



## Histopathological and Toxicological Study of Selenium Nanoparticles in BALB/C Mice

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### ABSTRACT

This study was aimed to evaluate the toxicity of biogenic Selenium nanoparticles (SeNPs) on hematological and some serum biochemical parameters and also its histopathological effects in liver tissue in mice. Forty-eight male BALB/c mice were also used to determine the toxicity effects of SeNPs (2.5, 5, 10, 20, and 30 mg/kg) on some serum biochemical parameters and also its histopathological effects in liver tissue in mice. Microscopic examination of tissue sections of liver under light microscope from mice treated with the Se NPs 2.5, 5, 10 and 20 mg/kg doses showed there is no significant pathological change in comparison with those of the control groups. However, there was occasional focal mononuclear lymphocytic cellular infiltration around the portal and hepatocellular degeneration in 30 mg/kg doses group. The findings demonstrated that orally administration of Se NPs at the doses of 2.5, 5, 10, 20, and 30 mg/kg for 14 days, had no significant toxicity on the serum hematological and clinical chemistry parameters. The obtained results confirmed that SeNPs at the tested doses had no significant toxicity on the liver and kidney organs and also against hematological parameters in mice. However supplementary investigations are required to determine other toxicity aspects such as genotoxicity, chronic toxicity.

**Keywords:** Selenium, Toxicity, Mice, Nanoparticles

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### INTRODUCTION

Nanotechnology sciences provide the improvement of experimental practice for the preparation of the nanoscale constituents with exceptional possessions [1]. Today, production of nanoparticles (NPs) using biosynthetic techniques, has been considered as a valuable method with increasing attraction [2-4]. One of the existing techniques is, green synthesis of

NPs using the microorganisms such as bacteria; which in accompanied with the techniques can produce nanoparticles with low toxicity and higher efficacy properties [5-8]. Selenium as a micronutrient element has shown a wide range of biological activities such as antioxidant, immune modulation, anti-cancer, and antimicrobial ones [9-12]. It has been proven that toxicity of selenium can be attributed to chemical forms; so that, the dangerous toxicity was observed as selenious acid or selenium oxide. One the other hand, reviews have shown that synthetic elemental selenium shows lower

toxicity compared with other oxidative states [13]. This study was aimed to evaluate the toxicity of biogenic Se NPs on hematological and some serum biochemical parameters and also its histopathological effects in liver tissue in mice.

## MATERIALS AND METHODS

### Biosynthesis and characterization of the Se NPs

In this study, biosynthesis of Se NPs was performed according to the methods explained previously with the present authors [13].

### Animals

Male BALB/c mice (6–8 weeks old) weighing from 25 to 30 g were used in this study. Male BALB/c mice (6–8 weeks old) weighing from 25 to 30 g, were prepared from the Pasteur Institute of Iran (Tehran, Iran) and were housed in plastic cages (four to six each) in a colony room with controlled temperature ( $22 \pm 1^\circ\text{C}$ ) and humidity ( $50 \pm 10\%$ ) with a 12/12 h light/dark cycle. The mice had free access to tap water and food. Here, the experimental procedures performed in compliance with the guidelines of the Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals.

### Study design

Forty-eight mice were divided into 6 groups (8 mice per group). The control group (group 1) was administrated 0.9% saline orally (PO), and groups 2 – 6 were orally received Se NPs at the concentrations of 2.5, 5, 10, 20 and 30 mg/kg, respectively, for two weeks.

### Hematological parameters

After 14 days administration, mice were anaesthetized by Ketamine-Xylazine, and blood sampling was done from the heart. Hematological parameters such as total count of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), red cell distribution width (RDW), platelet count (PLT), total white blood cells count (WBC), and differential count of each of the leukocytes were measured by an automatic hematology analyzer. Moreover, red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and Lymphocyte count were analyzed with the automatic analyzer [14, 15].

### Clinical chemistry parameters

The assays of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, BUN, cholesterol, bilirubin (direct and total) and creatinine (Cr) were performed using commercial kits on serums collected from each mouse [16, 17].

### Histopathological evaluations

To do this, the liver tissue were rapidly separated and used for the pathological study. Collected tissue samples from liver were immersion-fixed distinctly in 10% neutral buffered formalin at  $25 \pm 1$ . Preparation of paraffin blocks from the collected samples was carried out according to the method described elsewhere. The paraffin blocks were then sectioned with a Leica Rotary Microtome at 5–6  $\mu$  thickness. Then, sections were stained properly with Harris' hematoxylin and mounted by means of glass cover slips. Finally, microscopic slides were tested under a light microscope for any indication of histopathological changes compared with the control group [18].

### Ethical statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kerman University of Medical Sciences (Permit Number: 94/679).

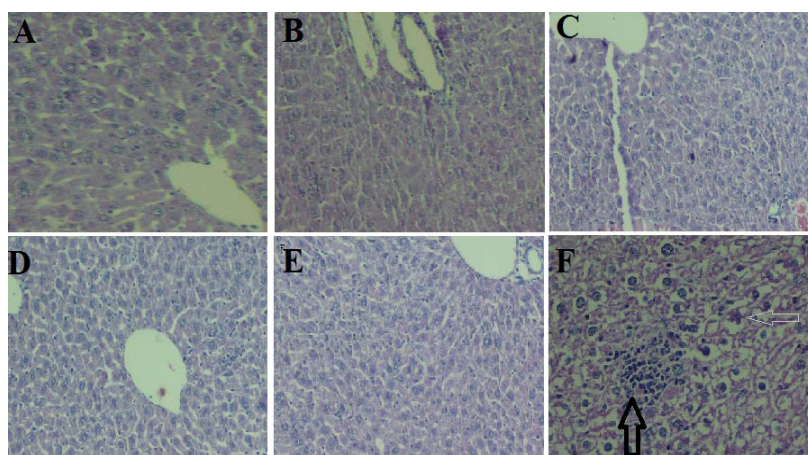
### Statistical analysis

The data in this study were indicated as the mean  $\pm$  SEM. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Turkey's post-hoc test was used to assess differences between experimental groups. In addition,  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

Microscopic examination of tissue sections of liver under light microscope from mice treated with the Se NPs 2.5, 5, 10 and 20 mg/kg doses showed there is no significant pathological change in comparison with those of the control groups. However, there was occasional focal mononuclear lymphocytic cellular infiltration around the portal and hepatocellular

degeneration in 30 mg/kg doses group as shown in Fig. 1.



**Fig.1.** Photomicrograph of Pathological liver section of mice after 14 days of treatment with the Se NPs 2.5, 5, 10, 20 and 30 mg/kg. Photomicrograph of liver sections of control mice (A), section of mice Liver after treatment with Se NPs 2.5 mg/kg (B), Se NPs 5 mg/kg (C), Se NPs 10 mg/kg (D), Se NPs 20 mg/kg (E) and Se NPs 30 mg/kg (f) The image F shows with a thick arrow shown the focal mononuclear lymphocytic cellular infiltration and thin arrow shown the hepatocellular degeneration.

As shown in Tables 1 and 2, the findings demonstrated that orally administration of Se NPs at the doses of 2.5, 5, 10, 20, and 30 mg/kg

for 14 days, had no significant toxicity on the serum hematological and clinical chemistry parameters.

**Table 1.** Comparison of hematological results among Se NPs treated groups, at doses of 2.5, 5, 10, 20 and 30 mg/kg body weight/day and control mice

Hematological parameter	Control (Normal saline)	Se NPs (2.5 mg/ kg)	Se NPs (5 mg/ kg)	Se NPs (10 mg/ kg)	Se NPs (20 mg/ kg)	Se NPs (30 mg/ kg)
RBC (M/UL)	5.78 ± 0.39	5.67 ± 0.83	5.52 ± 0.32	5.53 ± 0.51	5.21 ± 0.52	3.62 ± 0.21
WBC (K/UL)	6.79 ± 1.30	6.23 ± 2.12	6.51 ± 0.76	5.61 ± 0.95	4.77 ± 0.76	3.44 ± 0.36
Platelets (K/UL)	352.29±2.16	350.40±120.3	344.86±111.7	298.96±121.5	344.86±111.75	334.86±111.75
HGB (g/dl)	12.00 ± 0.71	13.33 ± 2.12	13.12 ± 2.32	13.43 ± 2.45	13.22 ± 2.43	10.24 ± 2.12
HCT (%)	40.05 ± 5.40	41.17 ± 3.69	39.64 ± 7.56	39.93 ± 6.66	38.77 ± 7.54	32.22 ± 5.36
MCV (fl)	85.12 ± 3.03	86.38 ± 3.55	83.06 ± 10.74	82.23 ± 12.65	82.33 ± 14.34	80.06 ± 13.34
MCH (pg)	28.33 ± 1.22	31.41 ± 4.12	29.86 ± 7.09	28.77 ± 5.09	29.75 ± 62.03	26.96 ± 6.19
MCHC (g/dL)	33.60 ± 2.04	34.12 ± 1.44	34.34 ± 1.65	34.44 ± 1.55	33.74 ± 1.85	33.64 ± 1.77
Lymphocyte (%)	46.26 ± 6.11	46.40 ± 14.77	49.23 ± 10.23	49.88 ± 12.44	49.77 ± 12.23	54.89 ± 14.13

**Table 2.** Clinical biochemistry parameters in serum of tested mice

Clinical biochemistry parameters	BUN (mg/dL)	Cr (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB
Control	31.4	0.24	130.6	38.7	133.3	0.15
Se NPs (2.5 mg/ kg)	34.2	0.27	132.4	38.9	135.8	0.15
Se NPs (5 mg/ kg)	33.5	0.27	141.8	41.2	137.2	0.16
Se NPs (10 mg/ kg)	34.1	0.29	144.3	42.7	139.1	0.18
Se NPs (20 mg/ kg)	34.8	0.32	144.6	43.5	139.8	0.18
Se NPs (30 mg/ kg)	36.2	0.37	151.4	49.6	144.7	0.28

BUN, Blood urea nitrogen; Cr, creatinine; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; TB, total bilirubin.

Reviews have reported a number of biological activities for SeNPs such as antimicrobial ones [19-21].

In recent decades, a very wide range of biosynthetic drugs and natural products have been used for medical purposes in all countries around the world. However, it seems obligatory to show the scientific validations for discovering their side effects in therapeutic goals [22, 23]. At the present time, one of the main unique experiments to determine the liver function and also inflammations and damage such as hepatitis and cirrhosis is measuring the serum liver enzymes. Measuring of serum BUN and creatinine is mostly used to evaluate the kidney function in a large number of conditions, to make possible detecting kidney diseases and to screen individuals with acute or chronic renal dysfunction or failure [24]. Regarding toxicity effects of SeNPs, Hey et al. [25] demonstrated that SeNPs had no noticeable toxic effects in rats, and might be applied as possible candidates for prevention of cancer chemoprevention, while doses higher than 2.0 mg Se/kg-bw induced chronic toxicity. In the other study conducted by Shakibaei et al. [13], it has been proven that the biogenic Se NPs has a much lower toxicity than synthetic Se NPs and is significantly (26-fold) less toxic than the SeO<sub>2</sub> [2]. The obtained findings demonstrated that although no significant pathological change was observed in liver of mice treated Se NPs 2.5, 5, 10 and 20 mg/kg doses; but, there was occasional focal mononuclear lymphocytic cellular infiltration around the portal and hepatocellular degeneration in 30 mg/kg doses group. Considering the histopathological effects SeNPs, Hey et al demonstrated that there are some lesions in the liver, kidneys, lungs, and thymus, and apoptotic liver cells in male rats after receiving SeNPs at the doses 4.0 and 8.0 mg/kg [25]. The difference between the previous investigation and present study might be due to some factors such as: animal type, method of preparation of SeNPs, used doses, etc.

### CONCLUSION

The obtained results confirmed that SeNPs at the tested doses had no significant toxicity on the liver and kidney organs and also against hematological parameters in mice. However

supplementary investigations are required to determine other toxicity aspects such as genotoxicity, and chronic toxicity.

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