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Comparison of the Histopathological Effects of Selenium Nanoparticles and Cerium Oxide Nanoparticles in Cadmium-intoxicated Rabbits

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Abstract

This study compared the effects of selenium nanoparticles and cerium oxide nanoparticles on biochemical and histopathological changes of cadmium-intoxicated rabbits. Forty white New Zealand rabbits were equally distributed into four groups. The Control group received saline orally while the negative control group received six-week oral administrations of cadmium 5 mg/kg. The third group received oral administration of selenium nanoparticles (0.8 mg/kg) and cadmium (5mg/kg). The fourth group received cerium oxide nanoparticles (0.8 mg/kg) and cadmium (5mg/kg). After six weeks, serum was obtained by the conventional methods, and then rabbits were sacrificed to obtain liver, kidney, and testis tissues for histopathological examinations. The oral administrations of Cadmium 5 mg/kg caused significant elevations in serum liver enzyme levels, serum BUN, serum creatinine, and lipid peroxidation levels ($P<0.05$). The histopathological investigations showed necrosis and inflammations in the liver, kidney, and testis of rabbits. Selenium nanoparticles oral administrations at a dose of 0.8 mg/kg significantly reduced liver enzymes, malondialdehyde (MDA) content, and histopathological changes. The cadmium intoxicated rats treated with Cerium oxide nanoparticles showed normal biochemical and histopathological parameters ($P<0.05$). The current study proved the hepatoprotective and nephroprotective effects of selenium nanoparticles and cerium oxide nanoparticles in cadmium intoxicated rabbits; however, cerium oxide nanoparticles were more effective.

Keywords: Selenium, Nanoparticles, Liver, Kidney, Toxicity

1 Introduction

Cadmium- a chemical element with the symbol Cd - is one of the most common environmental pollutants. Cadmium toxicity could induce detrimental effects on various organs of the body. Cadmium toxicity is a common heavy metal poisoning that occurs following chronic exposure to contaminated food, air, water, and dust (1). The kidney, liver, testis, heart, and brain are the main target organs of cadmium-induced toxicity. Cadmium hepatotoxicity and nephrotoxicity are linked to various illnesses such as headache, arthritis, nephritis, and Alzheimer's diseases (2). The economic impacts and health effects of lead toxicity made this heavy metal one of the most common environmental hazards. Previous studies have shown that minerals like selenium, potassium, Zinc, Magnesium, and Calcium are effective medical choices for the treatment of cadmium poisoning (3).

Selenium (Se) is an antioxidant element and plays an essential role in metabolism (4). Selenium plays a major role in reproduction and metabolism. Selenium is associated with the reduced risks of prostate cancer (5), heart diseases (6), and Alzheimer's (7). Selenium deficiency has been associated with a heart attack and immune imbalance (8). Selenium plays an important role in the body (9). The optimum dose of selenium is between 15 micrograms (mcg) to 60 micrograms (10). Nanoparticles or nano-carriers allows for Selenium delivery within the target organ. The physicochemical properties of selenium nanoparticles and cerium oxide nanoparticles are different from other materials (11). Nanoparticles have a wide range of biomedical and industrial applications (12). In recent years, the use of nanoparticles as efficient chemotherapy agents is increasing (13). Cerium oxide nanoparticles have been widely used in biomedicine due to the antioxidant, anticancer, and antioxidant potential. Cerium oxide nanoparticles are used in medical diagnostic imaging and medical engineering. Other applications of cerium oxide nanoparticles are antimicrobial wound-coatings, orthopedic surgical devices, and mineral supplements (14).

Cerium oxide nanoparticles can prevent cellular damage. However, there is a narrow margin between the therapeutic, and toxic doses of these nanoparticles. The high-dose of Cerium oxide nanoparticles showed toxicity in the long-term or short-term (15). Bio-distribution of Cerium oxide nanoparticles can easily be absorbed orally and could distribute in the respiratory system. Cerium oxide nanoparticles can be difficult to diffuse into tissues such as the testes, and brain (16). Cerium oxide nanoparticles can be metabolized by liver microsomal enzymes and could be reduced rapidly (17). This study aimed to investigate the effects of Cerium oxide nanoparticles and selenium nanoparticles following oral treatment. Also, there is little data regarding the protective effects of selenium nanoparticles against cadmium-induced reproductive toxicity and nephrotoxicity. Therefore, the present study was conducted to compare the therapeutic efficacy of cerium oxide nanoparticles and selenium nanoparticles against cadmium toxicity.

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2 Material and Methods

2.1 Materials

The assay kits for creatinine, ALT, BUN, and AST were purchased from Pars Azmoon Company. (Pars Azmoon, Tehran, Iran). Cadmium, selenium, sodium sulfite, and polyvinyl alcohol were purchased from Merck Company Germany.

2.1 Animal grouping

In the current work, forty male adult new Zeeland rabbits mean weight 1743 g were obtained from the anima laboratory breeding colony of the veterinary medicine faculty, University of Zabol. Following acclimatization, rabbits were randomly divided into four treatment groups. The first group (control group) was treated with normal physiological saline for six weeks. Negative control groups received cadmium (5mg/kg) orally for six continuous weeks. The third group received oral administrations of Selenium NPs at 0.8 mg/kg and cadmium 5mg/kg for six weeks. The fourth group received cerium oxide nanoparticles at 0.8 mg/kg and cadmium 5mg/kg.. The protocol of experiments was performed according to the approved protocols of the Laboratory Ethics Committee of veterinary medicine university of Zabol, Zabol, Iran.

2.2 Determination of biochemical parameters

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN, and creatinine were measured enzymatically by the Selectra pro, M autoanalyzer, (Vital Scientific, Span Neren, Netherlands) using Pars Azmoon reagents kit (Pars Azmoon. Co., Iran).

2.3 Determination of Lipid peroxidation assay

Serum levels of malondialdehyde (serum MDA) concentration was examined by the colorimetric method of Okhawa et al, with minor changes. The method of Okhawa is based on the chemical reaction between serum malondialdehyde (MDA) and thiobarbituric acid. The obtained optical density results were expressed as nmol/ml (18).

2.4 Histopathological examination

For histopathological analysis, rabbits were euthanized by the high doses of ketamine and xylazine. The animals were beheaded to remove the kidney, liver, and testis tissues for further histopathological investigations. The obtained liver and kidney sections were immediately stained by the hematoxylin-eosin staining method. The stained sections were investigated by the laboratory light Olympus microscope (Olympus, Tokyo, Japan) to detect histological changes.

2.5 Statistical analysis

To investigate the statistical difference between groups, collected data were examined by using the statistical software of SPSS (version 20.0). Multiple comparisons between experimental groups were determined by ANOVA and Bonferroni post-hoc test. The significance level was determined at a 5% level ($p<0.05$).

3 Results and Discussion

3.2.1 Biochemical results

The effects of six-week oral administrations of cerium oxide nanoparticles and Selenium nanoparticles on cadmium-intoxicated rabbits are presented in Table 1. The oral administering of Cadmium at a dose of 5 mg/kg significantly increased serum BUN, creatinine, serum AST, ALT, and serum MDA levels ($P < 0.001$) (Table 1). As shown in Table 1, the oral administration of cadmium caused a significant increase in serum ALT, AST, BUN, and creatinine levels and increased lipid peroxidation. There was also a significant decrease in serum catalase activity in this group ($P < 0.01$) (Table 1). Serum samples of rabbits treated with the 0.8 mg/kg dose of Selenium NPs and intoxicated revealed significant changes in serum BUN and creatinine. Other biochemical parameters were not significantly different ($P < 0.05$). Six weeks of treatment with cerium oxide NPs cadmium 5 mg/kg did not induce any significant changes in serum liver enzymes, serum BUN, and serum creatinine levels of cadmium intoxicated rabbits. The catalase activity and serum MDA levels also did not show significant alterations compared to those in the normal control rabbits.

3.2.2 Histopathological results

As shown in figure 1, the control group had normal cellular histology with normal hepatocytes and normal hepatic cords (figure 1A). The liver of rabbits cadmium intoxicated rabbits had severe fatty changes (figure 1B). Liver sections of cadmium intoxicated rabbits that were treated with selenium nanoparticles showed a decrease in histopathological lesions and fatty change (figure 1C).

The histopathological investigation of the liver of rabbits treated with cerium oxide NPs (0.8 mg/kg) and cadmium (5 mg/kg) revealed normal hepatocytes and normal central vein (figure 1D). The renal sections of the Control group had normal glomerulus, normal Bowman's capsule, well-observed distal tubules, and intact proximal tubules (figure 2A). The kidney histology of rabbits received the 0.8 mg/kg of selenium nanoparticles showed less histopathological changes, but cytoplasmic vacuolation was still present (figure 2B). The kidney of cadmium intoxicated rabbits treated with cerium oxide nanoparticles showed normal renal structure (figure 2C and D). The testis sections of normal control rabbits had a normal testicular appearance (figure 3A). Rabbits of the groups treated with 0.8 mg/kg of selenium nanoparticles along with cadmium 5 mg/kg also had normal kidney histopathology (figure 3C). Testis of cadmium intoxicated rabbits showed necrosis of germinal epithelium and reduction of diameter in the seminiferous tubules (figure 3B).

Table 1: Effects of Selenium nanoparticles and cerium oxide nanoparticles on serum biochemical parameters in rabbits.* indicate statistical significance from the control group ($P < 0.05$). ** indicate statistically significance from control group ($P < 0.01$). *** indicate statistically significance from control group ($P < 0.001$)

Item	Treatment			
	Control	Cadmium 5 mg/kg	Selenium NPs 0.8 mg/kg + cadmium 5 mg/kg	Cerium oxide NPs 0.8mg/kg +cadmium 5 mg/kg
CAT (U/L)	55.2 ± 6.9	62.3** ± 6.2	43 ± 5.2	48.7± 5
MDA (nmol/ml)	96.3 ± 9	124.7* ± 9.9	104.1 ± 13.1	113.1 ± 7.9
ALT (U/L)	62.4 ± 6.5	96.2*** ± 10.9	68.7 ± 8.4	65 ± 7.6
AST (U/L)	36.1 ± 6.2	52.3*** ± 7	39.4 ± 9.7	38.2 ± 4.1
BUN (mg/dl)	15.3 ± 2	23.5*** ± 3.0	19.1* ± 5.1	18.1 ± 3.1
Creatinine (mg/dl)	0.77 ± 0.2	1.2*** ± 0.2	0.91* ± 0.7	0.8± 0.2

Testis of rabbits treated with Cadmium and received cerium oxide nanoparticles had signs of testicular degeneration and necrosis of seminiferous tubules. Also, there were signs of congestion and inflammation in testis tissues (figure 3D).

4 Discussion

To the best of our knowledge, there are multiple experiments conducted on the testicular and hepatic toxicity of cadmium. These studies showed undeniable evidence regarding the renal and testicular toxicity of cadmium in various animal models. Selenium nanoparticles as potential antioxidant agents have been investigated in many prior studies for protecting against heavy-metal induced nephrotoxicity and reproductive toxicity. This study was in agreement with previous studies showing the hepatotoxicity of cadmium. Selenium NPs can cross the skin and gastrointestinal tract and distribute in the body (19). The toxicity of cadmium has been reported in earlier studies. In the current study, we observed significant histological alterations in different organs. Earlier experiments have shown that cadmium can induce histological changes in the liver and kidney of rats (20). Our histopathological investigation showed that cadmium could induce histological changes. Histopathological changes were less prominent in the selenium-treated rats. The effects of cadmium on testis indicate the ability of cadmium to cross the blood-testis barrier of rabbits that were in line with the previous studies. The current study showed the efficacy of Selenium nanoparticles in reducing cadmium side effects. Previous studies have shown that Selenium may reduce Arsenic or lead toxicity in rats and mice. The mechanism of the hepatoprotective effects of selenium and cerium oxide nanoparticles against cadmium could be due to the antioxidant effects of these materials. In the present study, selenium co-treatment with cadmium decreased lipid peroxidation. The decrease in lipid peroxidation can be one of the mechanisms of hepatoprotective effects of cerium oxide.

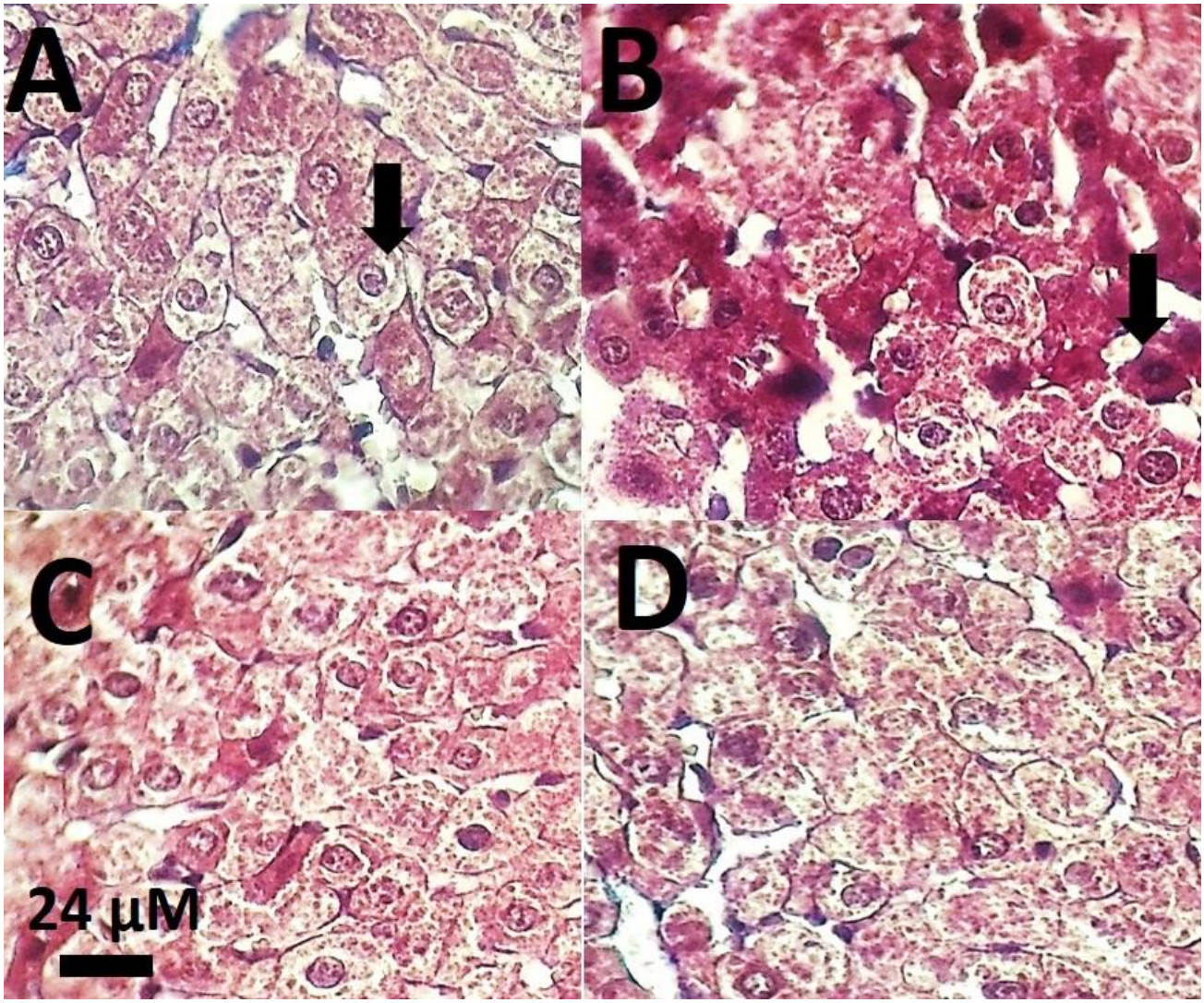

Figure 1: (A): Light micrograph of a liver section of a control rabbit; normal hepatocyte (arrow) and normal hepatic architecture. (B): Light micrograph of a liver section of rabbits treated with Cadmium and selenium nanoparticles (0.8 mg/kg). Hepatitis (arrow point), cytoplasmic vacuolation (arrow), and congestion. (C)**:** liver section of a rabbits treated with cerium oxide NPs (0.8 mg/kg) and cadmium 5 mg/kg. Normal hepatocytes (arrow), and normal central vein (CV). (D): liver section of a rabbits treated with Selenium NPs (0.8 mg/kg) and cadmium 5 mg/kg. Normal hepatocyte (arrow), and mild fatty change (arrow point) (H & E stain. Mic. Mag. × 40).

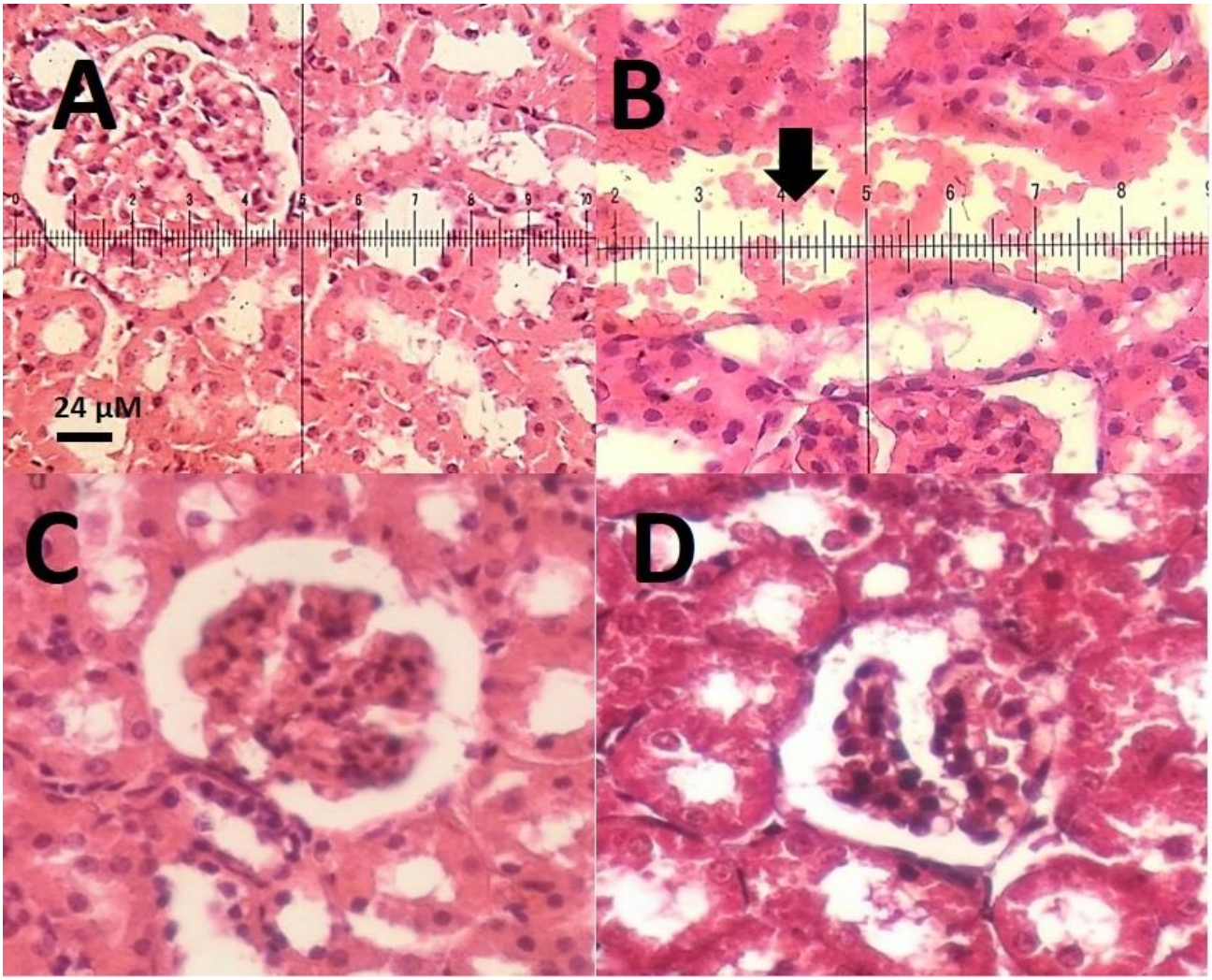

Figure 2: (A): Light micrograph of the kidney of a control rabbit; normal glomerulus (G), Bowman's capsule (Bc), proximal tubules (PT), and normal distal tubules (DT). Light micrograph of a kidney section of rabbits treated with Cadmium and selenium nanoparticles (0.8 mg/kg) (B): cytoplasmic vacuolation (arrow point), and hyaline cast (arrow) (C): Light micrograph of a kidney section of rabbits treated with Selenium NPs. D: Normal renal histopathology of a kidney of rabbits treated with cerium oxide NPs and cadmium 5 mg/kg. Normal renal structure (arrow point) (H & E stain. Mic. Mag. × 40).

Scientists are investigating the efficacy of nanoparticles to reduce the side effects of heavy metals. Selenium nanoparticles have a wide range of biological effects that make them widely available in the medical and healthcare industries. Selenium nanoparticles have unique biomedical applications due to the physical characterizations, biodegradability, and less toxicity (21). These studies showed conflicting results regarding the antioxidant/pro-oxidant activity of Selenium nanoparticles. Results of the present study showed that the Selenium nanoparticles, at low doses, had no toxic effects on serum biochemical parameters and histological alterations. Earlier studies showed that Selenium nanoparticles, at super nutritional levels, had no obvious toxic effects in rabbits (22). These results were in agreement with those of Hadrup (23*)* and in opposition to the results of Shakibaei (24) and Zhang (25). The difference in biological effects of Selenium nanoparticles could be due to the difference in physicochemical properties of Selenium nanoparticles. Results of Hasanin showed the antioxidant and anti-apoptotic effects of selenium nanoparticles in rabbit's thyroid (26). Recent studies have also shown that Selenium nanoparticles are less toxic than inorganic and organic selenium (27). In a previous study, the in vitro and in *vivo* toxicity assessment of Mk-Se NPs showed low cytotoxicity and good bactericidal activity (28). Effects of The dose-dependent toxicity of Selenium nanoparticles has been reported in the previous studies (29). Our histopathological investigations revealed prominent histological changes in the liver, kidney, and testis of rabbits. Previous studies have shown that Selenium nanoparticles can induce histological changes in the liver and kidney of rabbits (30). In the present study, the high dose of Selenium nanoparticles induced severe necrosis in the seminiferous tubules which indicates the ability of these nanoparticles to cross the blood-testis barrier. Previous studies have shown that Selenium nanoparticles can cross the blood vessels of the testis (31).

In the present study, the rabbits treated with the high dose of nanoparticles had elevated levels of MDA, liver enzymes, BUN, and creatinine levels; however, the elevations were not statistically significant. The increase in the lipid peroxidation levels indicates the pro-oxidant effects of Selenium nanoparticles, which is dose-dependent. Previous studies conducted on nanoparticles showed pro-oxidant effects of nanoparticles in laboratory rodent models (32). A previous experimental study showed the Selenium nanoparticles could reduce histological changes in the liver of thioacetamide-intoxicated rabbits (33). There are conflicting results regarding the pro-oxidant or antioxidant activity of Selenium nanoparticles and Cerium oxide nanoparticles.

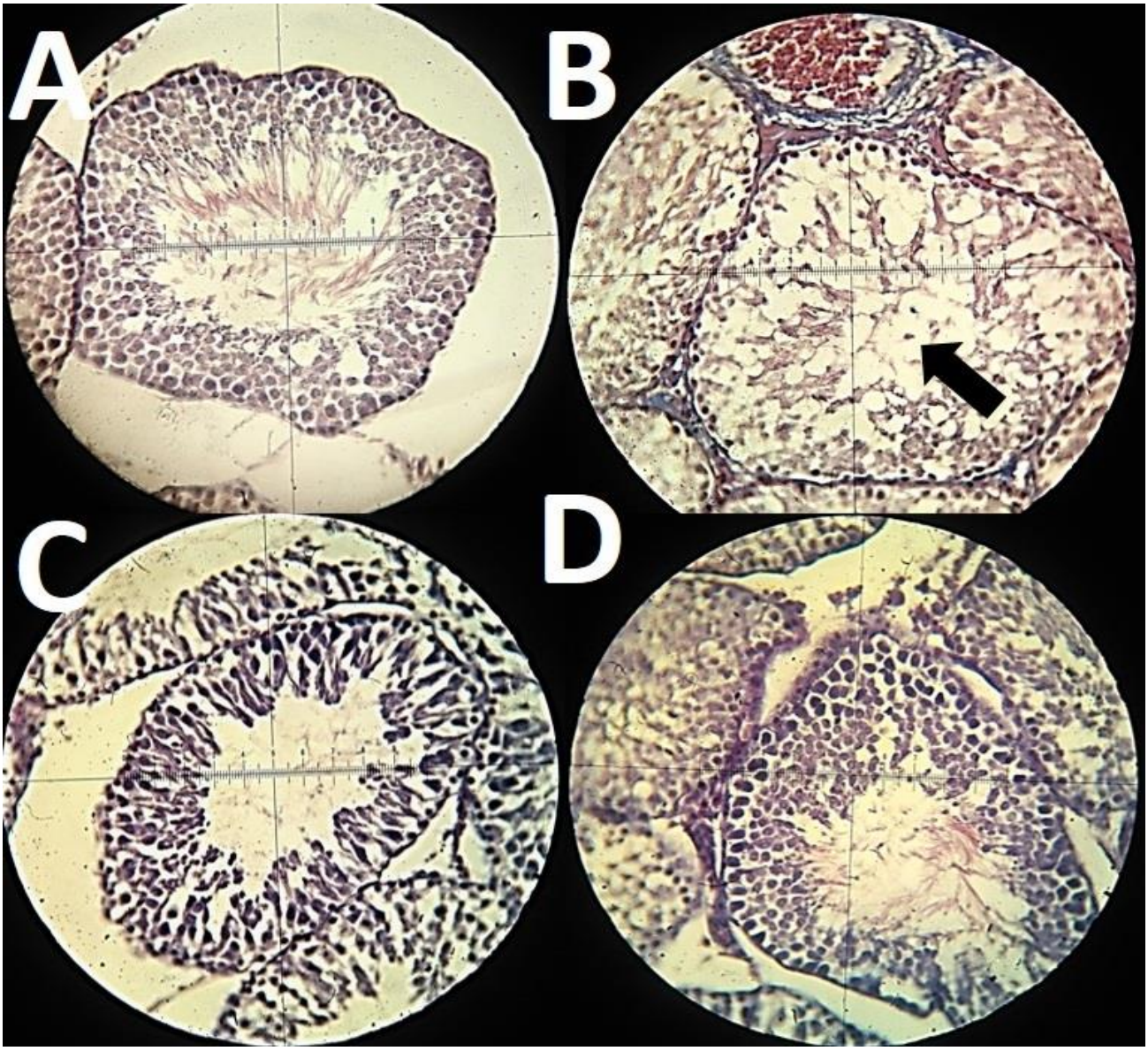

Figure 3: A: light micrograph of the testis of control rabbits with normal histology. B: testis of rabbits treated with Cadmium; arrow indicates testicular degeneration. Arrow point shows degeneration of seminiferous tubules. (PAS stain. Mic. Mag. × 40). C: Light micrograph of the testis of a selenium nanoparticles rabbits and a treated with Cadmium (D); arrow indicates congestion. (H&E staining. Mic. Mag. × 40).

Some studies have reported the anti-lipid peroxidation and antioxidant properties of Selenium nanoparticles, while other studies showed the pro-oxidant effects of Selenium nanoparticles and Cerium oxide nanoparticles. The present study showed the anti-fertility effects of Selenium nanoparticles, which was in contrast with those of other studies (34, 35).

5 Conclusion

The current study showed the potential therapeutic efficacy of selenium nanoparticles and cerium oxide nanoparticles against cadmium-induced renal, hepatic, and reproductive toxicity. Further studies are needed to understand the potential mechanisms of hepatoprotective activities of cerium oxide and selenium nanoparticles against heavy metal toxicity.

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Ethical issue

The experimental protocol of experiments was performed according to the ethical guidelines of Animal Ethics Committee of faculty of veterinary medicine university of Zabol, Zabol, Iran (Ethical code: ERC.UOZ.1399.001).

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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