



*Original Research Paper*

## Effects of silver nanoparticle (Ag NP) on oxidative stress, liver function in rat: hepatotoxic or hepatoprotective?

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Silver nanoparticles (Ag NPs) have been widely used as new potent antimicrobial agents in cosmetic and hygienic products. The purpose of this study was to investigate the possible protective role of Ag NP antioxidative biomarkers in rat liver. Rats received Ag NP, 5, 50, 250 and 500 mg/kg/day IP. After two week of treatment, the activity of catalase (CAT), total antioxidant capacity (TAC) and total thiol molecules (TTG) in liver tissue were measured. Then ALT (alanine aminotransferase) and ALP (alkaline phosphatase) activities in blood were measured. Ag NP in 5, 50mg/kg reduced activities of CAT, ALT and increased TAC and TTG in liver homogenate compared Ag NP 500mg/kg/day group. Also, in this study, Ag NP with 500mg/kg/day induced activities of CAT, AST and ALT compared control group. It is considered Ag NP beneficial effects for the protection oxidative liver injury in low doses and considered toxic in high doses.

**Key words:** Silver nanoparticle (Ag NP), oxidative stress, rat, liver

### INTRODUCTION

The small size of nanoparticles (NPs), defined as particles with at least one dimension between 1 and 100 nm, results in unique chemical and physical characteristics leading to advanced magnetic, electrical, optical, mechanical and structural properties compared to the original bulk substance (Korani et al., 2011). However, the same characteristics making NPs so attractive for their exploitation in new products have led to concerns that NPs may pose a risk for humans and the environment (Kim and Ryu, 2013). Nanosilver (Ag NP) is one of the most commonly used nanomaterials because of its strong disinfectant properties (Chen and Schluesener, 2008). As a result of Ag NP special characteristic of killing bacteria, antimicrobial materials containing Ag NP are becoming increasingly important because of their wide range of applications (Li et al., 2013; Jowkar et al., 2013).

However, it is the exceptional broad spectrum bacteriocidal activity of silver (6-8) 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 (Hsin et al., 2008).

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 reactive oxygen species (ROS), inflammation and cell death (Liu et al., 2006; Miura and Shinohara 2009). Some of these studies provided sample evidence that the cytotoxicity of AgNP may be partially due to their induction of cellular oxidative stress through the generation of free radicals and ROS (Miura and Shinohara 2009; Long et al., 2006). 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 AgNP increasing their market value (Korani et al., 2011; Stebounova et al., 2011; Chen et al., 2007).

It has been demonstrated that the harmful consequences of oxidative stress are associated with various diseases, such as cancer, cardiovascular diseases, ischemic injury, multiple sclerosis, rheumatoid arthritis, diabetes, neurological disorders, and senescence (Schluesener and Schluesener, 2013). Liver damage ranging from subclinical enteric hepatitis to necroinflammatory hepatitis, cirrhosis

and carcinoma has been proven to be associated with redox imbalance and oxidative stress (Wu and Cederbaum, 2009; Kaplowitz and Tsukamoto, 1996). Therefore, compounds that exhibit antioxidant properties, scavenging of free radicals and inhibition of lipid peroxidation are expected to show hepatoprotective activity (Halliwell, 2011). Despite the widespread use of Ag NP products, relatively few studies have been undertaken to determine the biological effects of Ag NP exposure. Therefore, this study aimed to examine hepatoprotective effects of AgNP in different doses in liver of rat by subchronic toxicity test in male rats.

## MATERIALS AND METHODS

### Reagents and Chemicals

Ethylenediamine tetra acetic acid (EDTA), dithiobis-2-nitrobenzoic acid (DTNB), tris base and 2, 4, 6-tripyridyl-S-triazine (TPTZ), hydrogen peroxide ( $H_2O_2$ ) were used in this study. All other chemicals were obtained from the Sigma.

The Ag NP (10 nm, 1000ppm, 95% purity) used in this study were supplied by Notrino company. 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 were used. Animals were randomly divided into six groups of five animals and treated for 2 week intraperitoneally (IP). The groups were as follows: control group, Ag NP (10 nm, 1000ppm), 5, 50, 250 and 500 mg/kg/day once day for 2 week.

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, serum and liver samples were collected.

### Sample collection

The liver were rinsed with normal saline solution, and then also stored at liquid nitrogen. Liver tissues were homogenized in 1: 5 volumes of PBS (pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min. And then, the supernatant was used as liver total homogenate sample. Then centrifuged at  $3000 \times g$  for 15 min. at  $4^\circ C$ , supernatant was kept at  $-80^\circ C$  for further biochemical measurements (18).

### Experimental Protocols

#### Estimation of marker enzymes

Levels of various liver marker enzymes such as ALT, AST in

serum were estimated according to the standard procedure of kits (Pars Azemon kit, Iran).

#### Measurement of biomarkers of oxidative stress

##### Measurement of total antioxidant capacity (TAC)

It was measured by the ferric reducing ability (FRAP) method. This method is based on the ability of plasma to reduce  $Fe^{3+}$  to  $Fe^{2+}$  in the presence of TPTZ reagent. The reaction of  $Fe^{2+}$  and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (Benzie and Strain, 1996).

##### Measurement of Catalase (CAT) activity assay

CAT activity was assayed in the samples by measuring the absorbance decrease at 240 nm in a reaction medium containing  $H_2O_2$  (10 mM), sodium phosphate buffer (50 mM, pH. 7.0). One unit of the enzyme is defined as 1 mol  $H_2O_2$  as substrate consumed/min, and the specific activity is reported as units/mg protein (Johansson and Håkan Borg, 1988).

##### Measurement of total thiol molecules (TTG)

To evaluate the liver total thiol molecules, DTNB reagent was used as a reagent. DTNB reacts with thiol molecules and creates a yellow complex which has good absorbance at 412 nm in spectrophotometer (Hu and Dillard, 1994).

##### Total protein

Protein concentrations in the samples were measured by the Bradford method using concentrated Coomassie blue reagent. Bovine serum albumin was used as the standard (Bradford, 1976).

##### Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as Mean $\pm$ 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

### Catalase

Ag NP caused a significant decrease in CAT activity in 5mg/kg when compared to 500 mg/kg ( $p < 0.05$ ). Ag NP 500mg/kg caused a significant increase in CAT activity when compared to control group ( $p < 0.05$ ); Table 1.

**Table 1:** Effect of Ag NP on liver oxidative stress biomarkers in rats

Groups	TAC ( $\mu\text{mol mg}^{-1}$ )	CAT( $\text{Umg}^{-1}$ )	TTG( $\text{mMol}$ )
Control	6.22 $\pm$ 0.62	0.5 $\pm$ 0.03	0.27 $\pm$ 0.040
Ag NP 5mg/kg	6.98 $\pm$ 0.41 <sup>b</sup>	0.47 $\pm$ 0.06 <sup>b</sup>	0.31 $\pm$ 0.051
Ag NP 50mg/kg	7.38 $\pm$ .51 <sup>b</sup>	0.48 $\pm$ 0.04	0.35 $\pm$ 0.030 <sup>b</sup>
Ag NP 250 mg/kg	5.75 $\pm$ 0.86	0.76 $\pm$ 0.05	0.25 $\pm$ 0.040
Ag NP 500 mg/kg	1.72 $\pm$ .90 <sup>a</sup>	0.88 $\pm$ 0.03 <sup>a</sup>	0.15 $\pm$ 0.012 <sup>a</sup>

Values are group means  $\pm$  SEM (n = 5 per group).

a Significantly different (P < 0.05) compared with control.

b Significantly different (P < 0.05) compared with Ag NP 500 mg/kg group.

**Table 2:** Effect of Ag NP on liver functions in rats

Groups	ALT(U/ml)	AST(U/ml)
Control	65 $\pm$ 4.50	1.36 $\pm$ 0.80
Ag NP 5mg/kg	61 $\pm$ 5.23	1.31 $\pm$ 0.60
Ag NP 50mg/kg	59 $\pm$ 3.67 <sup>b</sup>	1.24 $\pm$ 0.78
Ag NP 250 mg/kg	69 $\pm$ 4.70	1.38 $\pm$ 0.82
Ag NP 500 mg/kg	88 $\pm$ 6.23 <sup>a</sup>	2.11 $\pm$ 0.95 <sup>a</sup>

Values are group means  $\pm$  SEM (n = 5 per group).

a Significantly different (P < 0.05) compared with control.

b Significantly different (P < 0.05) compared with Ag NP 500 mg/kg group.

### Total thiol molecules

Ag NP caused a significant increase in TTG level in 50mg/kg when compared to Ag NP 500 mg/kg group (p <0.05). Ag NP caused a significant decrease TTG level in 500mg/kg when compared to control group (p <0.05); Table 1.

### Total antioxidant capacity

Ag NP caused a significant increase in TAC level in 5 and 50mg/kg when compared to Ag NP 500 mg/kg group (p <0.05). Ag NP caused a significant decrease TTG level in 500mg/kg when compared to control group (p <0.05); Table 1.

### ALT and AST activities

Also, Table2 shows the Mean $\pm$ SE of variables related to ALT and AST levels in animals test. Ag NP caused a significant decrease in AST activity in 50mg/kg when compared to 500 mg/kg (p <0.05). Ag NP 500mg/kg caused a significant increase in ALT and AST activities when compared to control group (p <0.05).

## DISCUSSION

The aim of this study was to determine Ag NPs are antioxidative and prooxidant properties. Our results demonstrate that Ag NP in 5 and 50 mg/kg decrease the oxidative stress, as shown by a decreased CAT activity and

increase TAC and TTG levels in these doses, but in 250mg/kg and 500mg/kg decrease TAC and TTG levels and increase CAT activity in this group compared the control group. Also liver enzymes such as AST and ALT increased in Ag NP 500 mg/kg group compared control group (Table1). AST activity in Ag NP 50mg/kg group decreased compared Ag NP 500 mg/kg group (Table 2).

AgNP have been found to increasing oxidative stress (Kim et al., 2009). These findings support the hypothesis (nanoparticle toxicity) that antioxidant/oxidant from the temporal AgNP exposed rats in lower doses which could ultimately lead to the observed hepatoprotective.

These results suggest that the observed decrease of spontaneous alternation in the liver function could result from AgNP induced or reduced oxidative stress. Altogether, these results suggest that AgNP in higher doses are capable of inducing oxidative stress, which is responsible for hepatotoxicity in rats. In addition, spontaneous alternation in the oxidative stress biomarkers indicates toxicity of nanoparticle accumulation such as AgNP.

Additionally, the investigators demonstrated increased ROS production and increased cell lethality in rat liver cells after exposure to NPs (Hunt et al., 2013). Many studies have implicated intracellular ROS in the signal transduction pathways leading to (Li et al., 2013). Recently, it was reported that apoptosis induced by exposure to Ag NP was mediated by oxidative stress in fibroblast, muscle and colon cells (Kim and Ryu, 2013; Jowkar et al., 2013). In the present study, antioxidant enzyme activity such as catalase (CAT) was used to measure the production of ROS in various dose of Ag NP (Table 1).

Importantly, after 14 days exposure, the doses which caused significant decreased in TAC and TTG in 500mg/kg dose (Table 1). 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 (Schluesener and Schluesener, 2013; Hunt et al., 2013).

The previous studies have shown that small dose Ag NP are more effective antioxidant than large NPs. Recently, studies reported that micro-sized particles are less toxic than their smaller counterparts (Schluesener and Schluesener, 2013; Rai et al., 2009; Asharani et al., 2008). In the present study Ag NP size is fixed but in different doses, high doses are more toxic.

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 and oxidative 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 (Li et al., 2013; Jowkar et al., 2013; Marambio-Jones and Hoek, 2010). It is considered NPs such as AgNP in large doses may be accumulate in tissues and capable toxicity in living organisms. Also, further studies are required to elucidate the cellular and molecular mechanism of action of these compounds, to ascertain the implication of their widespread use in various tissues.

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