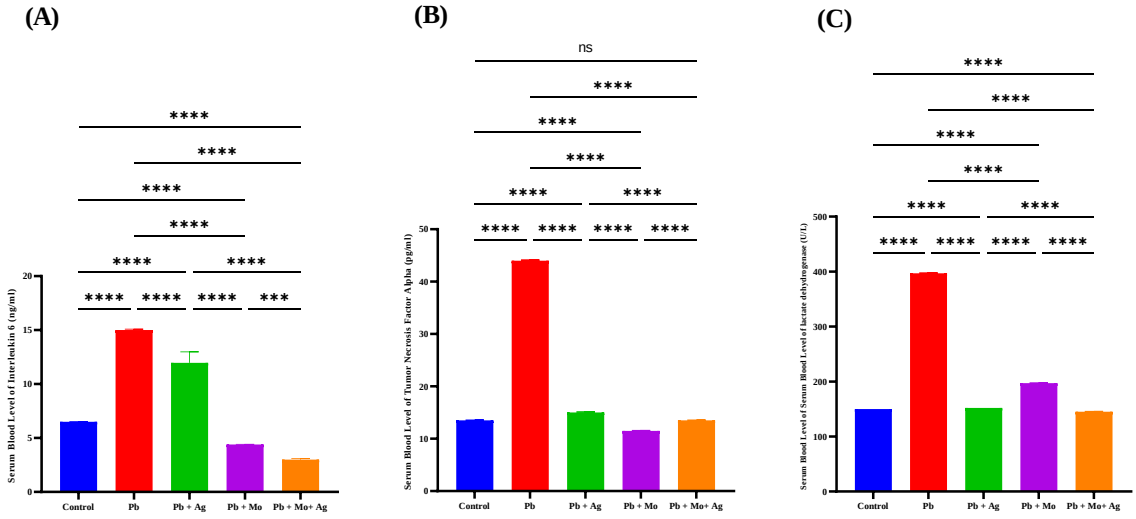

Fig. 5. Serum Blood Level (A) IL-6, (B) TNF-α and (C) LDH in different groups being studied. Data is presented as mean ± SD. Using One-way ANOVA (Tukey) test at $P < 0.05$.

Figure 6, shows the histological examination of liver tissues revealing notable differences among the experimental groups. In the control group (A), the liver exhibited normal architecture with a well-defined central vein (CV), intact hepatocytes (HC), and clear sinusoidal spaces (S). The lead-treated group (B) showed significant histopathological alterations, including congestion in the central vein, accumulation of red blood cells (RBCs), and structural disorganization. The group treated with M. oleifera + lead (C) demonstrated an improvement in liver histology, with reduced central vein congestion and a more preserved hepatic structure. Similarly, the AgNPs + *M. oleifera* + lead group (D) exhibited nearly normal hepatic architecture, with radially arranged hepatocytes around the central vein, distinct sinusoidal spaces, and the presence of Kupffer cells (KC), closely resembling the normal control group. These findings suggest a protective effect of M. oleifera and AgNPs against lead-induced hepatic damage.

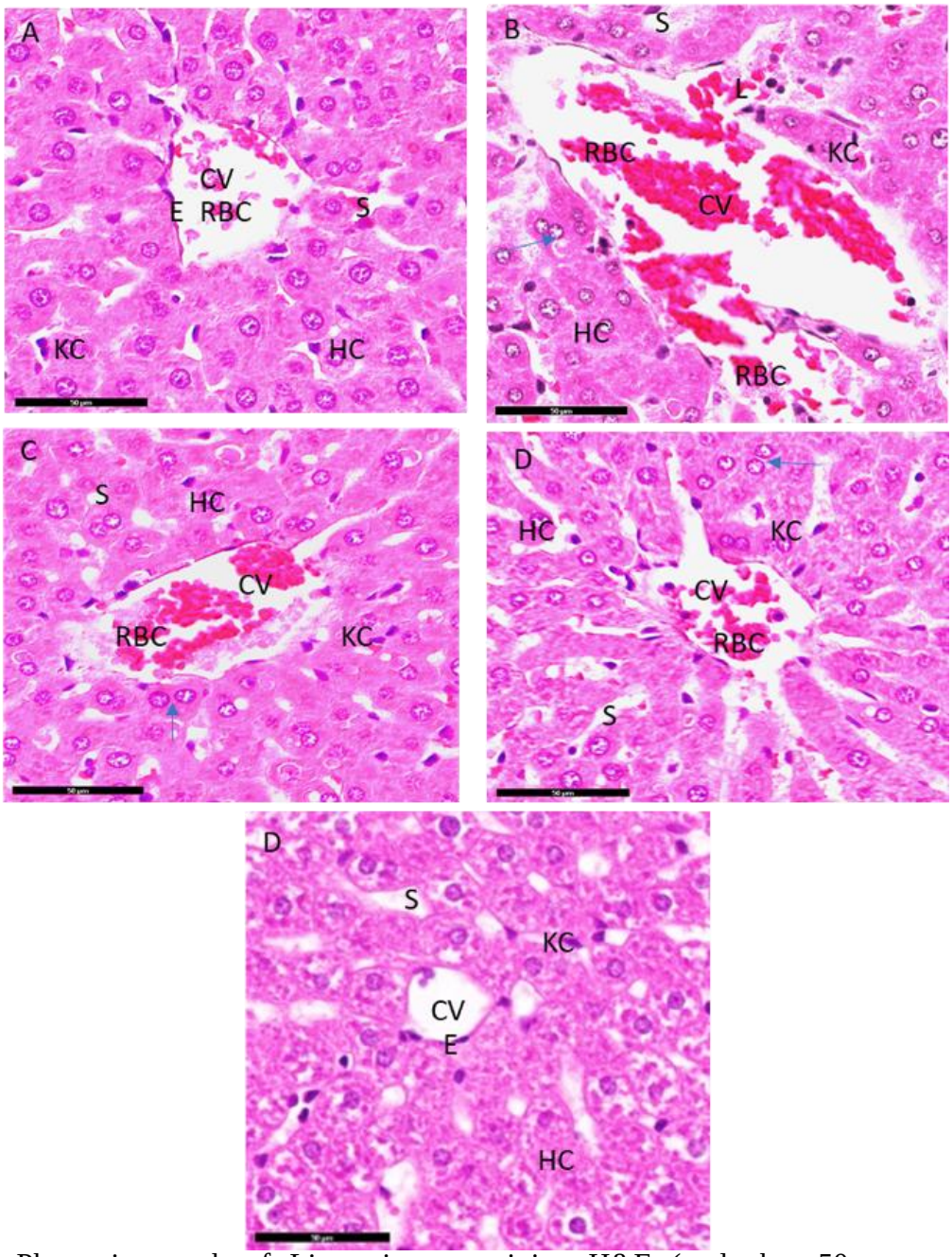

Fig. 6. Photomicrograph of Liver tissues staining H&E (scale bar 50 µm, and 400 magnification). **A)** Control group, **B)** Lead toxicity group (received Lead acetate), **C)** AgNPs + Lead toxicity. **D)** *M. oleifera* leaf extract + Lead toxicity. **E)** AgNPs + *M. oleifera* leaves extract + Lead toxicity.

Figure 7, shows the histological examination of kidney tissues revealing distinct structural differences among the experimental groups. The control group (a) exhibited normal renal architecture, with intact glomeruli (G), well-defined Bowman's space (BS), and properly structured proximal (PCT) and distal convoluted tubules (DCT). In contrast, the lead-treated group (b) displayed marked histopathological alterations, including glomerular shrinkage, narrowed Bowman's space, and tubular degeneration. The group treated with M. Oleifera + lead (c) showed notable improvement, with more preserved glomerular and tubular structures, although mild abnormalities persisted. Similarly, the AgNPs + M. oleifera + lead group (d, e) exhibited nearly normal renal histology, with well-maintained glomeruli, Bowman's capsule (BC), and tubules, closely resembling the control group. These findings suggest that M. oleifera and AgNPs exert a protective effect against lead-induced renal damage.

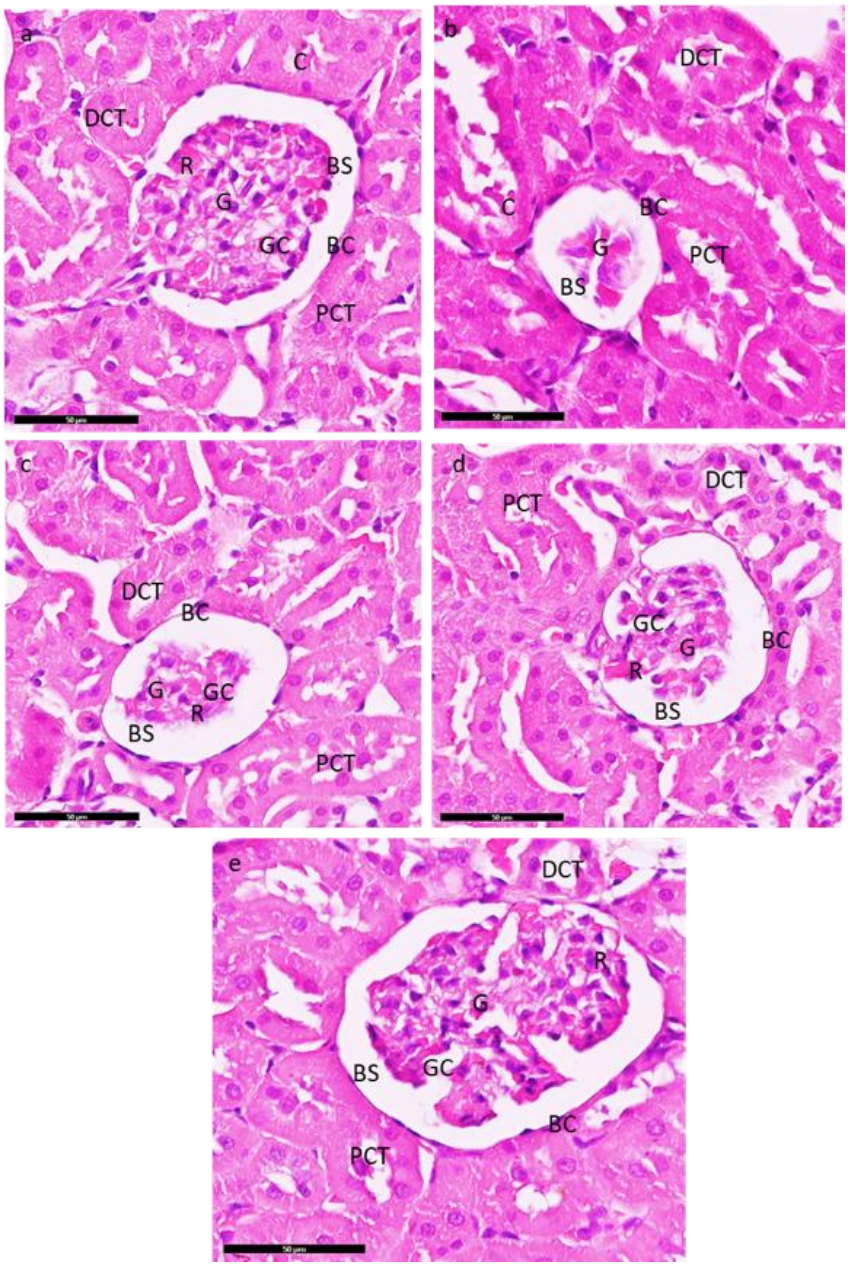

Fig. 7. Histological examination of hematoxylin and eosin-stained sections of rat Kidney for the study groups (scale bar 40 mm, 400X magnification). **A)** Control group. **B)** Lead toxicity group. **C)** Lead toxicity + AgNPs. **D)** *M. oleifera* leaf extract + Lead toxicity. **E)** AgNPs with *M. oleifera* leaf extract + Lead toxicity. BC: Bowman`s capsule, BS: Bowman`s space, PCT: proximal convoluted tubule. DCT: distal convoluted tubule, G: glomerulus, GC: glomerular capsule, R: red blood cells.

DISCUSSION

The present study results revealed that, in lead treated groups, serum levels of AST, ALT, ALP, TP and TBIL were significantly increased compared with negative control groups. Meanwhile, serum levels of albumin were significantly decreased in lead versus negative control. The treatment of the animals by M. Oleifera + lead caused significant decrease in serum levels of AST, ALT, ALP, TP and TBIL and significant increase in ALB versus positive control group but still showed significant changes versus negative control group. On the other hand, treatment of the animals, in the present work, by Ag-NPs + M. oleifera revealed significant decrease in serum levels of AST, ALT, ALP, TP and TBIL and significant elevation in ALB and TP versus Ag-NPs + lead groups but still showed significant changes versus negative control group.

The present results are in accordance with the findings of Sharma et al. (2015) who said that the ingestion of lead acetate stimulated a significant elevation of serum ALT, AST, ALP, TG, CHOL, and TBIL in the circulation, which lead to hepatic injury after lead exposure. The decrease in the levels of serum ALB by the exposure of lead might be a sign of a reduction in the capability of the liver to function, which resulting most likely from hepatocellular damage. Also, Mohammed *et al.* (2017) and Offor *et al.* (2017) concluded that lead was associated with an increase in serum ALP, ALT, AST activities, and TBIL level. After the administration of lead on adult Wistar rats on both sexes, there was a decrease in bodyweights. The biochemical analysis revealed an increase in the levels of ALP, AST, and ALT. The morphological change in the liver parenchyma due to lead acetate exposure caused distortion and degeneration (Ajibade et al., 2021). After lead application in the liver and kidney, there was a critical rise in serum AST, ALP, ALT, TBIL, CHOL, TG, and decreases in TP and ALB (Offor *et al.*, 2017).

In a study by Bozdağ and Eraslan (2020) they found that lead acetate exposure in rats caused significant changes in liver and kidney biochemical parameters as GLU, TG, CHOL, BUN, CREA, UA, LDH, AST, ALT, ALP, TP and ALB. They added that, administration of the antioxidant substance, Diosmin alleviated the harmful effects of lead toxicity.

The bioaccumulation of lead acetate in liver, kidneys, and brain showed increased UA, GPT, CREA, ALP, LDH, AST and ALT while on the other side GSH and SOD were found decreased due to lead acetate exposure. The concentration of lead acetate in the organs is as follows, liver, kidney, brain. The effects of lead accumulation in the brain, kidneys, and liver resulted in neurotoxicity, nephrotoxicity, and hepatotoxicity (Ali *et al.*, 2021).

Alao, administration of lead acetate can lead to significant disturbances in hepatic tissue, which can be attributed to its ability to induce reactive oxygen species (ROS), NF-kB activation, and TNF-α release, resulting in cellular injury, release of hepatic enzymes, lipid profile abnormalities, as well as reductions in total protein (TP) and albumin (ALB) levels, which are crucial liver components. These alterations were further evidenced by an increase in hepatic DNA breakage. In contrast, the administration of aqueous M. oleifera leaf extract with its diverse array of antioxidants and protective components effectively reversed the disturbances induced by lead acetate to a significant extent, surpassing the effects observed in the lead acetate group and sometimes even restoring values to levels comparable to those of the control group (Abdel Fattah *et al.*, 2020). Also, Parang and Moghadamnia, (2018) studied the effects of AgNPs on hepatic functional tests and alterations in liver tissues in adult male rats, and they reported that the mean serum levels of AST, TP, ALB, ALT and ALP increased significantly relative to the control group. These findings are in accordance with the present study results.

The results revealed the, administration of lead-induced abnormal lipid profile in treated rats. The levels of serum cholesterol (CHOL), Triglycerides (TG), and Low-density Lipoprotein-cholesterol (LDL-c) were significantly elevated, while High density Lipoprotein-cholesterol (HDL-c) decreased in Lead induced toxicity when compared with negative control rats. Administration of M. oleifera extract + Lead significantly decreased the levels of serum CHOL, TG and LDL-c compared to Lead treated positive control group. Restoration of HDL-c to approximately its normal range was at the dose level when compared with Lead treated group. In accordance with the present results are those of Offor *et al.* (2017) how declared that after lead application experimental animals, there was a critical rise in serum

CHOL, TG and LDL-c. Also, lead was found to cause a degree of alteration in the concentrations of HDL-c and LDL-c, with a significant decrease in the LDL-c fractions while a significant increase in HDL-c fractions. They added that the alterations of lead affecting lipid parameters vary according to doses (Okediran *et al.,* 2018).

It was observed that lead acetate contributed to the enhancement of the lipid profile, whereas pretreatment with M. oleifera resulted in a reduction in CHOL, HDL-C and LDL-C levels in animals that were exposed to lead acetate. Furthermore, lead acetate was found to elevate the total protein levels while decreasing total bilirubin and triglycerides following the administration of M. oleifera and lead acetate in comparison to the control group G1. The protective mechanism exhibited by *M. Oleifera* was attributed to its ability to lower TG and TBIL levels. They deduced that lead poses a toxic threat, and the utilization of M. oleifera serves as a partial remedy to ameliorate the adverse effects induced by lead (Melebary and Elnaggar, 2023).

Concerning the oxidative stress markers in the present work, administration of lead caused significant decrease in levels of antioxidant markers, Glutathione (GSH), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) compared to the negative control group. Whereas administration of AgNPs + Lead caused significant elevation in GSH and SOD compared to Lead group but still lower than negative control. On the other hand, the administration of *M. Oleifera* + Lead caused a significant decrease in antioxidants compared to Lead and *M. Oleifera* + Lead groups. Meanwhile, levels of oxidant marker, MDA was elevated versus control group. After the administration of AgNPs, the level of MDA was significantly decreased versus Lead group but still higher than control. Administration of Mo led to significant decreased levels of MDA versus Lead and M. oleifera + Lead groups but still level was higher than control.

In a study by Bozdağ and Eraslan (2020) they found that lead acetate exposure in rats caused significant changes in liver and kidney oxidative stress parameters such as MDA, NO, SOD, CAT, GSH-Px, GSH and they added that, administration of the antioxidant substance, Diosmin alleviated the harmful effects of lead toxicity. Hussain et al. (2019) said that the utilization of M. oleifera leaf extract was employed as a remedial approach to counteract the toxicity induced by Lead in a group of rats. The investigation revealed that M. oleifera leaves harbored substantial quantities of antioxidants effectively mitigated the deleterious impacts of lead poisoning and resulted in enhancements in the antioxidant parameters such as SOD, CAT and MDA as well as a decline in the accumulation of lead in both the liver and brain of the experimental rats. These findings are in accordance with the present work results. Also, the utilization of M. oleifera leaf aqueous extract was explored as a therapeutic intervention for cadmium-induced liver toxicity in albino rats.

The present results showed that oral administration of Lead-induced abnormal kidney function in treated rats. The Blood Urea Nitrogen (BUN), Uric Acid (UA) and creatinine (CREA) levels were significantly increased, in Lead treated rats when compared with negative control group. While administration of M. oleifera + Lead significantly decreased the levels of serum BUN, UA, and CREA compared to Lead-treated rats. Ansar *et al.* (2016) found that lead acetate exposure in rats led to increased UA, CREA, and BUN levels, along with decreased antioxidant enzyme activities, indicating renal inflammation. They added that, lead acetate exposure impairs kidney function by increasing UA, CREA, and BUN levels while decreasing antioxidant enzyme activities.

There has been no explanation of how Moringa oleifera leaves extract can reduce BUN and CREA levels in the blood. However, the role of that extract is likely to be indirectly protecting against damage to cells in the kidneys' proximal tubules, thus improving the kidneys' function (Rana *et al.,* 2018). Dwi *et al.* (2024) revealed that damages to the kidney proximal tubules cause impaired renal function, which can be evident as increased BUN and CREA in the blood and urine. They added that administration of the M. oleifera leaf extract significantly reduced the rats' serum BUN and CREA levels. These findings are consistent with the present study results and with those of Melebary and Elnaggar (2023), who reported similar results.

Silver nanoparticles (AgNPs) resulted in a significant elevation in the levels of various enzymes such AST, ALT, ALP, and UREA, CREAT, and MDA in the rat tissues, indicating increased oxidative stress. Conversely, there was a notable reduction in the concentrations of antioxidants like GSH, CAT, SOD, and total thiol groups. These alterations

in biochemical parameters suggest that the administration of AgNPs sub-dermally adversely affected the hepatic, renal, and cardiac functions in male Wistar rats, highlighting the potential toxicity of AgNPs on multiple organ systems (Olugbodi *et al.,* 2023).

The present results showed that intraperitoneal administration of lead-induced abnormal inflammatory biomarkers in treated rats. The levels of serum Interleukin-6 (IL-6), Tumor Necrosis Factor- (TNF-) and Lactate Dehydrogenase (LDH) were significantly increased, in Lead-treated rats when compared with negative control group. The administration of M. oleifera with Lead significantly decreased the levels of serum IL-6, TNF- and LDH decreased compared to Lead-treated rats. Restoration of inflammatory biomarkers to the normal range was at the dose level when compared with Lead-treated rats. These results are in accordance with the findings of Karthivashan et al. (2016) who said that the pathological alterations of serum IL-6, TNF- and LDH were reversed in a dose-dependent manner following treatment with M. oleifera leaf extract. This suggests that the M. oleifera leaf extract exhibits therapeutic efficacy against lead-induced nephrotoxicity by enhancing the endogenous antioxidant system and exerting a modulatory influence on specific inflammatory cytokines in the kidney tissues.

The present histopathological results revealed that, in lead administered group, the hepatocyte appeared with their vacuolated nuclei, blood sinusoids very huge dilation and congestion and the central vein contain a lot of RBCs and a little bet of the lymphocyte, degenerated liver cells, activated Kupffer cells, apoptotic and necrotic areas. Also, lead causes some histopathological changes in the kidney of rats displaying degenerative changes in renal epithelium, degenerative changes include the enlarged renal tubular epithelial, necrosis of the cells lining renal tubules, mesangial expansion, large Bowman's space, convoluted tubule displaying vacuolar degeneration, dilatation of the proximal and distal convoluted tubules, loss of the brush border. In accordance with these findings are those of Mohammed *et al.* (2017) who declared that lead caused numerous histological alterations in the liver, including chronic inflammation, biliary hyperplasia, edema, congestion, Kupffer cells hyperplasia and hemosiderosis, and in the kidney, including tubular dilation, atrophy of glomerular tuft, widening of urinary space and mild fibroblast. Also, Dehkordi *et al.* (2015) said that the harmful histological change in the liver tissues which lead is responsible for are vacuolation in some hepatocytes, fatty degeneration and in central veins and some sinusoids congestion appeared, and in the interstitial tissue there was hemorrhage and infiltration of inflammatory cells. In kidney tissue in male rabbits after a long exposure to lead acetate the histopathological effects are dilatation, congestion, nuclei heterochromatic effects and an increase in the urinary barrier thickness impacting kidney functions negatively (Karimfar *et al.,* 2016).

In silver nanoparticles + lead treated group, irregular degree of improvement was observed in hepatocytes, presented moderate hepatic sinusoids dilatation with a slightly moderate number of Kupffer cells and mild disintegrative changes, moderate dilation of central vein with mild RBCs. The histopathology of the kidney in AgNPs + lead group showed mesangial moderate expansion of the glomerulus, moderate Bowman's space, convoluted tubule showing mild vacuolar degeneration, mild dilatation of the proximal and distal convoluted tubules, few of the brush border. Elfaky *et al.* (2022) investigated and compared the effect of silver nanoparticles' particle size in terms of their potential hazard, as well as their potential protective effect in a lipopolysaccharide (LPS)-induced hepatotoxicity model. On the other hand, in group administered lead + *M. oleifera* leaves extract revealed a decrease in the histological alterations in the liver as there was less expansion of the central vein, sinusoidal gaps, congestion in the central vein, little endothelial cells. Also, the kidney tissues showed nearly normal glomeruli, simple squamous cells lining the Bowman's capsule, and roughly normal Bowman's space. Proximal and distal convoluted tubules are almost entirely normal with only a small degree of hyaline cast development.

M. oleifera leaves extract was utilized as a remedy for lead-induced liver damage in mature Wistar rats to assess its hepatoprotective and ameliorative effects on the liver's histology and hepatic reticular fibers. Microscopic examination uncovered changes in the liver histopathology such as hepatocytic vacuolations, sinusoidal congestion, and a reduction in reticular fibers post lead exposure. Conversely, the administration of *M. Oleifera* not only prevented but also reversed the hepatic damage induced by lead. To sum up, this investigation demonstrates that the extract derived from M. oleifera leaves possesses a

notable capability to shield against liver toxicity triggered by lead, partially due to its chemical components that exhibit hepatoprotective characteristics (Omotoso *et al.*, 2015).

Lastly, in groups treated with AgNPs + M. oleifera + lead approximately normal hepatocytes structure with radially oriented hepatocytes surrounding the central vein and the lack of any degenerated cells. Like the normal control group, the hepatic sinusoidal spaces and Kupffer cells were present. In the kidney tissues, proximal and distal tubules, Bowman's capsule, renal glomerulus, and renal cortex with a notable improvement and restoration of normal renal architecture are visible in lead + AgNPs + M. oleifera. Aboulthana et al. (2021) said that M. oleifera nano-extract was found to reverse the levels of hematological and biochemical parameters, as well as tumor and inflammatory markers, back to normal in both the groups of rats treated simultaneously and post-treatment with the nano-extract. Furthermore, it reduced the severity of histopathological changes in the group treated simultaneously and completely prevented it in the post-treated group.

Declarations

Ethics Approval: The experiments were processed using the animal ethical rules of King Abdulaziz University's Animal Care and Use Committee (ACUC). Furthermore, all tests adhered to the Arrive standards and the EU Directive 2010/63/EU regarding animal research.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contribution: Salim M. El Hamidy contributed to the paper by researching and editing the article.

Data Availability Statement: The collection of data developed and/or assessed throughout the present work is available through the corresponding author upon reasonable request.

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