Disease and Diagnosis

Dis Diagn. 2021; 10(2):75-81

10.34172/ddj.2021.15

Original Article

Comparing the Histopathological Effects of Selenium Nanoparticles and Selenium Nanocomposites in Rat Models

Mohammad Reza Hajinezhad[1*], Abbas Jamshidian[2], Motahareh Abdollahi[1], Alireza Samzadeh Kermani[3]

[1]Department of Basic Veterinary Science, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.
[2]Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.
[3]Department of Basic Science, Faculty of Science, University of Zabol, Zabol, Iran.

Abstract

Background: Selenium nanoparticles (Se NPs) and selenium nanocomposites (Se NCs) have different biological effects. The current study aimed to compare the effects of newly synthesized Se NPs and Se NCs on biochemical and histopathological parameters of rats. The synthesized Se NPs were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy dispersive X-ray analysis (EDAX), and scanning electron microscopy (SEM) techniques.

Materials and Methods: Adult male Wistar rats were divided into four equal groups to examine the biological effects of Se NPs. Control rats received saline intraperitoneally while experimental rats were received four-week intraperitoneal injections of Se powder, Se NPs, and Se NCs at the dose of (0.4 mg/kg). After four weeks, serum was obtained by the conventional methods, and then rats were sacrificed to separate liver, kidney, and testis tissues for histopathological examinations.

Results: The intraperitoneal injection of Se powder caused significant elevations in serum liver enzyme levels, serum blood urea nitrogen (BUN) lipid peroxidation, and serum creatinine levels ($P<0.05$). The histopathological investigations showed necrosis and fatty change in liver. Kidney sections showed cytoplasmic vacuolation and hyaline casts, and the testis sections showed degeneration of seminiferous tubules. Se NPs intraperitoneal injections at a dose of 0.4 mg/kg caused no significant effects on liver enzymes, malondialdehyde (MDA) content, and histopathological changes while significantly increased serum BUN and creatinine levels ($P<0.05$). The group treated with Se NCs showed normal biochemical and histopathological parameters ($P<0.05$).

Conclusion: The current study proved the toxicity of Se powder; however, nano-formulations of Se showed fewer side effects.

Keywords: Selenium, Nanoparticles, liver, kidney, Toxicity

***Correspondence to**
Mohammad Reza Hajinezhad, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.
Tel: +98-5424822250,
Email: hajinezhad@uoz.ac.ir

Received: February 10, 2021, Accepted: May 23, 2021, ePublished: June 28, 2021

Introduction

Selenium (Se) is an antioxidant element with an influential role in preventing free radical formation. Previous experiments have demonstrated that Se can reduce the risk of cardiovascular diseases and can prevent Alzheimer's disease (1). This essential trace mineral affects glucose metabolism, hormone secretion, and mental health. Also, Se could reduce the risks of prostate problems (2), cardiac infarction (3), mental problems (4), thyroid dysfunction, and immune imbalance (5). Based on the health guidelines, Se deficiency and Se overburden are associated with health problems (6). The daily dose of Se is between 30 micrograms per day (7). Nanoparticles (NPs) and nano-carriers are used for the controlled drug release and delivery. Se NPs, due to unique physiochemical effects, are valuable materials for drug delivery. Properties of nanomaterial are different from the bulk form of the materials (8). NPs are used in biomedicine, bioengineering, environmental monitoring (9), and cosmetic products (10). Other applications include surgical devices, minerals, wound-dressing, and diagnostics tools (11). Se NPs can reduce free radicals and prevent cellular damage. However, the safety margin of Se NPs could limit the biomedical applications. In recent studies, the high dose of Se NPs showed long-term toxicity (12). Previous studies on the bio-distribution of Se NPs showed that these NPs could enter the body via the gastrointestinal tract. Se NPs could distribute mainly from the skin and respiratory system, and subsequently diffuse into different parts of the body. However, they can be difficult to diffuse into the eye, thymus, and brain (13). The absorption of Se NPs is highly size-dependent (14).

Se NPs can be metabolized by liver microsomal enzymes and excreted along with biliary excretion (15). Currently, there are few studies regarding the reproductive effects of Se NCs. Furthermore, Se NPs and Se NCs have not been compared in rats. There is little data regarding the reproductive effects of Se NCs. Accordingly, the present study aimed to investigate potentials of a newly synthesized Se NP on rats. We also examined the effects of polyvinyl alcohol (PVA)-stabilized Se NPs following injection.

Material and Methods

Reagents

All materials were of laboratory grade chemicals. Se powder, sodium sulphite Na_2SO_3, and PVA were purchased from Merck Company, Germany.

Instruments

The synthesized Se NPs and Se/PVA NCs were analyzed by Fourier transform infrared (FTIR) spectrometer (Bruker Optics Ft Tensor 27, Germany) using KBr discs. The spectral range was from 400 to 4000 cm^{-1}. The X-ray diffraction (XRD) patterns of the NPs were recorded with a Phillips X-ray diffractometer, model PW 1710, using a Cu Kα source (λ=0.15406 nm). Scanning electron microscope (SEM) was applied to observe the surface morphology of Se NPs by using a Hitachi S4160 apparatus, and a uniform surface structure was observed when Se NPs were analyzed by SEM. EDAX elemental analysis map of Se NPs was recorded by FESEM MIRA II TESCAN (SAMX detector) Czech Republic instrument and the chemical composition of synthesized Se NPs was confirmed by EDAX map.

Synthesis of Se NPs

Synthesis of Se NPs by Chemical Method

To synthesize Se NPs, Se powder was added to the solution of sodium sulfate (0.50 M) in 100 mL of double-distilled water. The appearance of a pink color preliminary confirmed the formation of Se NPs. This color indicated the synthesis of Se NPs into the solution. After synthesizing Se NPs, the whole supernatant was collected and centrifuged at 11 500 rpm for 20 min at 4°C. The pellet was washed with distilled water thrice. This method is capable of producing spherical Se NPs of size 20 to 50 nm, under ambient conditions. To this purpose, Se NPs were mixed with PVA in water and stirred for 30 minutes. In this step, Se NPs were distributed in the polymer matrix and stabilized.

Animal Grouping

In this experimental study, 40 male rats 225 ± 12 g were used. Rats were obtained from the animal house of the Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran. After two weeks of acclimatization, animals were randomly divided into four equal groups (n=10 each). The first group (control) was treated with normal physiological saline for 28 days. The experimental groups were treated with intraperitoneal injections of Se NPs at 0.4 mg/kg Se.

Determination of Biochemical Parameters

The laboratory chemical kits for determination of creatinine (CREA), blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were purchased from Pars Azmoon diagnostic Inc. (Pars Azmoon, Tehran, Iran). Se powder and PVA were purchased from Merck Company Germany. Serum levels of ALT, AST, BUN, and CREA were measured enzymatically by the Selectra Pro M autoanalyzer (Vital Scientific, Span Neren, the Netherlands). Serum lipid peroxidation was determined according to the Okhawa method (15). For histopathological analysis, rats were euthanized. Liver, kidney, and testis specimens were sliced and preserved in formalin.

Statistical Analysis

The biochemical and histopathological results were analyzed using SPSS software (version 18.0). Multiple comparisons were performed by ANOVA followed by Tukey's post hoc test. The level of significance was set at a 5% level of confidence ($P<0.05$).

Results

The XRD pattern, FT-IR Spectrum, SEM image, and energy dispersive x-ray diffraction (EDS) analysis of Se NPs are depicted in Figures 1-4.

The XRD Pattern of Se NPs

The XRD pattern of Se NPs suggests the sample to be nano-crystalline (Figure 1). Also, the crystallite size, D, was calculated from the XRD data using Scherrer's formula. The average particle size of Se NPs was nearly 30 nm.

The FT-IR Spectrum of Se NPs

The spectral range was from 400 to 4000 cm−1. The results of FT-IR spectrum of Se NPs showed that there was no significant absorption in the spectrum of Se NPs, which confirms the synthesis of Se NPs (Figure 2).

The SEM Image of Se NPs

SEM was applied to observe the surface morphology of Se NPs by using a Hitachi S4160 apparatus. The uniform surface structure was observed when Se NPs were further analyzed by SEM. The topographic structure of Se NPs surface was found to be spherical agglomerated and uniform structure everywhere in Figure 3.

Energy Dispersive X-ray Spectrum of Se NPs

Chemical composition of synthesized Se NPs was confirmed by using elemental analysis EDS spectrum. As shown in Figure 4, the spectrum indicates that Se NPs were only synthesized. In this spectrum Se signal is observed.

In Vivo Experiments

Biochemical Results

The effects of 4-week intraperitoneal injection of Se NPs are presented in Table 1.

The intraperitoneal injection of Se powder at a dose of 0.4 mg/kg significantly increased serum malondialdehyde (MDA) levels ($P<0.01$), as shown in Table 1. The intraperitoneal injection of Se powder caused a significant increase in serum ALT, AST, BUN, and CREA levels. ($P<0.05$) (Table 1). Rats treated with the 0.4 mg/kg dose of Se NPs increased serum BUN and CREA ($P<0.05$). Four weeks of treatment with Se NCs did not induce any significant changes in serum liver enzymes, serum BUN, and serum CREA levels. The catalase activity and serum MDA levels also did not show significant alterations than those in the normal control rats.

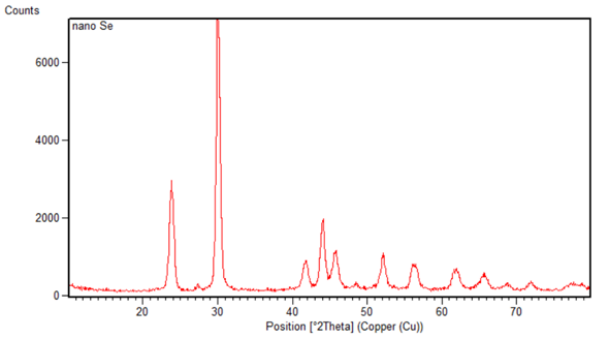

Figure 1. XRD Pattern of Se NPs.

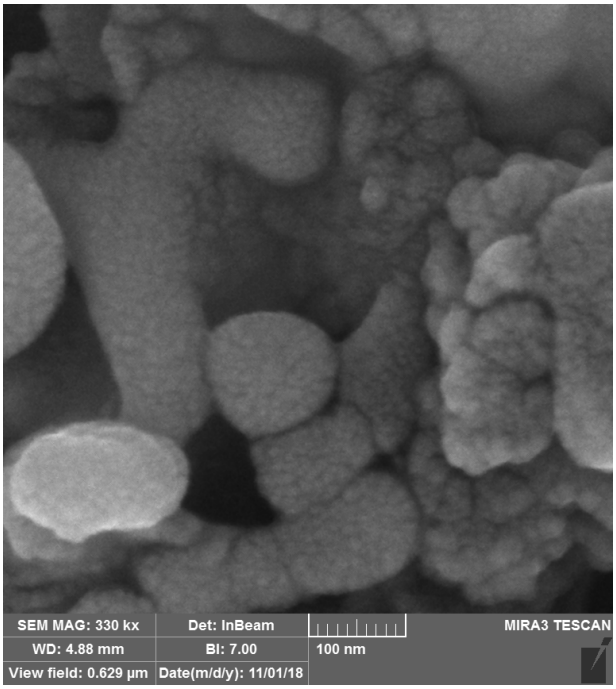

Figure 3. The SEM Image of Se NPs.

Histopathological Results

The histopathological changes of the liver in the control and experimental groups are shown in Figures 5-7. The liver of saline-treated rats (control) had normal cellular histology with normal hepatocytes and radial arrangement of hepatic cells around central veins. Also, the portal triad area and the central veins were normal (Figure 5A). A light micrograph of a liver section of rats treated with Se powder (0.4 mg/kg) showed hepatitis, cytoplasmic vacuolation, and congestion (Figure 5C). The liver

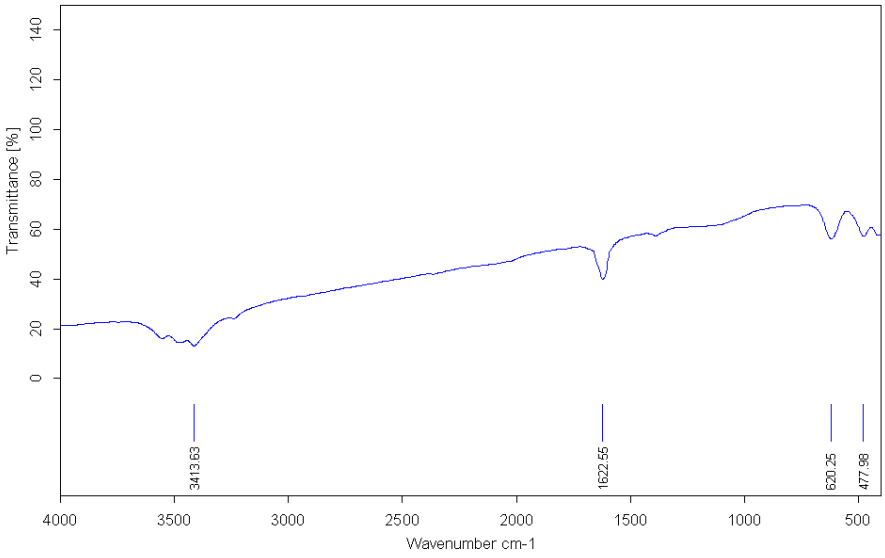

Figure 2. FT-IR Spectrum of Se NPs.

Table 1. Effects of Se NPs on Serum Biochemical Parameters in Rats

Item	Treatment			
	Control	Selenium Powder 0.4 mg/kg	Selenium NPs mg/kg 0.4 mg/kg	Selenium NCs 0.4 mg/kg
MDA (nmol/mL)	44.1 ± 7.1	58.1± 5.1**	42.9 ± 4.1	49.2± 4.8
CAT (U/L)	113.8 ± 10.8	97.2± 8.4*	103.3 ± 12.8	112.8 ± 8.2
AST (U/L)	61.3 ± 7.4	95.3 ± 11.2***	69.9 ± 8.5	64.9 ± 7.7
ALT (U/L)	35.7 ± 5.1	51.2 ± 6.9***	35.3 ± 9.6	37.4 ± 3.2
BUN (mg/dL)	14.2 ± 1.8	24.6± 2.9***	20.2 ± 4.6*	17.2 ± 2.6
Creatinine (mg/dL)	0.67 ± 0.19	1.1± 0.1***	0.87± 0.8*	0.79± 0.11

Data are presented as Mean ± SD.
* indicates a statistical significance compared to the control group ($P<0.05$). ** indicates a statistical significance compared to control group ($P<0.01$). *** indicates a statistical significance compared to control group ($P<0.001$).

section of rats treated with Se NCs (0.4 mg/kg) showed normal hepatocytes and central vein (Figure 5D). The rats receiving 0.4 mg/kg dose of Se NPs showed hyaline cast formation and cytoplasmic vacuolation (Figure 6B). Light micrograph of a liver section of a rat treated with Se NPs showed normal renal corpuscles with normal Bowman's space (Figures 6C and 6D). The histological analysis of testis showed that the control group had normal testicular histology. The groups treated with 0.4 mg/kg also had normal histological appearance (Figure 7A). Rats treated with Se powder showed necrosis of germinal epithelium in the seminiferous tubules (Figure 7B). The testis of control rats was healthy (Figure 7C). Testis of a rat treated with Se powder showed testicular degeneration of seminiferous tubules, congestion, and inflammation (Figure 7D).

Discussion

Recently, scientists have investigated the effects of Se NPs on biochemical parameters of different experimental animals. Se NPs have a wide range of biological effects that make them widely available in the medical and healthcare industries. Se NPs have unique biomedical applications due to the physical characterizations, biodegradability, and less toxicity (16). They have good bioavailability and could penetrate to liver, kidney, brain, and testis. Results of the present study showed that treatment with Se NPs, at nutritional levels, could not induce any toxic effects on serum liver enzymes, serum BUN, and serum CREA. The

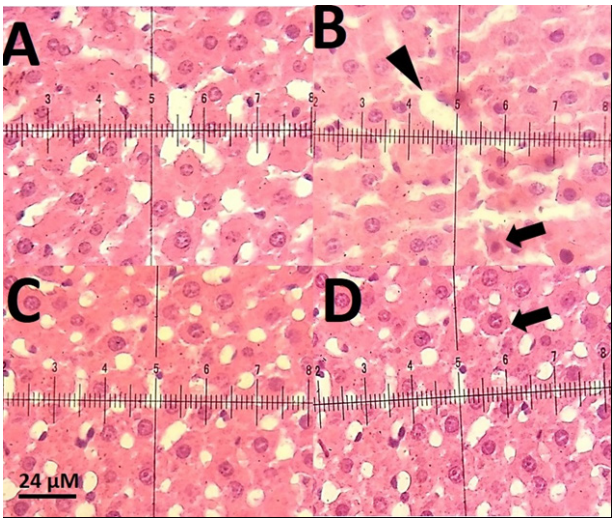

Figure 5. (A): Light micrograph of a liver section of a control rat; normal hepatocyte (arrow) and normal hepatic architecture. (B): Light micrograph of a liver section of rats treated with Se powder (0.4 mg/kg). Hepatitis (arrow point), cytoplasmic vacuolation (arrow), and disarrangement of hepatic cords. (C): Liver section of a rat treated with Se NCs (0.4 mg/kg). Normal hepatocytes (arrow). (D) Liver section of a rat treated with Se NPs (0.4 mg/kg). Normal hepatocyte (arrow) (PAS stain. Mic. Mag. × 40).

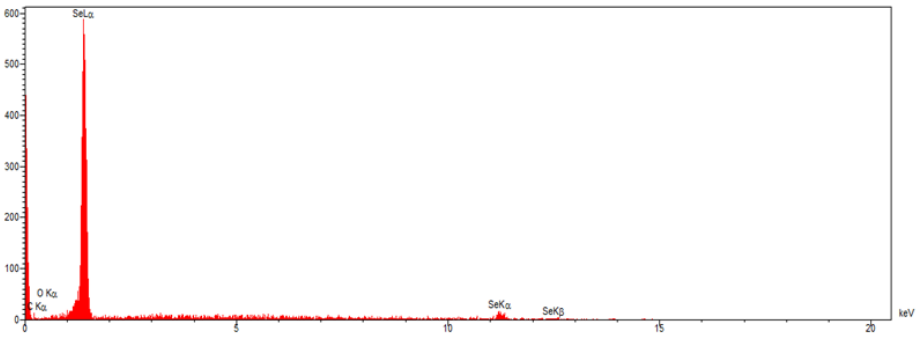

Figure 4. EDS Analysis of Se NPs.

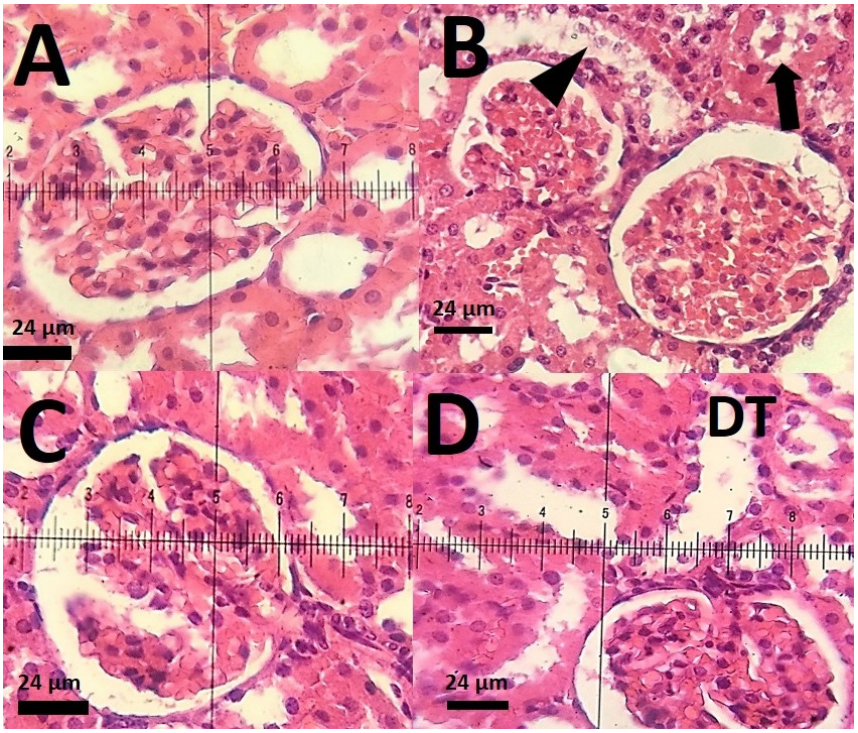

Figure 6. (A): Light micrograph of the kidney of a control rat; normal glomerulus (G), Bowman capsule (Bc), proximal tubules (PT), and normal distal tubules (DT). Light micrograph of a kidney section of a rat treated with Se powder (B): Cytoplasmic vacuolation (arrow point), and hyaline cast (arrow) (C): Light micrograph of a kidney section of a rat treated with Se NPs. D: Normal renal histopathology; Light micrograph of a kidney of a rat treated with Se NCs. Normal renal structure (arrow point) (H & E stain. Mic. Mag. × 40).

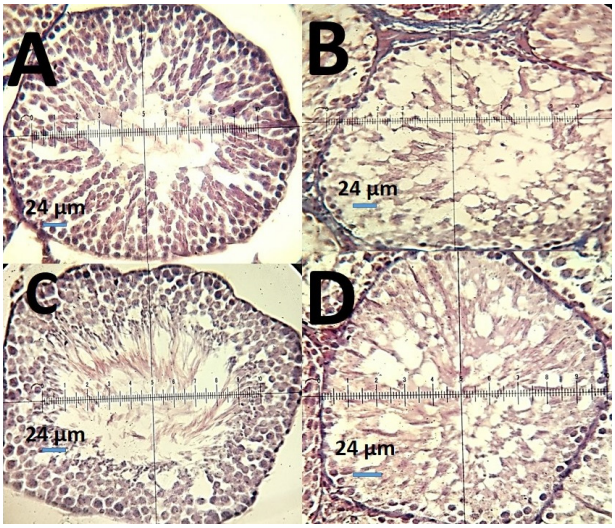

Figure 7. (A): Light micrograph of the testis of a control rat with normal histology. B: Testis of a rat treated with Se powder; arrow indicates testicular degeneration. Arrow point shows degeneration of seminiferous tubules (PAS stain. Mic. Mag. × 40). C: Light micrograph of the testis of a control rat and a treated with Se powder (D) (H&E staining. Mic. Mag. × 40).

histopathological investigations also showed the low dose of Se NPs did not caused any histopathological changes in liver and kidney tissues. The results of He et al showed that Se NPs, at super nutritional levels, had no obvious toxic effects in rats (17). Although Se potential as an antioxidant for the prevention of cardiovascular diseases is promising, additional long-term intervention trials are necessary. These results were in agreement with those of Hadrup et al (18) and inconsistent with the results of Shakibaie et al (19) and Chandramohan et al (20). Previous studies showed the antioxidant and anti-apoptotic effects of Se NPs in rats' thyroid (21). Se NPs are less toxic than inorganic and organic Se (22). In vitro and in vivo toxicity assessment of Se NPs showed low cytotoxicity and good bactericidal activity (23). Effects of the dose-dependent toxicity of Se NPs have been reported in previous studies (24). Our histopathological investigations revealed prominent histological changes in the liver, kidney, and testis of rats. Se NPs can induce histological changes in the liver and kidney of rats (25). Se powder induced severe necrosis in the seminiferous tubules. Previous studies have shown that Se NPs can cross the blood vessels of the testis (26). Our results showed that intraperitoneal injections of Se NPs powder could increase liver enzymes and renal markers. The increase in the lipid peroxidation levels indicates free-radical-increasing effects of Se powder (27). Se NPs could deteriorate histological changes in the liver of thioacetamide-intoxicated rats (28). Some studies indicated that Se could have oxidant effects and other studies have shown that Se powedr could have prooxidant effects (29, 30). Several studies have reported the anti-lipid peroxidation and antioxidant properties of Se NPs; and others showed the pro-oxidant

effects of Se. The present study showed the anti-fertility effects of Se. These results were in contrast with those of some other studies (31, 32). Despite the prominent role of Se in the reproductive system, this trace element has a narrow margin of safety. Earlier experiments showed that Se could have a protective effect on the male reproductive system based on histopathological and antioxidant status evaluations (33). Testis, like the brain and the pancreas, is highly dependent on normal Se levels for the proper function. The testis is vulnerable to free radicals due to the high polyunsaturated acid ratio. The antioxidant enzymes work together to reduce oxidative stress in the testis; therefore, Se deficiency could increase the testis susceptibility to oxidative damages. Further studies might investigate the effects of Se NPs and Se NCs on lipid peroxidation in liver, kidney, and testis. Investigating the effects of Se NPs and Se NCs on sperm motiloity and sperm count were the main limitaions of the current study.

Conclusion

The Se powder induced toxic effects on different tissues; however, Se NPs showed less toxicity. Further studies are required to determine the safe doses of Se NPs for biomedical applications.

Conflict of Interest Disclosures

There was no conflict of interests.

Acknowledgments

The authors would like to thank Dr mahmood Salehi Moghadam for his excellent animal handling and technical supports.

Ethical Statement

The experimental protocol of experiments was performed according to the ethical guidelines of the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran (Ethical code: ERC.UOZ.1399.001).

Authors' Contributions

All authors equally contributed to writing the manuscript.

Founding/Support

This study received a grant from the University of Zabol (grant number: 9618-15).

Informed Consent

Not applicable.

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