

Antioxidant and hepatoprotective role of selenium against silver nanoparticles

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Abstract: Silver nanoparticles (AgNPs) have attracted the most interest in terms of their potential biomedical and industrial applications. However, these nanoparticles have shown their toxic behavior toward environment, living tissues, and organisms. Selenium (Se), an essential trace element, is necessary for various metabolic processes, including protection against oxidative stress and immune function. The present study was undertaken to evaluate the effect of Se against AgNP-induced hepatic oxidative stress. AgNPs were synthesized and then prepared nanoparticles were characterized using various analytical techniques such as UV-visible spectroscopy, X-ray diffraction, and transmission electron microscopy. Rats were administered AgNPs intraperitoneally (5 mg/kg/day) and Se (0.2 mg/kg) was given by gavage. AgNP administration induced hepatic damage as indicated by the serum marker enzymes with reduction in levels of glutathione, and decrease in activities of SOD, CAT, and GSH-peroxidase ($P < 0.05$). Decrease in levels of total antioxidant capacity (TAC) and increase in level of C-reactive protein (CRP) was also observed in AgNP-treated group compared to control group. However, Se markedly attenuated AgNP-induced biochemical alterations, levels of TAC, CRP, and serum transaminases (AST, ALT) ($P < 0.05$). Taken together, these findings suggest that administration of AgNPs produces hepatotoxicity in rats, whereas Se supplementation attenuates these effects.

Keywords: silver nanoparticles, selenium, antioxidant enzymes, hepatotoxicity, oxidative stress

Introduction

Silver nanoparticles (AgNPs) undergo several changes when released into the environment. Other than aggregation and agglomeration, there is formation of silver chloride and silver sulfide. The chemical species which are actually present determine the bioavailability and toxicity of silver in the environment. Bioavailability of AgNPs in soil depends on both particle and soil properties. The release of ionic silver has been found to be the main cause of toxicity in humans, in the environment and in other applications.

AgNPs are known for their antibacterial effect and used in various biomedical applications.¹ However, there is increasing concern related to the biological impact of the use of AgNPs on a large scale, and the possible toxicity to the environment and health. AgNPs' toxicity can accumulate in several organs, thereby causing toxicity, in part via oxidative stress including pulmonary and germ cell toxicity, embryo toxicity, and cell death.²⁻⁹ Nanoparticle-induced oxidative stress is hypothesized to be the main mechanism regulating the biological effects of these nanoparticles.^{10,11} Toxicity of AgNPs could also be due to release of Ag ions in the system or extensive systemic distribution of Ag in tissues causing oxidative stress, protein or DNA damage, and apoptotic cell death.¹²⁻²¹

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AgNPs' toxicity is based on surface parameters like shape, size, surface charge, purity, and bioavailability.^{22,23} Moreover, nanoparticles like silver are known to induce ROS in various cell types.^{24,25} In addition, several investigations have also revealed that metal nanoparticles show toxicity and cause a wide range of abnormal manifestations in animals and plants.^{26–28} With increased probability of exposure to AgNP, understanding the toxicological mechanisms is very relevant in this technology-driven society. Under normal circumstances, the body is endowed with antioxidant systems which help in combating oxidative stress and toxicity. However, under extreme oxidative challenge, like exposure to toxins or nanoparticles, the antioxidant machineries may lose their specificity and become overwhelmed. Nanoparticle-induced oxidative stress is hypothesized to be the main mechanism regulating the biological effects of AgNPs.¹¹

Selenium (Se) is an essential micronutrient, which is well-known for its detoxifying effects in heavy metal exposure.²⁹ Se deficiency is associated with many diseases including chronic degenerative diseases,³⁰ impaired cognitive function, and neurological disorders.³¹ Se supplementation also protects from various diseases, such as viral infections,³² immune system dysfunction,³³ and neurodegenerative disease.³⁴ Se can also restore the activity of important antioxidant enzymes such as glutathione peroxidase (GSH-Px) and Trx reductase.^{35–38}

Thus, the protective properties of Se against AgNP-induced hepatotoxicity were investigated in this study, in the hope of shedding light on potential biochemical alterations, the biological responses induced by acute dosing, and to assess cellular responses. To our knowledge this is the first study to show that Se may be an effective agent for protecting cells from AgNP-induced hepatotoxicity.

Materials and method

Materials

Silver nitrate (AgNO₃), sodium selenite, chitosan, succinaldehyde, polyvinyl alcohol (molecular weight range: 85,000–124,000), and trisodium citrate (NaBH₄) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other chemicals were of laboratory grade and were used as received. All other necessary reagents were of analytical grade and were purchased from Sigma-Aldrich Co.

Preparation of AgNPs

Chitosan (2.5 g) and polyvinyl alcohol were dissolved in 30 mL of 1% CH₃COOH aqueous solution with continuous

sonication until a homogeneous mixture was obtained, and then 1 mL of succinaldehyde was added dropwise with continuous stirring to ensure homogeneous cross-linking by the polymerization process. Then, 10 mL of 1 mM AgNO₃ was added. The hydrogel containing AgNO₃ was transferred into glass tubes and deoxygenated with nitrogen gas for 30 min at 5 mL/min, and then 3 mL of 1 mM NaBH₄ was added. The tubes were irradiated in a microwave (Discover® SP System, CEM Corporation, Matthews, NC, USA) under ambient conditions. The AgNPs prepared in the polymer mixture were separated by centrifugation in aqueous solution and dried at room temperature.

Characterization

AgNPs exhibit unique and tunable optical properties due to their surface plasmon resonance which is dependent on shape, size, and size distribution of the nanoparticles. UV-visible absorption spectrum of AgNPs was recorded on UV-visible spectrophotometer (Shimadzu 1650; Shimadzu Corporation, Kyoto, Japan) using distilled water as a blank solution. The X-ray diffraction (XRD) pattern of the AgNPs was recorded using an X'pert Pro X-ray diffractometer (PANalytical, Almelo, the Netherlands) with Cu-Kα radiation. Surface morphology of prepared AgNPs was examined on a field emission scanning electron microscope at 200 kV (JSM-7600F; JEOL, Tokyo, Japan). Transmission electron microscopy (TEM) studies were carried out with a field emission-transmission electron microscope (JEM-2100F; JEOL). The elements present in the samples were determined through energy dispersive spectrometry (EDS) analysis.

Animals

Male Wistar rats (weighing approximately 150–200 g) were acclimatized for a month prior to experiments. All the animals used in this study were kept in cages in an air conditioned room (72°F–74°F) with a 12 h light/dark cycle. The rats were fed commercial pellet feed and given water ad libitum. The study was approved by the Ethical Committee of King Saud University and was carried out in compliance with the declarations of National Research Council, USA for animal care guidelines. The rats were divided into four groups, each group consisting of six animals as follows: control group (distilled water), Se-treated group (0.2 mg/kg/day), AgNP-treated group (5 mg/kg/day), and AgNPs-Se-treated group (5 mg/kg/day for AgNPs and 0.2 mg/kg/day for Se). AgNPs in 0.5 mL of distilled water were injected intraperitoneally and sodium selenite, as a source of Se, was administered orally once daily for 7 days before AgNPs injection.

These doses were selected on the basis of an acute toxicity study for AgNPs and sodium selenite (data not shown), well below the LD50 values. Blood samples were collected for biochemical analysis and serum was centrifuged for 10 min at 3,000 rpm.

Livers were carefully removed for biochemical studies, washed in ice-cold 50 mM Tris-HCl, pH 7.4 and homogenized. The homogenates were centrifuged and the supernatants were used for further experiments. The total protein content in the homogenized tissue was determined by using previously reported method of Lowry et al.³⁹

Biochemical assays

The liver homogenates were used to determine glutathione (GSH) level,⁴⁰ SOD,⁴¹ CAT activity,⁴² and GSH-Px activity.⁴³ The levels of total antioxidant capacity (TAC) were quantified using an ELISA kit (CYT 294; EMD Millipore, Billerica, MA, USA), C-reactive protein (CRP) was quantified by ELISA kit (MBS 733414, MyBioSource, San Diego, CA, USA) and serum transaminases (ALT, AST) were determined using Sigma-Aldrich Co. kits (MAC052, MAK055).

Histopathological analysis

Samples of fragments of fresh livers were fixed in buffered 10% formalin solution. Thereafter the tissues were dehydrated and cleared using a graded series of ethanol and xylene respectively and embedded in paraffin (58.6°C). Sections of paraffin blocks were cut by a rotary microtome (5 µm) and the sections were stained with hematoxylin and eosin, and were examined and photographed using a photomicroscope (Nikon Corporation, Tokyo, Japan).

Statistical analysis

Results were analyzed using SPSS software and expressed as the mean ± SEM. One-way ANOVA was applied to test for the significance of biochemical data of the different groups followed by parametric paired *t*-test and Duncan's multiple range test wherever appropriate. Results were considered significant when $P \leq 0.05$.

Results

Characterization of the AgNPs

A UV-visible spectrum was used to determine the formation and stability of AgNPs in the polymer matrix as shown in Figure 1A. The result revealed that the maximum absorbance of yellow AgNPs was at 412 nm due to the surface plasmon absorption of small-sized AgNPs.⁴⁴ The nanoparticles were stable in size and shape, supported by UV-visible spectra which was repeated weekly for 2 weeks.

An XRD method was used to characterize the synthesized AgNPs and the result is shown in Figure 1B. The XRD AgNPs exhibit characteristic peaks at scattering angles (2θ) 39.86°, 44.23°, 64.47°, and 77.33° corresponding to (1,1,1), (2,0,0), (2,2,0), and (3,1,1) planes, respectively and support the face-centered cubic (fcc) structure of AgNPs. The crystalline fcc structure of AgNPs can be assigned to standard JCPDS Card 89-3722. The lattice plane reflection at 39.86° was used to calculate the average size of AgNPs according to the Debye–Scherrer equation and the crystallite size of AgNPs was estimated to be approximately 18 nm, which is also further supported by TEM results.

The TEM and high-resolution transmission electron microscopy (HRTEM) images of AgNPs are shown in Figure 2.

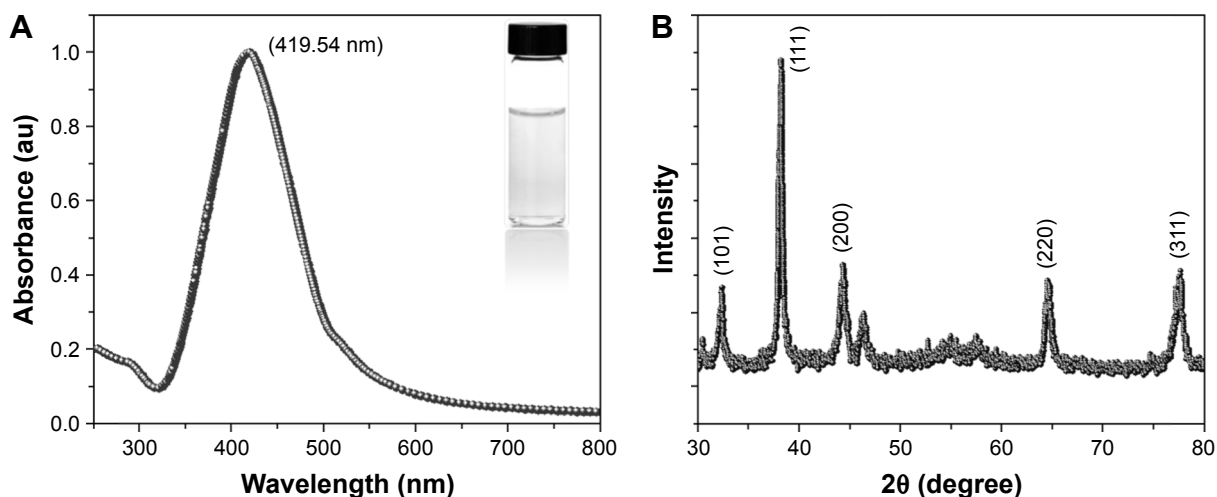


Figure 1 (A) UV-visible spectra of AgNPs, (B) XRD pattern of AgNPs.

Abbreviations: AgNPs, silver nanoparticles; UV, ultraviolet; XRD, X-ray diffraction.

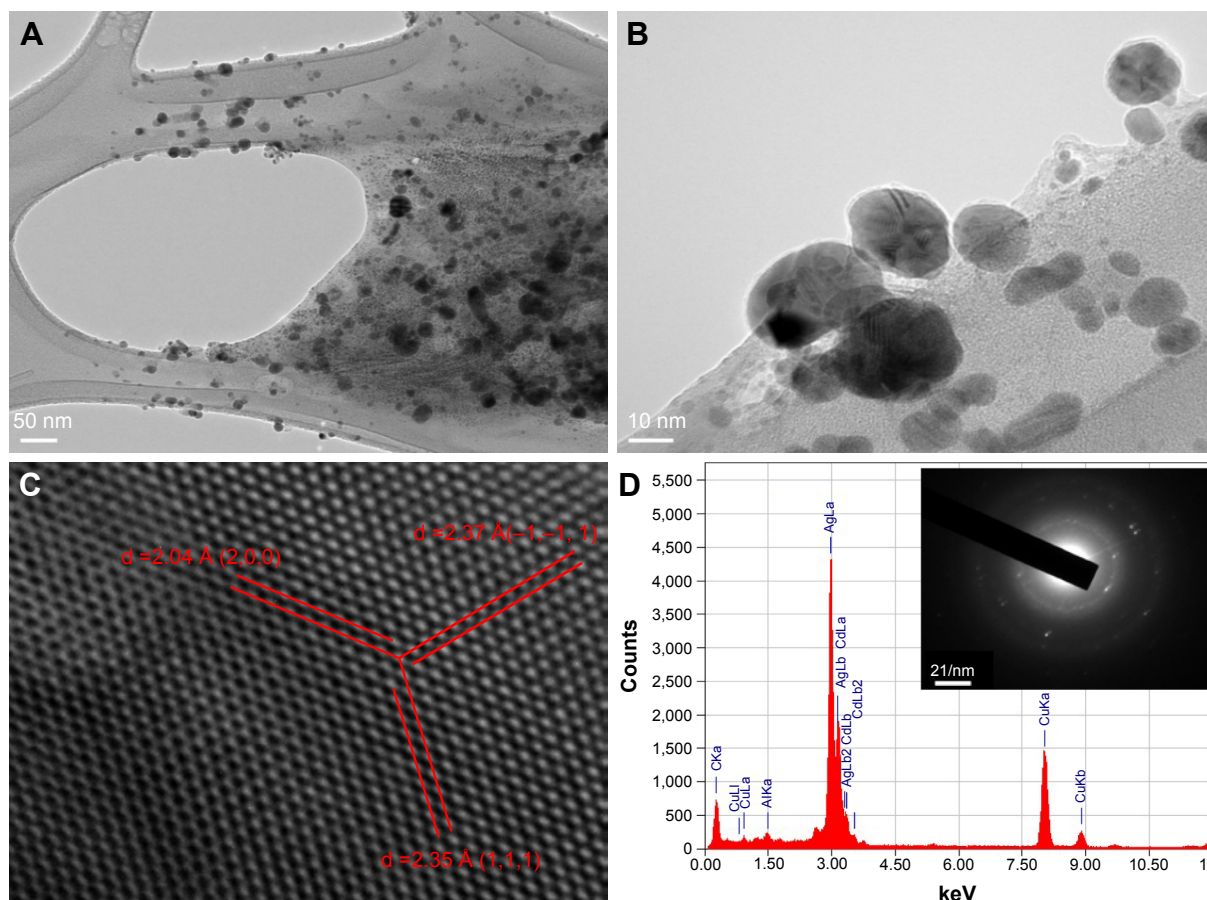


Figure 2 (A, B) TEM images of AgNPs, (C) HRTEM of AgNPs, (D) SAED pattern of AgNPs.

Abbreviations: TEM, transmission electron microscopy; AgNPs, silver nanoparticles; HRTEM, high-resolution transmission electron microscopy; SAED, selected area (electron) diffraction.

In Figure 2A and B, the AgNPs were seen to have a diameter of approximately 15–20 nm, the particle size distribution histograms of AgNPs were almost Gaussian and the mean particle size was 17.5 nm, calculated from 120 nanoparticles. The results of TEM revealed that the synthesized AgNPs were almost monodispersed. The lattice fringes in processed high-resolution TEM images (Figure 2C) show that the AgNPs are single crystals, and also reveal that the particles have a lattice d-spacing of 2.04, 2.35, and 2.37 Å and their indices (hkl) are assigned to {2,0,0}, {1,1,1}, and {−1,−1,1} planes of Ag, respectively, indicating an fcc crystalline structure.^{45–48}

The elemental composition of sample over the grid by EDS (Figure 2D) revealed that the main component of the

sample is Ag, although the C and Cu peak could primarily come from the lacey-carbon of the TEM grid. On the other hand, the presence of lightest elements such as H are not detected by EDS technique.⁴⁹ The insert of Figure 2D shows a representative selected area (electron) diffraction (SAED) pattern of AgNPs. In this pattern, spotty rings ascribed to the {111}, {200}, {220}, {311}, and {331} planes of fcc metallic silver (JCPDS 87-0720) are clearly observed.^{46,47}

Effect of Se on serum ALT and AST

The present study explored the effect of Se on AgNP-induced hepatic toxicity. The activities of AST and ALT were significantly increased in serum of rats treated with AgNP compared

Table 1 Effect of Se on AgNP-induced serum AST and ALT levels

Experimental groups				
Parameters	Control (1)	AgNP (2)	Se (3)	Se + AgNP (4)
AST (IU/L)	205.18±24.12	341.12±32.32*	203.43±37.37	242.11±36.45**
ALT (IU/L)	125.34±21.32	250.40±31.25*	120.12±31.35	167.55±23.12**

Notes: All values are mean ± SEM, n=6. Group 1 vs 2 (*P<0.05); group 2 vs 4 (**P<0.05).

Abbreviations: Se, selenium; AgNP, silver nanoparticle.

to their respective control ($P < 0.5$) (Table 1). Pretreatment of rats with Se in AgNP-treated group caused reduction in the levels of AST and ALT ($P < 0.5$). This indicates that supplementation of Se normalized the AST and ALT activity in serum compared to AgNP-treated rats. However, activities of AST and ALT did not show any change in group 3 rats (Se-treated only) compared to control group.

Effect of Se on hepatic antioxidant defense system

AgNPs' treatment led to a decrease in the activity of GSH level, as shown in Figure 3A. However, pretreatment with Se (group 4) led to an increase in the levels of GSH as compared to group 2 ($P < 0.05$). As shown in Figure 3B–D, AgNPs' administration (group 2) also led to a decrease in activities of antioxidant enzymes: SOD, CAT, and GSH-Px relative to the control rats ($P < 0.05$). On the other hand, Se pretreatment (group 4) increased the activities of SOD,

CAT, and GSH-Px significantly ($P < 0.05$) compared to the AgNP group. No change in activities of enzymes was observed in Se pretreated group 3 vs control group. Results indicate that AgNP causes oxidative stress by decreasing levels of antioxidant enzymes compared to control groups. Treatment with Se successfully elevated the activities of SOD, CAT, and GSH-Px significantly, compared to the AgNPs treated group.

Effect of Se on TAC and CRP levels in serum

AgNPs caused a significant decrease in TAC level compared to control group as shown in Figure 4A. However, Se pretreatment (group 4) elevated TAC significantly ($P < 0.05$) compared to the AgNP group. No change in TAC was observed in Se pretreated group 3 vs control group 1. AgNPs' effect on CRP levels in the serum was also studied (Figure 4B). AgNP treatment in rats resulted in a significant increase in

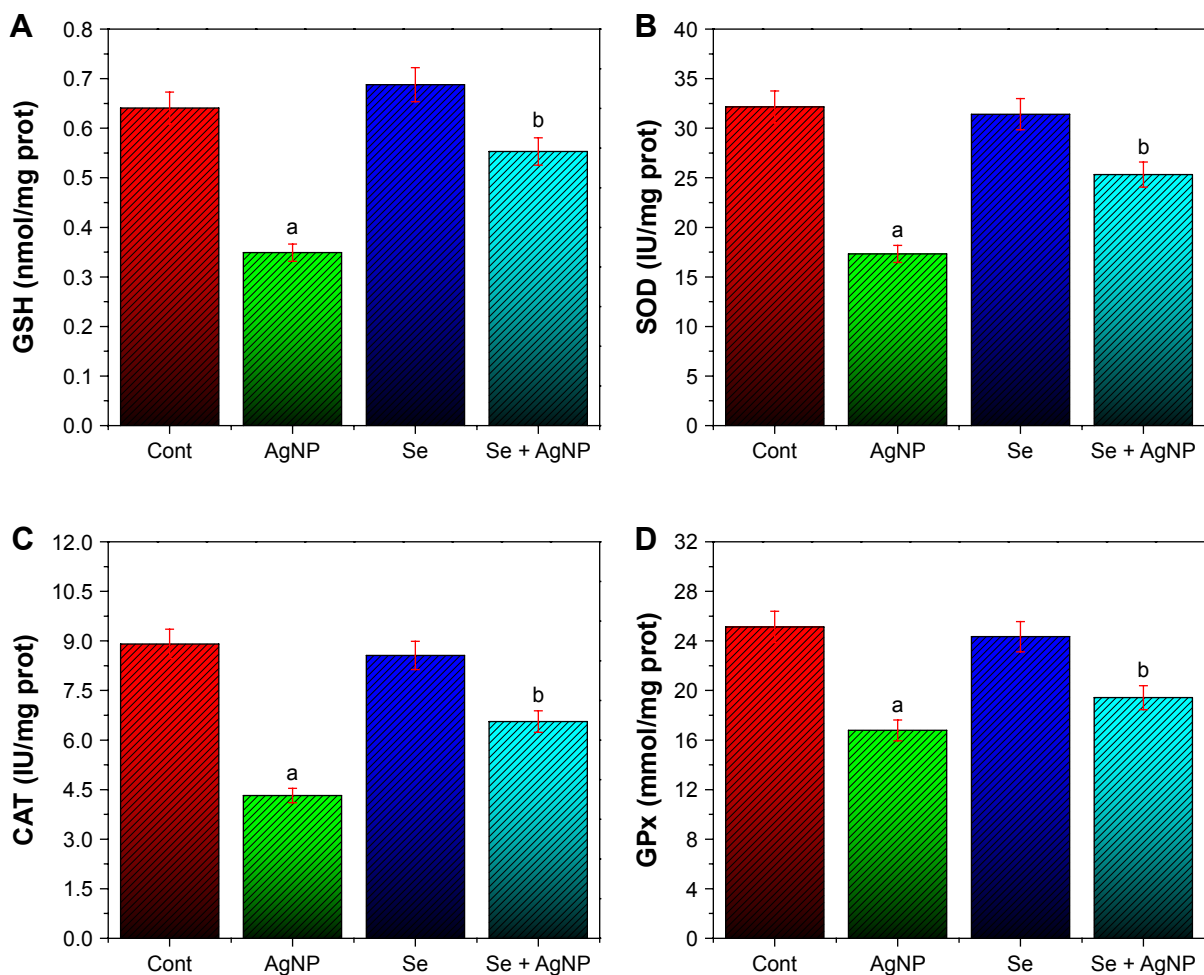


Figure 3 (A) Effect of Se on AgNP-induced GSH levels, the data represent means \pm SEM. **(B–D)** Influence of Se on AgNP-induced antioxidant levels in the liver compared with their respective control groups, the data represent means \pm SEM. a: $P < 0.05$ with respect to the control group (significant). b: $P < 0.05$ with respect to AgNP group (significant).

Abbreviations: Se, selenium; AgNP, silver nanoparticle; Cont, control; GSH, glutathione; prot, protein.

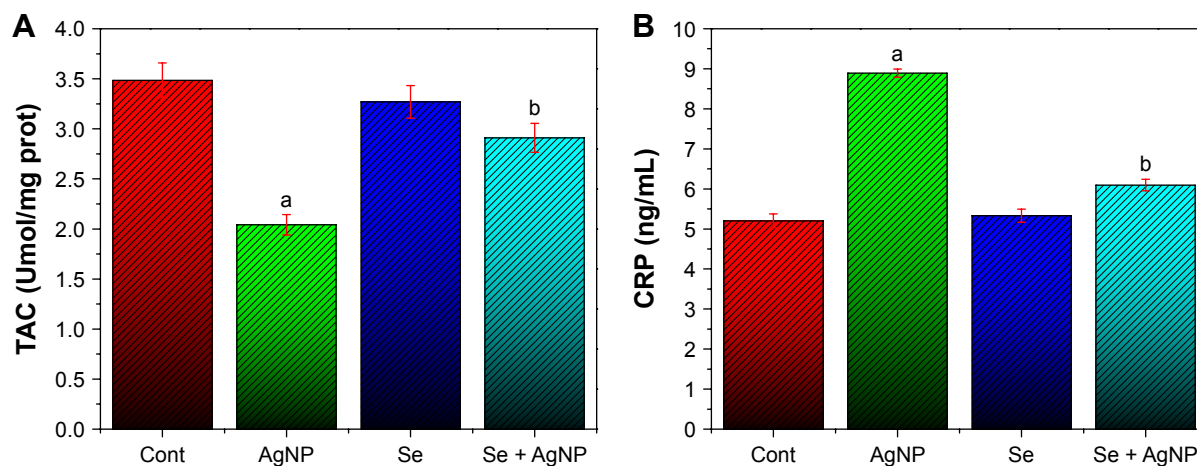


Figure 4 (A, B) Influence of Se on AgNP-induced TAC and CRP levels in serum compared with their respective control groups. The data represent means \pm SEM. a: $P < 0.05$ with respect to the control group (significant). b: $P < 0.05$ with respect to AgNP group (significant).

Abbreviations: Se, selenium; AgNP, silver nanoparticle; TAC, total antioxidant capacity; CRP, C-reactive protein; Cont, control; prot, protein.

this inflammatory biomarker (group 2 vs group 1). However, pretreatment with Se resulted in a significant decrease in the CRP biomarker compared to group 2 ($P < 0.05$). Se by itself in group 3 did not affect the CRP levels compared to group 1. These results suggest that AgNPs-induced increase in the inflammatory marker was significantly prevented with the Se pretreatment in these rats ($P < 0.05$).

Histopathological effects on the liver

The sections of the control liver showed a normal hepatic structure with hepatic lobule and central vein (Figure 5A). Sections of rat livers exposed to AgNPs alone (group 2) showed a disorganized hepatic structure with dilated central vein, hepatocytes with pyknotic nuclei, and Kupffer cells (Figure 5B). Liver sections from Se-treated rats (group 3)

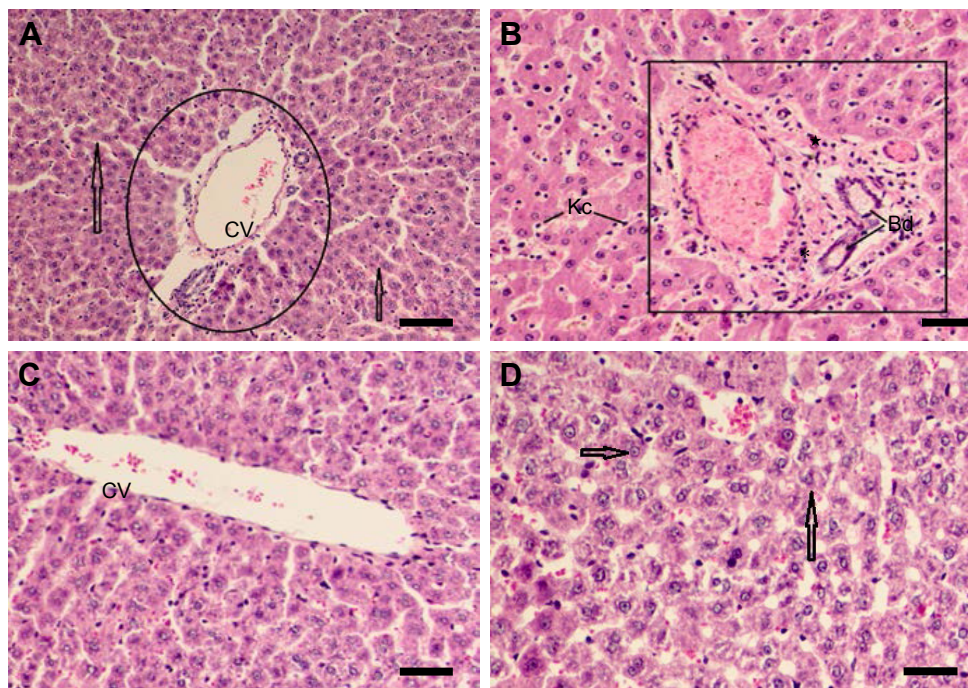


Figure 5 Histological evaluation of Se and AgNPs treatment of rats. **(A)** Control group, normal liver architecture showing portal area (circle), hepatic strands of eosinophils (arrows), and central vein. **(B)** AgNPs treated group showing portal tract expansion (square), dense infiltration of mononuclear cells (star), increase in Kupffer cells (Kc), proliferated bile duct (Bd), and accumulation of cells around central vein. **(C)** Section of rat liver (Se-treated) showing normal hepatocytes and central vein similar to control. **(D)** Section of rat livers (Se + AgNPs treated) showing normal cellular appearance and decrease in toxicity (arrows). Scale bar 50 μ m.

Abbreviations: Se, selenium; AgNPs, silver nanoparticles; CV, central vein.

showed more organized hepatic strands than group 2 and better hepatic architecture (Figure 5C). Sections from rats of group 4 (Figure 5D), treated with Se + AgNPs showed normal architecture of cells and fewer Kupffer cells in comparison to group 2.

Discussion

Nanoparticles are used on a daily basis and have several applications in the medical and electronic fields.⁴⁹ The unique physical and chemical properties of AgNPs make them excellent candidates for many purposes in the medical field, including their antimicrobial and anti-inflammatory properties. However, there are reports which suggest that nanosilver can allegedly cause adverse effects on humans as well as the environment. Organisms tend to incorporate nanoparticles within their bodies mainly through the gut but can translocate within the body. Increased ROS production after exposure to nanoparticles has been linked in several studies.⁵⁰⁻⁵² It has also been reported that oxidative stress seen in different types of cells like fibroblast, muscle, and colon was due to apoptosis induced by exposure to AgNPs.⁵⁰ The formation of free radicals and other toxic oxidizing agents can be prevented using some enzymatic antioxidants such as GSH reductase, CAT, Px, etc.

In the present study, AgNPs treatment led to a decrease in the activity of GSH, and pretreatment with Se increased its levels. GSH is an antioxidant which can quench free radicals or serve as a substrate for other antioxidant enzymes, such as GSH-Px and GSH reductase. The decreased levels of GSH after exposure to AgNPs may be due to complexing of AgNPs with thiol groups or to increasing use of GSH to downplay the effect of free radicals after exposure to the nanoparticles.^{52,53} These nanoparticles have a strong affinity for thiol groups and may therefore predispose to a decrease in GSH content, thereby leading to the formation of complexes between radical species and cellular proteins or other biomolecules. Se is an essential element with beneficial antioxidant property and is a structural component of several enzymes like GSH-Px and Trx reductase, and has been found to have detoxification effects on various heavy metals.

CAT levels were inconsistently altered after exposure to AgNPs, as also observed in previous studies.⁵⁴ Also, AgNPs caused inconsistent reductions in the levels of GSH-Px in serum and tissues, perhaps because the nanoparticles have an affinity for thiol groups.⁵⁵ The alterations in the levels of these enzymes may represent an adaptive mechanism to offset the stress of exposure. Earlier studies have shown that exposure of rats to AgNPs can alter biochemical processes

and AgNPs can sequester and accumulate in vital organs, including the kidney, lung, testes, and brain.⁵⁶⁻⁵⁸ These data suggest that AgNPs can induce oxidative damage through an ROS-mediated process. However, it remains to be investigated whether AgNPs induce free radicals directly or indirectly through depletion of antioxidant defense mechanisms depending on dose eg, caused by interactions with antioxidant systems.⁵⁹ This may explain why the biochemical alterations caused by AgNPs became more pronounced with longer exposure, and indicates that caution should be exercised in their use for biomedical purposes.

Se treatment, however, reduced CRP level compared with the AgNP intoxicated group. Also, AgNPs caused a significant decrease in TAC level compared to control group. However, Se pretreatment increased TAC levels significantly compared to the AgNP group. Nanoparticles can be toxic and can elicit a lot of tissue responses such as cell activation, generation of ROS, inflammation, and cell death.^{59,60} The elevated levels of CRP indicate the presence of reactive species related to the incidence of many pathological conditions such as coronary heart disease, hypertension, and inflammation. However, pretreatment with Se can effectively counteract oxidative damage caused by scavenging reactive oxygen radicals and protect membrane integrity.

The liver damage as observed in the current study, has been consistent with previous studies reporting toxicity by AgNPs in the hepatic Kupffer cells and endothelial cells lining the sinusoidal spaces.^{61,62} However, it has been concluded that deposition of AgNPs in tissue samples is size-dependent in the liver and kidneys of male and female rats.^{63,64} In this study, Se treatment of AgNPs exposed rats showed alleviation of the histopathological changes in rat livers. Se pretreatment of AgNPs showed a mild restoration of hepatic cellular organization in comparison to the AgNPs treated group. Recently, Se has been shown to interact in the body of mammals, reducing toxicity of certain compounds.⁶⁵⁻⁶⁷ It could be that Se administration prevents free radical formation by increasing antioxidant activity of tissue, as evident in our studies, and prevent AgNPs' toxicity.

Conclusion

AgNPs are used in an increasing number of applications and these compounds are already used in several products like toothpaste and antibacterial gels, without a profound understanding of how the human body will react and respond to sustained exposure. Therefore, future investigations should be conducted to explore the effects of particle size, different

doses, and chemical composition on cellular and molecular responses in various tissues. Se has been known to be important for the antioxidant defense system of the organism, and has been shown to be protective against various toxic insults. Thus, Se can be used as a therapeutic agent in improving the antioxidant system and reducing tissue toxicity. However, the beneficial effect of Se was expressed in a very narrow dosage range, and the high and low doses of Se may be connected with pathological manifestations.

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Disclosure

The authors report no conflicts of interest in this work.

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