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Original Research

Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat

Akram Ranjbar[1*], Zahra Ataie[2], Farzad Khajavi[3], Hassan Ghasemi[4]

[1]Department of Toxicology and Pharmacology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Pharmacology and Physiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[3]Department of Chemistry, Bu-Ali Sina University, Hamedan, Iran
[4]Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, *Hamadan, Iran*

Abstract

Objective(s): Nanotechnology and nanoparticles are increasingly recognized for their potential applications in aerospace engineering, nanoelectronics, and environmental remediation, medicine and consumer products. More importantly is the potential for the application of silver nanoparticles (Ag NPs) in the treatment of diseases that require maintenance of circulating drug concentration or targeting of specific cells or organs the aim of this study was to investigate the possible protective role of Ag NP antioxidative biomarkers in rats. Ag NPs are used to investigate the potential risks for the environment and health.
Materials and Methods: Rats received Ag NP, 5, 50, 250 and 500 mg/kg/day IP. After two week of treatment, the activity of enzymatic scavengers such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and total antioxidant capacity (TAC) of blood samples were measured.
Results: Ag NP in 5, 50, 250 and 500 mg/kg reduced activities of CAT, SOD and increased TAC in plasma.
Conclusion: In this study, Ag NP with 500mg/kg induced activities of CAT, SOD and decreased TAC. It is concluded that antioxidative properties of Ag NP is dose dependent.

Keywords: Enzyme antioxidant, Oxidative stress, Silver nanoparticle (Ag NP)

***Corresponding authors:** *Akram Ranjbar, Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran*
Tel: +98 811 838003, Email: akranjbar1389@yahoo.com

Introduction

Silver nanoparticles (Ag NPs), are clusters of silver atoms that range in diameter from 1 to 100 nm. attracting interest as antibacterial and antimicrobial agents for applications in medicine (1). Ag NP is a blossoming field of research and has been highly commercialized. Clothing manufacturers have incorporated Ag NP into fabrics for socks and exploit the antibacterial activity for neutralization of odor-forming bacteria (2-4). The medical industry has been slow to exploit the potential of Ag NP in infection prophylaxis, but this field is now gaining momentum (5). Ag NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, atalytic activity and non-linear optical behaviour (6-9).However, it is the exceptional broad spectrum bacteriocidal activity of silver (10-12) and relatively low cost of manufacturing of Ag NP, that has made them extremely popular in a diverse range of consumer materials, including plastics, soaps, pastes, metals and textiles (13).Moreover, nanoparticles (NPs) like silver is known to induce reactive oxygen species (ROS) in various cell types (14, 15). In spite of this, the link between AgNP and oxidative stress is not well established. Most often, the harmful effects of ROS may be manifested through damage of DNA, oxidations of polyunsaturated fatty acids in lipids and oxidations of amino acids in proteins (16, 17).Also, NPs can undergo a series of processes like binding and reacting with proteins, phagocytosis, deposition, clearance and translocation. On the other hand NPs can elicit a spectrum of tissue responses such as cell activation, generation of ROS, inflammation and cell death (18, 19). Some of these studies provided sample evidence that the cytotoxicity of AgNPs may be partially due to their induction of cellular oxidative stress through the generation of free radicals and ROS (19, 20).

This is of clinical significance because certain pathological conditions such as inflammation is associated with elevated oxidative stress and this may in turn alter the sensitivity of cells and tissues to potentially cytotoxic AgNPs increasing their market value (21, 22).

Therefore, this study aimed to examine antioxidant effects of AgNP in different dose in blood of rat by subchronic toxicity test in male rats.

Materials and Methods

Reagents and chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), from Fluka, Italy, was used in this study.GPx and SOD (Ransel kit, Randox Laboratories Ltd, Crumlin, UK), bioxytech GSH kit (Oxis Research, USA), were used in this study.

All other chemicals were obtained from the Sigma.

The Ag NP (10 nm, 1000ppm) used in this study were supplied by Notrino compony. The nanoparticle was suspending in deionized water, the stock concentration of Ag NP was 250ml.

Animals and treatments

Adult male Wistar rats weighing 180–250 g maintained on a 12-hour light/dark cycle with free access to tap water and standard laboratory chow and randomly divided into five groups of five animals each. AgNPs suspension at 0,5,50,250 and 500 mg/kg of body weight per day for groups 1-5 respectively was prescribed intraperitoneally (IP) for two weeks**.** The groups were as follows: control group, Ag NP(10 nm, 1000ppm), 5, 50, 250 and 500 mg/kg/day once day.

One group of animals received only normal saline and was assigned as control. Treatment was carried out for 14 days. At the end of the treatment, 24 hours post the last dose of treatment, animals were killed, blood samples were collected from heart in tubes and serum was isolated.

The experiments were conducted according to the ethical rules approved by Institutional Review Board (IRB).

Measurement of Cu/Zn- SOD activity

The activity of Cu/Zn- SOD was measured using a commercial kit (Ransod kit, Randox Laboratories Ltd, Crumlin, UK).

Measurement of the enzyme was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase and reacted with 2-(4-iodophenyl)-3-(4-nitrofenol) 5-phenyltetrazolium chloride (INT) to form a red formazan dye.

The formazan was read at 505 nm.

One unit of Cu/Zn- SOD was defined as the amount of enzyme necessary to produce 50% inhibition in the INT reduction rate.

Measurement of GPx activity

The amount of GPx was determined using a commercially available kit (Ransel kit, Randox Laboratories Ltd, Crumlin, UK) by measuring the rate of oxidation of NADPH at 340 nm.

A unit of enzyme was expressed as the amount of enzyme needed to oxidize 1 nmol of NADPH oxidase/minute.

Measurement of total antioxidant capacity (TAC)

It was measured by the ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma to reduce Fe^{3+} to Fe^{+2} in the presence of TPTZ.

The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (23).

Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as Mean±SE. All data were analyzed with SPSS Version 16 employing one-way ANOVA followed by Tukey post hoc test. Differences between groups was considered significant when $P < 0.05$.

Results

Superoxide dismutase

Ag NP caused a significant decrease in SOD activity in 250 mg/kg when compared to control, 5 and 50 mg/kg ($p < 0.05$).

Ag NP caused a significant increase in SOD activity when compared to 500 mg/kg kg ($p < 0.05$); Figure 1.

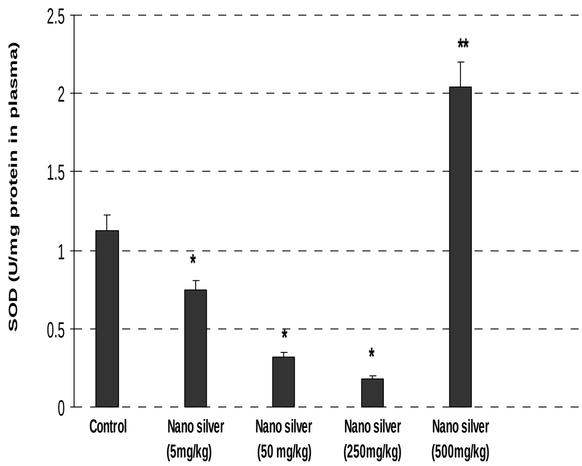

Figure 1. Superoxide dismutase (SOD) activity in plasma of rats.
*Significantly different from control group at p < .05. **Significantly different from Ag NP 250 mg/kg group at p < .05

Glutathione peroxidase

Ag NP caused a significant decrease in SOD activity in 250 mg/kg when compared to control,5 and 50 mg/kg ($p < 0.05$).

Ag NP caused a significant increase in SOD activity when compared to 500 mg/kg ($p < 0.05$); Figure 2.

Total antioxidant capacity

Ag NP caused a significant increase in TAC level in 250 mg/kg when compared to control, 5 and 50 mg/kg ($p < 0.05$). Ag NP caused a significant decrease in TAC level in 500 mg/kg when compared to 250, 5 and 50 mg/kg ($p < 0.05$); Figure 3.

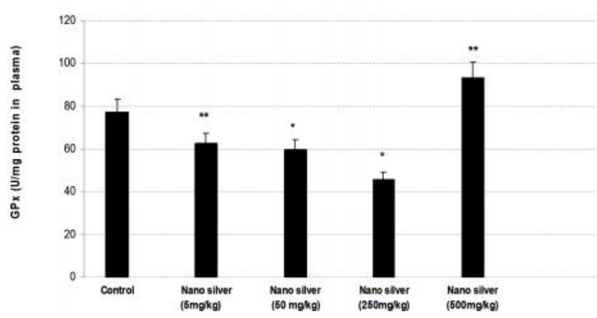

Figure 2. Glutathione peroxidase (GPx) activity in plasma of rats.
*Significantly different from control group at p < .05. **Significantly different from Ag NP 250 mg/kg group at p < .05

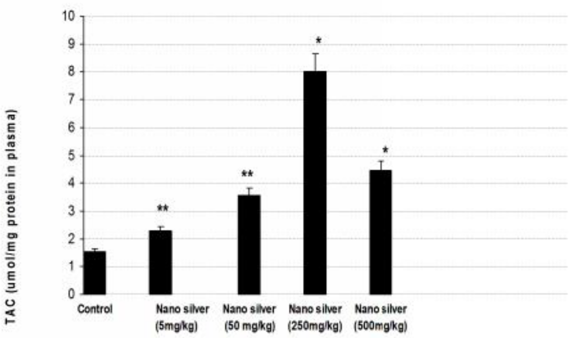

Figure 3. Total antioxidant capacity (TAC) in plasma of rats.
*Significantly different from control group at p < .05. **Significantly different from Ag NP 250 mg/kg group at p < .05

Discussion

The aim of this study was to determine Ag NPs are antioxidative properties.

Our results demonstrate that Ag NP decrease the oxidative stress, as shown by a decreased SOD and GPx activities and increase TAC level in 5,50 and 250 mg/kg, but in 500 mg/kg decrease TAC level and increase SOD and GPx activities in this group compared the other groups.

Ag NPs are already in use in several industries, exposing humans on a daily basis.

From the textile to the food industry, from the production of sunscreen to cosmetics, with applications in the medical and electronic fields (24).

Additionally, the investigators demonstrated increased ROS production and increased cell lethality in rat liver cells after exposure to NPs (25).

Many studies have implicated intracellular ROS in the signal transduction pathways leading to (26).

Recently, it was reported that apoptosis induced by exposure to Ag NP was mediated by oxidative stress in fibroblast, muscle and colon cells (27).

In the present study, antioxidant enzymes activity such as GPx and SOD were used to measure the production of ROS in variouse dose of AG NP (Fig. 1,2). Importantly, after 14 dayes exposure, the doses which caused significant increases in TAC expect 500 mg/kg NS dose. (Fig. 3). These data suggest that Ag NP can induce oxidative damage through a ROS-mediated process However, it remains to be investigated whether Ag NP induce free radicals directly or indirectly through depletion of antioxidant defense mechanisms depending dose e.g. caused by interactions with antioxidant systems (28).

The previous studies have shown that small Ag NP are more toxic than large NPs. Recently, this effect was also reported (29).

Other studies reported that micro-sized particles are less toxic than their smaller counterparts (30, 31). In the present study Ag NP size is fix but in different dose.

This implies that size, dose and chemical composition are properties of great importance when evaluating nanotoxicity.

This was also reported in a number of comparative studies (32-34).

We recommend that future studies should be conducted to explore the importance of particle size, different doses and chemical composition on cellular and molecular responses in various tissues to Ag NP.

In the present study ,we investigated antioxidative properties in different dose and we found in high dose Ag NP is toxic.

Since Ag NP are used in an increasing number of applications and these compounds are already used in several products (from toothpaste to antibacterial gels and aerosolized deodorants) without a profound understanding of how the human body will react and respond to sustained exposure (35).

Future investigations could also elucidate the mechanism of action of these compounds, to ascertain their widespread use.

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