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Effects of Selenium Nanoparticles on Biochemical Parameters and Histopathological Changes in Lead-intoxicated Rats

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ABSTRACT

Background: Lead pollution -a major environmental problems in industrial countries- is associated with health problems. The present study aimed to investigate the protective effects of selenium nanoparticles in a lead-induced testicular toxicity model.
Methods: In total, 30 Wistar rats were divided into three groups and treated (except the normal control) with lead acetate in drinking water (1,000 mg/l) for five weeks. The negative and positive control rats received saline intraperitoneally, and the third group received intraperitoneal injections of selenium nanoparticles (0.5 mg/kg).
Results: The lead-treated group showed a significant increase in blood urea nitrogen, serum creatinine, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and malondialdehyde levels ($P < 0.01$). The lead-intoxicated rats treated with selenium nanoparticles showed a significant decrease in serum AST and ALT compared to the untreated negative controls ($P < 0.5$). The histopathological examination of liver and kidney tissues indicated lead-induced injuries (e.g., necrotic cells in liver and kidneys). The selenium-treated group showed reduced histopathological signs of lead-induced injuries. Lipid peroxidation levels were also lower in the selenium-treated rats compared to the negative controls ($P < 0.05$).
Conclusion: This experimental study confirmed the protective effects of selenium nanoparticles in the rats exposed to chronic lead-induced toxicity. However, further experiments are required to evaluate the possible side-effects and complications.

1. Introduction

Lead (Pb) is the most common environmental pollutant, which could exert detrimental effects on human health. Lead poisoning may occur following long-term exposure to lead-contaminated air, food, water, and soil [1]. The liver, heart, kidney, and brain are the most susceptible organs to lead toxicity. Lead-induced neurotoxicity is associated with central nervous system disorders, constipation, headache, arthritis, and memory loss. The numerous health effects and economic impacts of lead toxicity have rendered this heavy metal the most prevalent environmental pollutant [2].

Chelation therapy is frequently used for the treatment of lead poisoning. According to the literature, minerals such as zinc, magnesium, and calcium could prevent the complications caused by lead toxicity [3]. Selenium is an essential element with a key role in metabolism, liver function, and reproduction, which make this element

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significant in various areas. Selenium is an antioxidant with diverse antioxidant effects on the body, which could prevent cancer. However, conflicting data have been proposed regarding the biological effects of this element [4].

Selenium has been reported to be effective in the prevention of prostate cancer, breast cancer, and cardiac diseases. Data are scarce regarding selenium toxicity although selenium deficiency is linked to thyroid dysfunction, memory loss, and impaired sperm production. Selenium also plays a pivotal role in the regulation of the immune system, as well as the production of immune-enhancing agents [5].

The use of selenium nanoparticles (NPs) is progressively growing across the word. Nanotechnology could affect the biomedical application of numerous drugs and elements. Furthermore, evidence suggests that nanoparticles from the bulk forms of materials have a wide range of biological effects [6]. NPs have unique biomedical and industrial properties, which makes them proper candidates for biomedical and pharmacological experiments. Notably, NPs have unique physicochemical properties compared to conventional drugs and minerals [7].

Selenium NPs affect various aspects of daily life due to their diverse industrial functions in electronics, medical devices, medical engineering, and orthopedic devices [8]. Selenium NPs have attracted the attention of researchers for the manufacturing of novel drugs. Selenium NPs are used in laboratories worldwide to prepare antioxidant, anticancer, and energizing formulations given their enhanced effects and low toxicity [9].

Animal studies have indicated that selenium NPs could reduce the toxicity of several metals, such as cadmium and lead [10]. The mechanism of the hepatoprotective effects of selenium has been attributed to the antioxidant effects of this element. Furthermore, these NPs could prevent the absorption of lead and other toxic metals by the digestive system, while the hypothesis requires further scientific evaluations. These NPs could also be metabolized by the microsomal enzymes of the liver and biliary excretion. Data are scarce regarding the protective effects of selenium NPs against lead-induced reproductive toxicity.

To the best of our knowledge, there is little data regarding the protective effects of selenium nanoparticles on lead acetate-induced toxicity. The present study aimed to investigate the protective effects of selenium nanoparticles in a lead-induced testicular toxicity model.

2. Materials and Methods

2.1. Experimental Materials

Serum creatinine, serum blood urea nitrogen (BUN), and serum liver enzymes were measured using the commercial diagnostic laboratory kits manufactured by Pars Azmoon Company (Pars Azmoon, Tehran, Iran). The biochemical analysis of serum ALT, serum AST, serum BUN and serum creatinine were performed using the Selectra Pro M autoanalyzer (Vital Scientific, Spankeren, Netherlands).

2.2. Synthesis of Selenium NPs

To synthesis selenium nanoparticles (SeNPs), selenium powder (0.25 M) was added to the solution of sodium sulfate (0.50 M) in 100 milliliters of double-distilled water, and the mixture was stirred at the temperature of 70°C for nine h. A transparent Na_2SeSO_3 solution was obtained and used as a precursor for the synthesis of the SeNPs. The preliminary appearance of a pink color confirmed the formation of the SeNPs; the color indicated the synthesis of the SeNPs into the solution. After the synthesis of the SeNPs, the supernatant was fully collected and centrifuged at 11,500 rpm and the temperature of 4°C for 20 min. The supernatant was discarded, the pellet was washed with distilled water thrice, and the final pellet was suspended into distilled water and lyophilized. Afterwards, the powder was collected for performing the analytical techniques. To stabilize the SeNPs, polyvinyl alcohol (PVA) or other biopolymers (e.g., starch, chitosan, and cellulose acetate) were considered. The synthesized SeNPs were stabilized using 1% aqueous polyvinyl alcohol (0.05 ml). To this end, the SeNPs were mixed with PVA in water and stirred for 30 min. Following that, the SeNPs were distributed in the polymer matrix and stabilized, and the aggregation of the NPs was delayed.

2.3. Animal Grouping

To perform the biological experiments, 30 adult Wistar rats were equally divided into three groups. The experimental animals were obtained from the animal breeding colonies of the laboratory animal house of the University of Zabol (Zabol, Iran). Prior to the experiments, the rats were housed in the experiment room to adapt to the conditions and received lead acetate for five weeks in drinking water (1,000 mg/l). The control and negative control rats received saline intraperitoneally, and the lead-intoxicated group received 0.5 ml of SeNPs intraperitoneally (0.5 mg/kg) for seven days before and during lead intoxication. The serum was obtained from the retro-orbital sinus of the eye using an EDTA-free microhematocrit tube. After blood sampling, the animals were euthanatized by sodium pentobarbital. Animal handling and injection procedures were performed in accordance with the Ethical Committee codes of the Institutional Animal Research (UOZ.REC.1399.003).

2.4. Lipid Peroxidation

Serum and liver malondialdehyde (MDA) levels were measured using the method of Okhawa *et al.* (1979) with slight modifications [9]. The method is based on the chemical

reaction between MDA and thiobarbituric acid powder. After the chemical reaction, the absorbance of the final solution was determined using a spectrophotometer, and the final optical density was expressed as nmol/ml.

2.5. Histopathological Examination

For histopathological examinations, the animals were sacrificed to remove the abdominal and thoracic organs (liver, kidney, and testis). The specimens were cut into two separate pieces; one piece was frozen for the further analysis of lipid peroxidation, and the other piece was preserved in 10% neutral buffer formalin. Following that, the specimens were sent to the pathology laboratory for paraffin embedding and serial block making, and the paraffin blocks were sliced using a microtome device. After paraffin embedding, the obtained liver, kidney, and testis sections were stained using the Hematoxylin-Eosin method for further investigations via light microscopy (Olympus, Tokyo, Japan).

2.6. Statistical Analysis

Data on serum MDA, serum BUN, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and creatinine were statistically analyzed in SPSS version 18.0 using and one-way analysis of variance (One-way ANOVA). In addition, multiple comparisons were performed using Tukey's post-hoc test at 5% confidence interval ($P < 0.05$).

3. Results and Discussion

3.1. Biochemical Results

Table 1 shows the serum biochemical changes in the lead-intoxicated rats and normal control rats.

According to the obtained results, lead intoxication significantly increased liver AST, serum ALT, serum creatinine, serum MDA, and serum BUN compared to the healthy animals ($P < 0.01$). In addition, liver MDA significantly increased in the lead-intoxicated rats compared to the control group ($P < 0.01$). On the other hand, SeNP treatment decreased serum liver enzymes and lipid peroxidation in the lead-treated rats. However, serum BUN and serum creatinine were lower in these animals compared to the untreated lead-intoxicated rats ($P < 0.05$). Serum AST level, serum lipid peroxidation, and serum ALT levels showed no significant changes compared to the normal control rats ($P > 0.05$).

Table 1: Serum biochemical indices and serum lipid peroxidation in experimental groups.

Item	Treatment		
	Control	Lead-intoxicated rats	Selenium NPs + Lead
Serum MDA (nmol/ml)	50.3 ± 11.2	85.7** ± 14.2	63.1 ± 4.1
Liver MDA (nmol/mg/ tissue)	145.2 ± 15.6	176.4** ± 17.4	153.1 ± 12.2
AST (U/L)	97.5 ± 8.5	129.1** ± 24.6	113.1 ± 8.1
ALT (U/L)	37.2 ± 5.2	55.2** ± 10.6	42.2 ± 8.8
BUN (mg/dl)	14.8 ± 2.4	25.6** ± 3.3	17.8 ± 2.2
Creatinine (mg/dl)	0.72 ± 0.19	1.1** ± 0.11	0.84 ± 0.09

*Significance difference with control group [$P < 0.05$], **significant difference with control group [$P < 0.01$], *** significant difference with control rats [$P < 0.001$].

The investigation of the paraffin sections of the control group indicated the healthy structure of the liver with distinct hepatocytes, and normal portal triad (Figure 1A). The liver micrographs of the animals receiving lead acetate only showed the disarrangement of the hepatic cords with signs of pyknotic nuclei, resembling the early stages of necrosis (Figure 1B). However, these changes were not evident in the group treated with SeNPs and intoxicated with lead (Figure 1C). Masson trichrome staining of the liver sections also revealed similar histopathological results (Figure 1B). The control group had a normal structure, while the lead-treated rats showed congestion and cytoplasmic vacuolation (Figure 2B). On the other hand, the control group had healthy glomerulus, renal corpuscle bodies, and normal Bowman's space (Figure 3A).

According to the findings, the rats treated with lead acetate had massive and diffuse hyaline cast generation in the distal and proximal tubules (Figure 3B). The kidney section of a selenium-treated, lead-intoxicated rat showed decreased hyaline cast formation. Therefore, it could be inferred that treatment with SeNPs could reduce lead-induced renal histological changes. However, selenium treatment cannot restore the renal histology to the normal state.

Our findings indicated that the lead-intoxicated group treated with SeNPs showed lower toxicity compared to the lead-intoxicated rats (Figure 3C). Furthermore, selenium treatment decreased lead-induced testicular toxicity, and the groups treated with lead acetate showed signs of testicular toxicity (figures 4C & 4B). However, the control rats had normal testicular histopathology with distinct seminiferous tubules and normal spermatogonium cells (Figure 4A). The testis of the rats treated with lead acetate showed the necrosis of germinal epithelium in the seminiferous tubules (Figure 4B).

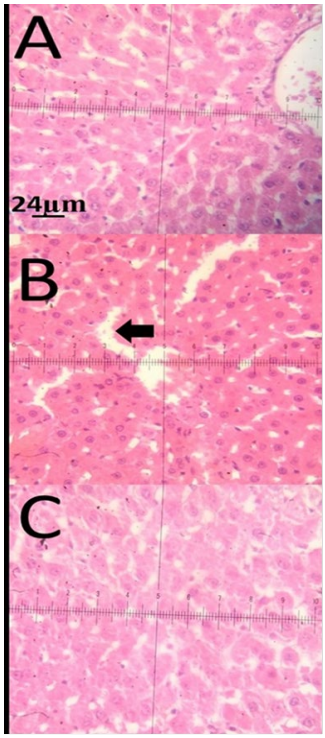

Figure 1: Light micrograph of liver section of experimental groups; A) liver section of normal control rats, B) liver section of lead-treated rats (arrow shows disarrangement of hepatic cords), C) liver micrograph of lead-intoxicated rats after treatment with selenium nanoparticles [H&E stain; microscopic magnification 40X; bar=24 µm])

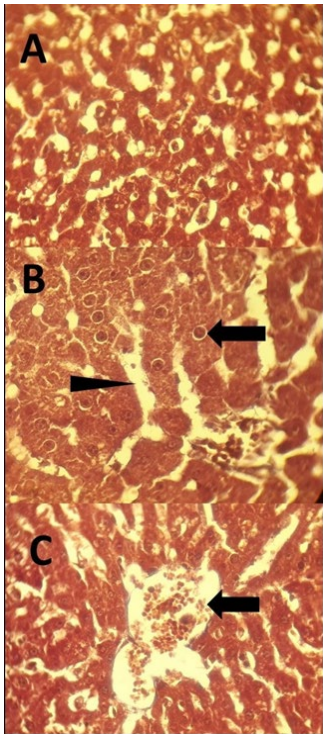

Figure 2: Masson trichrome staining of liver sections; A) liver section of normal control rats, B) liver micrograph of lead-treated rats (arrow shows vacuolation in hepatocyte cytoplasm), C) liver micrograph of lead-intoxicated rats after treatment With selenium nanoparticles (arrow shows mild blood congestion [H&E stain; microscopic magnification 40X]; bar=24 µm)

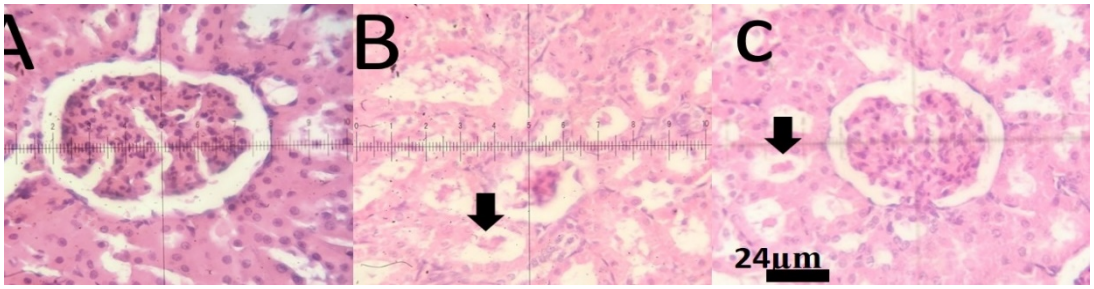

Figure 3: Light micrographs of kidney sections; A) control rats, B) lead-treated rats (arrow shows hyaline casts), C) kidney section of selenium-treated lead-intoxicated rats (arrow shows decreased hyaline cast formation [H&E stain; microscopic magnification 40X])

Environmental lead exposure and toxicity are major health issues in developing and industrial countries. Children are susceptible to lead toxicity due to higher metabolism rates and higher dietary lead absorption. Recently, serum lead concentrations have been reported to decrease in industrial countries; nevertheless, environmental lead exposure remains a significant health concern. The environmental accumulation of lead acetate in soil, air, and water is associated with the high concentrations of lead in the food chain. Chemical drugs are the first choice for the treatment of lead toxicity although they have unwanted adverse effects. SeNPs have been investigated in previous studies as potential antioxidant agents [10]. The results of the present study indicated that selenium treatment could reduce lead toxicity in the rats, which is consistent with the previous findings regarding the hepatotoxicity effects of lead acetate. According to the current research, the proper dose of the SeNPs exerted no toxic effects on the histopathological and biochemical parameters of the rats. Furthermore, the results of the histopathological analysis confirmed the biochemical results. Previous experiments have also indicated that at super nutritional levels, SeNPs have no toxic effects on rats

[11]. Our findings in this regard are consistent with the previous studies [12, 13].

SeNPs are absorbed through the skin, respiratory system, and gastrointestinal tract and could rapidly distribute throughout different parts of the body. However, they cannot easily penetrate specific organs, such as the testes, thymus, and brain. The results obtained by Hasanin demonstrated the antioxidant and anti-apoptotic effects of SeNPs in the thyroid of rats [14]. Recent findings have also indicated that SeNPs are less toxic compared to inorganic and organic selenium [15]. The *in -vitro* and *in -vivo* toxicity assessment of Selenium nanoparticles has also confirmed low cytotoxicity and proper bactericidal activity [16]. In the current research, the kidney section of a selenium-treated, lead-intoxicated rat showed decreased hyaline cast formation. Therefore, it could be concluded that SeNP treatment could reduce the lead-induced renal histological changes. However, selenium treatment could not restore the renal histology to the normal state.

Previous studies have elaborated on the toxicity of lead acetate in various organs [17, 18]. In the current research, significant histological changes were also observed in various organs, including the liver, testis, and kidneys. The SeNPs could induce histological changes in the liver and kidneys of rats [19]. Histopathological investigations have also shown that lead may induce severe histological changes. Our findings in this regard are consistent with the previous studies [20, 21]. In the present study, histological changes were less significant in the selenium-treated, lead-intoxicated rats. On the other hand, the effects of lead acetate and SeNPs on the testis of the animals indicated the ability of these materials to cross the blood-testis barrier in rats. In this regard, previous experiments have also confirmed that SeNPs could cross the blood vessels of the testis [22].

Previous studies regarding NPs have shown the pro-oxidant effects of SeNPs in laboratory rodent models [23, 24]. The results of the present study indicated the effectiveness of the SeNPs in the reduction of the intoxication complications caused by lead acetate. According to the literature, selenium could also reduce cadmium toxicity by activating the nuclear factor erythroid 2-related factor 2 pathway [25]. The hepatoprotective effects of selenium against lead toxicity could be attributed to the antioxidant and anti-lipid peroxidation properties of this element. In the current research, co-treatment with selenium and lead acetate was observed to decrease lipid peroxidation.

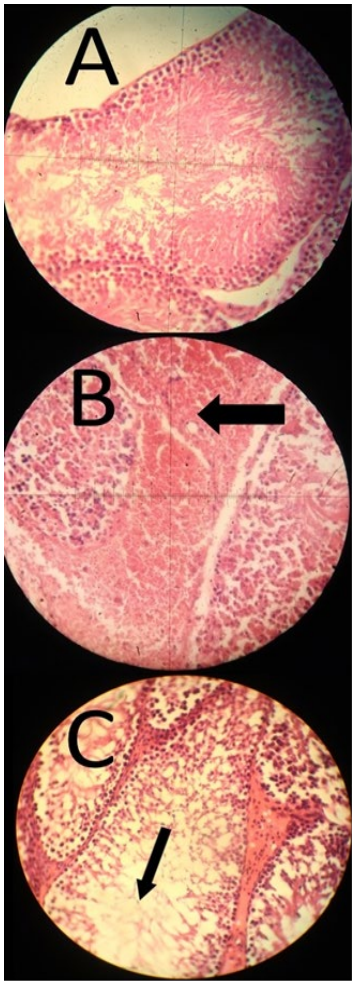

Figure 4: Testis micrographs of experimental rats; A) testis section of normal control rats, B) testis section of lead-treated rats (arrow shows hemorrhage in testis), C) testis micrograph of lead-intoxicated rats after treatment with selenium nanoparticles (H&E stain; microscopic magnification 40X; bar=24 µm)

4. Conclusion

According to the results, the SeNPs reduced the lead-induced complications in the liver, kidneys, and testis of the rats. Further investigations are required to determine the interaction of SeNPs with other heavy metals.

Authors' Contributions

M.R.H., supervised the research project and drafted the manuscript.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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