

Histopathological assessment of protective effects of selenium nanoparticles on rat hepatocytes exposed to Gamma radiation

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Abstract

Gamma radiation are used in many medical and technical applications, however, it is one of the most dangerous kinds of radiation and can be harmful to the body. The present study was designed to clarify the protective effects of the selenium supplementation as selenium nanoparticle and selenite selenium in rat liver against Gamma irradiation with different intensities of 2.00 and 8.00 Gy. A total number of 45 healthy male Wistar rats were randomly divided into nine groups of five each. The radiation procedure was carried out in the Cobalt 60 equipment in Omid hospital, Urmia. The animals were simultaneously immobilized in a transparent acrylic plate and exposed to different intensities of 2.00 and 8.00 Gy radiations on day 7th and 14th of the experiment. After 72 hr after the last radiation, the animals were euthanized, and blood and liver tissue were collected. Histological analyses revealed the radiation-induced hepatic injury in rats, which included vacuolated cytoplasm, liver necrosis, fibrosis, and vascular lesions followed by a significant increase in alanine transaminase, alanine transaminase, alkaline phosphatase, and Gamma-glutamyl transferase. Selenium nanoparticles bear a more potent antioxidant effect in comparison with selenium selenite and can effectively protect the liver cell against Gamma radiation at a dose of 8.00 Gy.

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Introduction

Gamma radiation is used in cancer treatment and radiotherapy. Gamma radiation and X-rays are the most dangerous radiation.¹ Gamma radiation are ionizing rays to liberate electrons from atoms or molecules, thereby, generating free radicals known as reactive oxygen species (ROS) which can react with and damage complex cellular molecules such as fats, proteins, and especially DNA.² The extent of damage depends on the amount of radiation and the sensitivity of tissue. Body cells that divide more rapidly are more radiosensitive which include the ovary, testes, bone marrow, and the epithelial cells in the intestine.³ Because there are a growing concern and awareness regarding the fatal effects of radiation therapy on healthy cells of the human body, there is numerous research to find therapeutic agents to help protect human health. The substances that reduce the harmful effect of radiation are called radioprotective. These compounds include vitamins

A, C, and E.⁴ natural products, and herbs⁵ such as curcumin,^{6,7} melatonin^{8,9} and chemicals like amio-phystin.¹⁰ Although these substances believed to be safe, few have efficacy of treatments. Amifostine is the only Food and Drug Administration (FDA) approved radio-protector being used clinically. Due to its high cost and side effects such as cephalgia, nausea, vomiting, and hypotension this drug is not used widely.¹¹ Therefore, there is an urgent need for the acquisition of a safe, efficient, less dangerous, and more useful drug.¹² Nano-technology, a known field of research for the last several decades, has attracted attention in different branches of science from physics, chemistry, and natural science to practical sciences like electronics¹³ and removing environmental pollution.¹⁴ They are tiny materials having sizes ranges from 1 to 100 nm and can be classified into different classes based on their properties, shapes, or sizes. Swedish chemists Jons Jakob Berzelius discovered thorium in 1815, and working with Johan Gahn,

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discovered selenium in 1817.¹⁵ At that time little was known on its biological function, until its nutritional requirement was revealed about 140 years later by Schwarz and Foltz in 1960. They found out that low levels of selenium would protect the liver from vitamin E deficient mice against necrosis. In the late 1960s, the desire to identify the role of selenium in human health has increased dramatically and most researchers have focused on human diseases that respond to selenium treatment in animals. The interest to understand the real role of Selenium in human health has been increased and most of the researchers have focused on diseases that respond to selenium treatment.¹⁶

Research in recent years has led to increased knowledge on selenium functions so that the short and long-term effects of selenium on health have been somewhat challenged.¹⁷ Nanoparticles of selenium, as a strong antioxidant, have much less toxicity than selenium and have a higher power in scavenging free radicals.¹⁸ This nanoparticle detoxify hydroperoxidase and lipid peroxides that accumulate on cytoplasm and mitochondria.¹⁹ Therefore, because of the occupational exposure of the radiation workers and cancer patients who are at the risk of the harmful effects of radiation therapy, this study was designed to detect the protective role of Selenium nanoparticle and selenite selenium against the harmful effect of Gamma radiation with the severity of 2.00 and 8.00 Gy, in rat liver.

Materials and Methods

Animals: A total number of 45 healthy male Wistar rats were obtained from the Laboratory Animal Center (Faculty of Veterinary Medicine, Urmia University). The rats were near of the same age (12 weeks old) and weighing 220-240 g. The rats were maintained on standard laboratory rodent diet pellets and were housed in humidity and temperature-controlled ventilated cages on a 12 hr day/night cycle. The research and animal care procedures were approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University (AECVU-171-2018) and were performed by the National Research Council Guide for Care and Use of Laboratory animals.

Nanoparticle synthesis and characterization. Sodium selenite (99.99%), L-cysteine (99.99%), was used with patent citation of Gao and Sun.²⁰ Sodium selenite (99.99%), L-cysteine (99.99%), peptone from soybean (80.00%) were purchased from Merck Chemicals, Darmstadt, Germany, and stored in a dry box. Peptone from soybean (5.00 g) was added to 100 mL of 100 mM sodium selenite solution and ascorbic acid was added to reach a final concentration of 400 mM. The resulting mixture was stirred at 25.00 °C for 10 hr. Then sodium ions and oxidized ascorbate were removed by dialysis and

stayed overnight against ultrapure water in a dialysis bag (MWCO 2 kDa; Biodee Biotechnology Co., Beijing, China), and a solution consisted of amorphous selenium particles and peptone was obtained. Dried powders of the synthesized nanoparticle were sent to Sharif Central Lab for Services (Sharif University of Technology, Tehran, Iran) to further characterization. X-ray diffraction (XRD) patterns of Se-NPs were obtained by X'Pert PRO MPD PANalytical Company X-ray diffractometer using Cu K α (1.54059 Å) radiation with the X-ray generator operating at 45.00 kV and 40.00 mA. The XRD patterns of Se nanoparticles are shown in Figure 1. All definite peaks of Nano-Se are indexed as Nano-Se structure, which is matched and compared to the standard data. Moreover, the EM900 transmission electron microscope (Zeiss, Oberkochen, Germany) was used to assess the morphology and particle size of Se-NPs (Fig. 2). The SEM micrograph of the prepared Nano-Se is shown in Figure 3. The average grain size of selenium nanoparticles was calculated and found between 0.30 μ m. The grain size for nano compounds was calculated according to Scherrer's formula:²¹

$$\beta = 0.87\lambda / D \cos \theta$$

where, D is the crystalline grain size (nm), θ is the half of the diffraction angle in degree, λ is the wavelength of X-ray source (Cu-K α) in nm, and β is the degree of widening of diffraction peak which is equal to the difference of full width at half maximum (FWHM) of the peak at the same diffraction angle between the measured sample and standard one.

Radiation facility. Whole-body Gamma irradiation was performed using Gamma cell Co-60 unit installed at the Omid hospital, Regional Radioisotopes center for cancer therapy. Rats were exposed to fractionated Gamma-irradiation delivered as 2.00 Gy and 8.00 Gy once in a week.

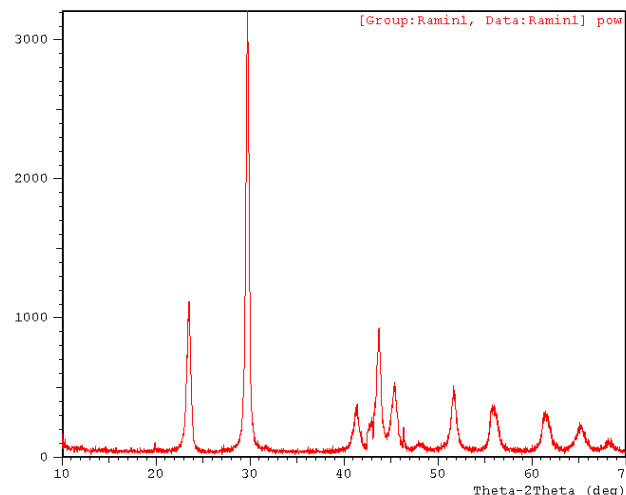


Fig. 1. XRD spectrum of the selenium nanoparticles synthesized by chemical method.

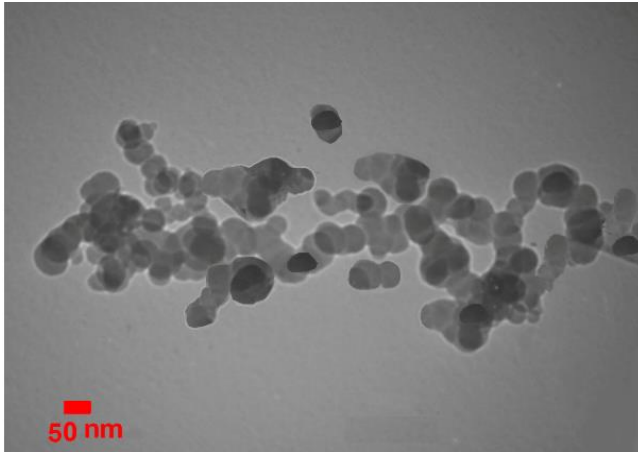


Fig. 2. Transmission electron microscopy (TEM) micrographs on the nano-selenium obtained from synthesis.

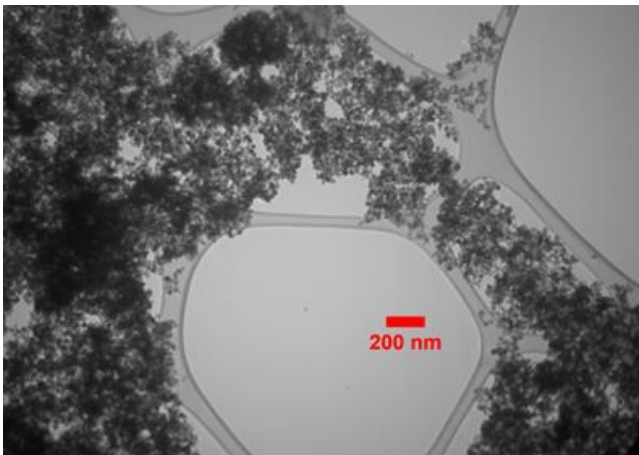


Fig. 3. Scanning electron microscope (SEM) micrographs on the nano-selenium obtained from synthesis.

Experimental groups. All rats used in this study were allowed to adapt to the new environment for one week before the commencement of the study. Animals were divided into nine groups. Group 1: Rats were neither treated with selenium compound nor irradiated as control. Group 2: Rats received daily intraperitoneally (IP) injection of 0.10 mg kg^{-1} body weight of selenium nanoparticle for two consecutive weeks and exposed to Gamma irradiation 2.00 Gy . Group 3: Rats received daily IP injection of 0.10 mg kg^{-1} body weight of selenium nanoparticle for two consecutive weeks and exposed to Gamma irradiation of 8.00 Gy . Group 4: Rats received daily IP injection of 0.10 mg kg^{-1} body weight of selenite selenium for two consecutive weeks and exposed to Gamma irradiation 2.00 Gy . Group 5: Rats received a daily intraperitoneal injection of 0.10 mg kg^{-1} body weight of selenite selenium for two consecutive weeks and exposed to Gamma irradiation 8.00 Gy . Group 6: Rats did not receive any drug but were exposed to 2.00 Gy radiation. Group 7: Rats did not receive any drug but were exposed to 8.00 Gy radiation. Group 8: Rats received only selenite

selenium without radiation, and Group 9: Rats were treated with selenite selenium without Gamma radiation. At the end of the experimental periods (72 hr after the last radiation), rats were fasted overnight and were anesthetized by intraperitoneal ketamine (60.00 mg kg^{-1} ; Alfasan, Woerden, Netherlands) and xylazine (10.00 mg kg^{-1} Alfasan), and blood samples were collected from the heart in a clean centrifuge tube and left to incubate at $37.00 \text{ }^\circ\text{C}$ and centrifuged at $3,000 \text{ g}$ for 10 min. The clear nonhemolyzed supernatant sera were quickly removed and immediately stored at $80.00 \text{ }^\circ\text{C}$ till been used for the biochemical analysis. The animals were euthanized.

The liver tissue sample was fixed in 10.00% formalin then dehydrated, through an ascending series of ethyl alcohol. Dehydration was then followed by clearing the samples in two changes of xylene, embedded in paraffin, and cut into $5.00 \text{ }\mu\text{m}$ and stained with Hematoxylin and Eosin (H & E) and Masson's trichrome for identifying collagen fibers by blue color and scoring fibrosis.²³ Stained sections of control and treated rats were examined for alterations in the architecture based on scores 0 to 4.²⁴

Special Flogenes staining to determine the damage of DNA nuclei was performed.²⁵ Glycogen granules in liver sections were detected using Periodic acid Schiff reagent (PAS). Cryosections of the samples were stained with Oil red O or Sudan black B to demonstrate different kinds of lipids. The extent and intensity of staining was evaluated semi-quantitatively.

Biochemical analysis. The activities of the alanine transaminase (ALT), alanine transaminase (AST), alkaline phosphatase (ALP), and Gamma-glutamyl transferase (GGT) enzymes were performed using the Pars Azmon test kit (Karaj, Iran) according to the manufacturer's protocol.

Determination of liver tissue selenium content. Selenium concentrations in liver tissue samples were measured using atomic absorption spectrophotometer (AA-6800; Shimadzu, Kyoto, Japan). Graphite-furnace atomic absorption spectrophotometry method described by Jacobson and Lockitch (1988) used for determination of selenium.²²

Statistical analysis. The computer software, SPSS (version 22.0; SPSS Inc., Chicago, USA) was used for analysis. The histopathological data were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Kruskal-Wallis. The data generated on enzyme activities were subjected to statistical analysis for reporting group means and standard error with significance between the controls and the treated groups. All the parameters characterized by continuous data were subjected to Bartlett's test to meet the homogeneity of variance before analyzing variance (ANOVA) and Dunnett's *t*-test. Mann-Whitney U- test was performed to calculate the significance. The differences were considered significant at $p < 0.05$.

Results

Histopathological results. Light microscopic examination of control group showed, the normal structure of the liver, each lobule had a central vein and hepatic cords in a radiating shape. The administration of Gamma radiation with an intensity of 2.00 Gy without medication showed obvious signs of hepatic alterations. These included congested blood vessels that some were extravasated. Rimac columns were disarrayed with mono-nuclear inflammatory cell infiltration. Hepatocyte vacuolar degeneration with liver necrosis and apoptosis were extensive. In the control group exposed to 8.00 Gy, the changes were more severe. The liver architecture was disturbed with loss of radial arrangement of hepatic cords and fibrinoid necrosis in portal vessels. Hepatocytes were extensively apoptotic.

Infiltrating lymphocytes at the portal space with evidence of increased collagen fibers were noticed. Hepatocytes were large and appeared empty, with the loss of cytoplasmic contents. There was a significant difference in the severity of lesions with other groups. In the group of selenium selenite at a dose of 2.00 Gy, congestion with a dilated sinusoid with increased vascular permeability and edema were observed. The apoptosis and necrosis continued to be present in selenite selenium and 8.00 Gy radiation. In the group receiving selenium nanoparticles with the radiation dose of 2.00 Gy, the damaging effects of radiation were reduced sharply. The cellular structure of hepatocytes was preserved and degeneration of fat and cell apoptosis was significantly decreased. In the group receiving selenium nanoparticles with 8.00 Gy radiation dose, the architectural distortion was not changed significantly and overall apoptotic and necrotic cell numbers were appeared to decrease. The special staining with Oil red O and Sudan black showed very little fatty vacuoles and no collagen was present in liver parenchyma (Fig. 4).

Biochemical results. The results obtained from the evaluation of the enzyme activities are shown in Figure 5. The present study showed that exposure to Gamma radiation at the dose of 2.00 and 8.00 Gy displayed a significant ($p < 0.05$) increase in serum ALP activity compared to healthy control rats in a dose-dependent manner. However, the supplementation of selenium as sodium selenite and selenium nanoparticles significantly decreased the activity of ALT, AST in the serum of irradiated rats. Treatment of irradiated rats with selenium nanoparticles was more effective than selenite selenium in the reduction of the activity of ALT and AST ($p < 0.05$). The ALP activity changes were exactly similar to ALT and AST. Treatment with selenium nanoparticles significantly induced ALP activity depression in the serum of irradiated rats. A

significant increase in serum GGT was observed in the irradiated rat. Administration of selenium compound effectively reduced GGT in irradiated rats and no significant difference between them was found.

Selenium content in liver tissue. The results of this study revealed that selenium administration as nanoparticles and salt form restored liver selenium concentration significantly, however, an amount close to normal range and Gamma radiation decreased liver tissue selenium content dose-dependently (Table 1). Administration of Se-NPs to the animals under radiation (2.00 and 8.00 Gy exposure) led to higher amounts of selenium in liver tissue, significantly exceeding the control group.

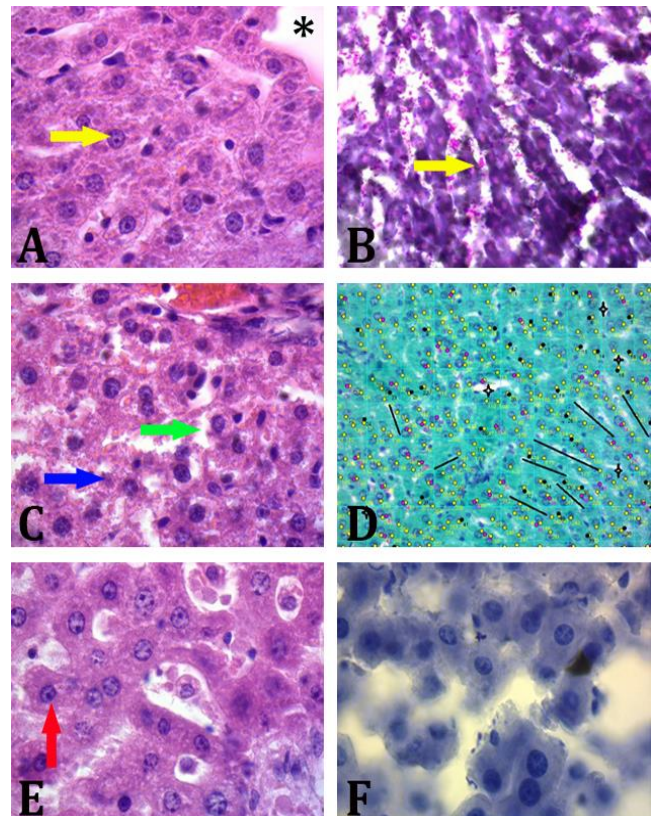


Fig. 4. **A)** Photomicrograph of a section in the liver of control rats showing radially arranged polyhedral hepatocytes, nuclei are distinctly round, with one or two prominent nucleoli (arrow) and central vein (star), (H & E, 400×); **B)** Oil red O staining of lipid droplets in hepatocytes (arrow) and sinusoidal space; **C)** Photomicrographs of the sections of rat liver of experimental groups treated with Selnite selenium and exposed to 8.00 Gy radiation, which shows hepatocytes with vacuolated cytoplasm (green arrow) and some karyolytic nuclei (blue arrow), (H & E, 400×); **D)** Feulgen staining, from 264 counted cells, 37.00% were apoptotic which are marked with dark and violate dots (100×); **E)** Photomicrographs of the sections of rat liver of experimental groups treated with nano selenium. Hepatocytes with round nuclei and distinct nucleoli (arrow), (H & E, 400×) and **F)** Sudan black staining did not show cytoplasmic inclusion of lipid in nano selenium treated group.

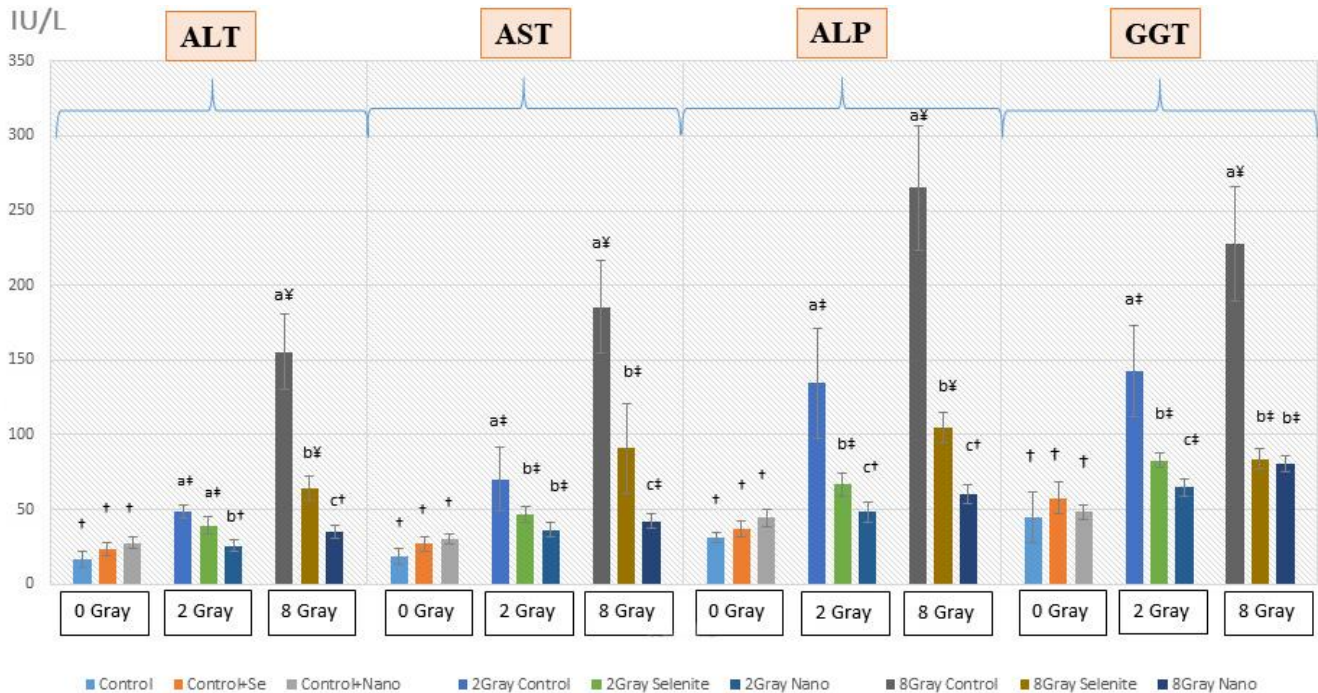


Fig 5. Bar graph shows the effect of different doses of Gamma radiation on hepatocyte serum enzyme in control and groups treated with selenium nanoparticles.

abc in each radiated therapy group and symbols of †, ‡, ¥ indicate significant differences among the groups of rats in different groups.

Table 1. Liver selenium concentration ($\mu\text{g g}^{-1}$) in various animal groups. Data are presented as mean \pm SD.

| Groups | Radiation (Gy) | | | p-value |
|------------------------|--------------------------------|-------------------------------|-------------------------------|---------|
| | 0.00 | 2.00 | 8.00 | |
| Control | 3.70 \pm 0.46 ^{†a} | 1.39 \pm 0.22 ^{†b} | 0.93 \pm 0.18 ^{†b} | 0.000 |
| Selenium | 10.46 \pm 0.84 ^{‡a} | 4.83 \pm 1.02 ^{‡b} | 2.61 \pm 0.54 ^{‡c} | 0.000 |
| Selenium nanoparticles | 9.19 \pm 1.20 ^{‡a} | 6.74 \pm 1.12 ^{†b} | 5.66 \pm 0.86 ^{†b} | 0.001 |
| p value | 0.000 | 0.000 | 0.000 | - |

^{abc} Means within a row with different superscript letters denote significant differences compared to 0.00 Gy irradiation values ($p < 0.05$).

^{†‡‡} Means within a column with different superscript characters denote significant differences compared to the control values ($p < 0.05$).

Discussion

Radiation therapy has long been established as a kind of cancer treatment that causes the ionized cancer cells to shrink and the tumor cells die. However, the radiation reacts with the healthy living cells and causes severe biochemical reactions and death of normal tissue. The intensity of tissue damage depends on parameters including the sensitivity of the tissue to radiation, duration of exposure, radiation source, radiation dosage, length of exposure, and, importantly, the genetic and epigenetic makeup of the exposed individual.²⁶ Thus, this study was designed to evaluate the damaging effects of Gamma rays in the liver and the protective role of selenium nanoparticles in comparison with selenium selenite. The results of H & E staining showed that while Gamma radiation in dose with 2.00 Gy, caused severe pathological lesions in hepatocytes, there was a more devastating liver injury in the group that received 8.00 Gy radiation.

The results showed that both materials selenium nanoparticles and selenium selenite at a dose of 2.00

Gy decreased liver damage, however, the destructive effects of Gamma-ray were effectively reduced more efficiently by selenium nanoparticles ($p < 0.05$) that represents more powerful antioxidant properties of nano selenium particles. Furthermore, the evaluation of hepatocytes of the two treatment study groups with Feulgen stain showed that the hepatocytes from selenite selenium had a significantly higher level of nuclear anomalies, which were resulted from apoptosis. Moreover, selenium nanoparticles protective effect to reduce the amount of 8.00 Gy induced apoptosis and DNA damage was evident. The extracellular and intracellular structure of the liver appeared to be preserved. The lobulation and the large vessels and ductular structures were normal. Clinical signs of disease, particularly gastrointestinal syndrome (intestinal bleeding) was not observed. The protective role of nano selenium has been well proven in cancer treatment,²⁷ and has shown to be less toxic than inorganic selenium and certain organoselenium compounds.^{28,29}

These rays generate ROS by the action of radiation on water, which comprises 80.00% of human tissue. The free radicals react rapidly either directly or by secondary reactions with the cellular component that produce biochemical lesions that initiate a series of physiological symptoms that ultimately will lead to cell death.³⁰ This study has created hope that the use of nano selenium as a strong antioxidant can prevent the oxidative stress generated by radiation.

Increased serum transaminases are related to the extensive breakdown of liver parenchyma.³¹ Transaminases are involved in protein and amino acid metabolism. They are present in most of the cells of the tissues in the body, however, ALT and AST levels are the most specific markers for hepatic cell destruction and loss of hepatocyte integrity. Since they are present in cytoplasm, they are released into the circulation following diseases or injuries.³²

A higher concentrations of these enzymes were found in the serum of rats exposed to 2.00 and 8.00 Gy radiation in this study. Makhlof and Makhlof, showed that levels of liver enzymes (ALT and AST), and creatinine and urea were increased in serum of rats after Gamma-irradiation.³³ Kafafy reported similar results in rats exposed to radiation.³⁴ Some investigators reported different findings.³⁵ Both hypotheses are plausible. The increase or decrease in the activity of liver enzymes may be related to the time that antioxidant treatment was started.³⁶

Our study revealed that biochemical parameters were in consistent pathological findings and the liver enzymes were increased significantly ($p < 0.05$) in untreated groups. The present findings are consistent with those found previously³⁷ who showed a significant increase in enzyme levels in rats exposed to 8.00 Gy Gamma radiation.³⁸

The present data showed that treatment of irradiated rats with nano selenium appears capable of reducing the response recorded in enzyme activity of AST, ALT, ALP, and GGT and destruction in liver tissue of untreated group. The chemoprotective efficiency role of the nano selenium could be attributed to its action in scavenging free radicals produced after radiation exposure.³⁹

The results of this study showed that treatment with selenium nanoparticles at a dose of 10.00 mg kg⁻¹ effectively reduced the damaging effects of Gamma radiation with a dose of 8.00 Gy. Nano selenium particles bear a more powerful antioxidant effect in comparison with selenite selenium and can effectively protect the liver cell against Gamma radiation at a dose of 8.00 Gy. Furthermore, selenite selenium was too close to the control groups and no significant difference was observed between them.

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Conflicts of interest

The authors declare no conflict of interest.

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