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The Effect of Silver Nanoparticles on the Biochemical Parameters of Liver Function in Serum, and the Expression of Caspase-3 in the Liver Tissues of Male Rats

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Abstract

Background: Silver nanoparticles have antibacterial properties and their use is growing in different industries. Since the toxicity of nanosilver is not well known, it is essential to examine its safety.
Objectives: This experiment was undertaken to study the effects of nanosilver on rat liver function with an evaluation of blood biochemistry parameters and caspase-3 expression in the liver.
Materials and Methods: In this experimental study, 40 adult male Sprague-Dawley rats were divided into five groups. In the four experimental groups, nanosilver particles were given orally for 28 consecutive days at doses of 30, 125, 300, or 700 mg/kg. Rats in the control group received equal volumes of deionized water. To evaluate the expression of caspase-3 in liver tissue, the real-time PCR method was used. Albumin, total protein, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were measured with an RA-1000 autoanalyzer.
Results: The results indicated that caspase-3 was upregulated in the treated groups compared to the control group ($P < 0.05$). No considerable changes in serum biochemical parameters were observed ($P > 0.05$).
Conclusions: Based on the present study, it can be concluded that oral administration of silver nanoparticles for 28 days had no effect on rat liver function, but probably led to early apoptotic stages.

Keywords: Apoptosis, Caspase-3, Liver, Nanoparticles, Silver

1. Background

Nanotechnology is a rapidly growing science that involves the production of engineered nanoparticles (1). Among the various nanomaterials, silver nanoparticles (Ag-NPs) are used generally in medical, industrial, and home products (2). Their unique characteristics, including antibacterial properties, have resulted in their widespread use in medical applications, such as wound-care products (3), silver-coated catheters, and implantable medical devices (4). Despite the growing applications for products containing Ag-NPs, there is little information about their potential toxicity and side effects (5). In vitro evidence supports the suggestion that Ag-NPs induce strong cytotoxicity and pro-inflammatory effects (4) in a broad spectrum of cells (6).

Production of reactive oxygen species (ROS) and the release of cytokines are considered to be the mechanisms by which metal nanomaterials induce toxicity (3). ROS are continually generated and eliminated in biological systems by endogenous or exogenous antioxidants (7-12), but excessive production of ROS can lead to apoptosis and cause oxidative DNA damage (3). Apoptosis is initiated by the sequential activation of caspases, which are a group of cysteine proteases that exist in cells as inactive proenzymes (13). Caspase-3 is a key effector caspase involved in the apoptotic cascade within cells (14), cleaving different cellular substrates and inducing apoptotic cell death (15).

Ag-NPs can be found in products related to food and beverages, such as food-packing materials, kitchen appliances, and health supplements. Therefore, it can be expected that the gastrointestinal tract is an important site

of exposure for consumers (16). Orally absorbed Ag-NPs can enter the bloodstream (17) and aggregate in the liver, spleen, kidney, lung, and brain (4). The liver in particular is one of the major organs of accumulation of Ag-NPs (18).

2. Objectives

Given the increased introduction of new nanoscale products in everyday life (19), this experiment was carried out to evaluate the effect of Ag-NPs on biochemical parameters and the expression of caspase-3 in the liver tissues of male rats.

3. Materials and Methods

3.1. Ag-NP Solution

Ag-NPs (CAS No. 7440-22-4) were purchased in powder form from US Research Nanomaterials, Inc. (Houston, TX, USA). The size distribution of the Ag-NPs was analyzed using dynamic light-scattering (DLS; Malvern, Nano ZS ZEN-3600, UK). Deionized water was used for the dispersion of Ag-NPs into concentrations of 30, 125, 300, and 700 mg/kg by vigorous vortexing, followed by sonication for 5 min.

3.2. Animal Model and Administration of Ag-NPs

In this study, 40 male Sprague-Dawley rats with weights of 180 - 200 g were used. The rats were purchased from Hamadan Medical University (Hamadan, Iran), and were kept in the animal house under a natural light/dark cycle with standard conditions of 21 ± 2°C and 50 ± 5% humidity. The rats were fed a standard chow diet. All experiments in this study were approved by the ethics committee of Hamadan University of Medical Sciences. The rats were randomly divided into five groups (n = 8). Four experimental groups were treated with 30, 125, 300, or 700 mg/kg Ag-NPs for 28 days by oral gavage. The control group received equal volumes of deionized water. The day after the last administration, the animals were anaesthetized with chloroform, and blood samples were collected from their hearts. The liver tissues were removed and stored at -80°C.

3.3. Blood Biochemistry

Blood samples were allowed to clot for 45 min at room temperature. After coagulation, the serum was separated by centrifugation at 1500 × g for 10 minutes. Albumin (Alb), total protein (TP), total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using an autoanalyzer (Hitachi 7180, Hitachi, Japan).

3.4. Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using RNX-Plus reagent (CinnaGen Co., Iran). The RNA samples were then quantified with a NanoDrop spectrophotometer (BioTek, USA). Reverse transcription was synthetized (5 μg of total RNA) with an AccuPower RT PreMix Kit (Bioneer, Korea) according the manufacturer's protocol. After that, cDNA was stored at -80°C until use.

RT-PCR was performed using cDNA and gene-specific primer pairs mixed with SYBR Green PCR master mix in a final volume of 25 μL for each tube. The primers for amplification of cDNA coding for caspase-3 were designed from the GenBank databases using the Allele-ID 6 software. The primer sequences and PCR product sizes are listed in Table 1.

Table 1. Primer Sets Employed in RT-PCR Analysis

mRNA	Accession No.[a]	Primer Sequence (5' to 3')
Caspase-3	NM_012922.2	
		sense: 5'-TTTGGAACGAACGGACCTGT-3'
		anti-sense: 5'-CACGGGATCTGTTTCTTTGC-3'
β-actin	NM_ 031144.3	
		sense: 5'-ATCCTCTTCCTCCCTGGAGAA-3'
		anti-sense: 5'-TGTTGGCATAGAGGTCTTTACGG-3'

[a]GenBank accession numbers (http://www.ncbi.nlm.nih.gov).

PCR analyses were performed with a C1000 Thermocycler, CFX96 Real-Time System (BioRad, USA) using a Quanti-Fast SYBR Green PCR Kit (Bioneer, Korea) according the manufacturer's protocol. The samples were first denatured for 5 min at 95°C, followed by 30 cycles for caspase-3 and 35 cycles for β-actin for denaturation at 95°C for 15 seconds, annealing at 63.9°C for caspase-3 and at 49.4°C for β-actin for 30 seconds, and extension at 72°C for 30 seconds. All of the PCR reactions were duplicated for each sample.

The threshold cycles (Ct) in each sample were measured and normalized to β-actin (housekeeping gene). An average Ct of duplicate detection for each gene was obtained. The results were calculated with the Livak Method ($2^{-\Delta\Delta Ct}$) (20) and expressed as the ratio of the Ct value of cDNA concentrations of target genes relative to that of β-actin. To confirm the expected molecular weight (size of amplification product), sequencing was performed (Bioneer, Korea).

3.5. Statistical Methods

The values were presented as the mean ± standard deviation (SD). Statistical evaluation between the groups was

performed with a one-way analysis of variance (ANOVA). Post-hoc comparisons were done using Tukey's test. A significant difference was considered at a P value of < 0.05.

4. Results

4.1. Characterization of Ag-NPs

DLS was used to determine the size distribution of the Ag-NPs in the water-based solution. The nanoparticles showed peak sizes ranging from 200 to 300 nm, with a maximum at 292.5 nm and a width of 78.59 nm (Figure 1).

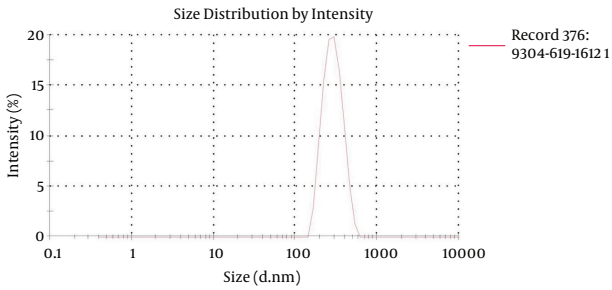


Figure 1. Size Distribution of the Ag-NPs as Determined by DLS

4.2. Effects on Blood Biochemistry

There were no significant changes in the serum biochemical parameters, including total bilirubin, TP, Alb, AST, ALT, and ALP, among the treated groups and the controls ($P > 0.05$) (Table 2).

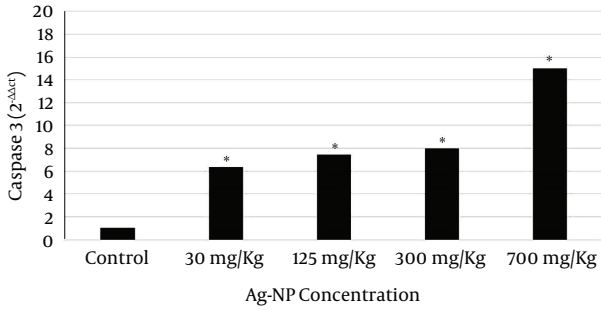
4.3. Expression of Caspase-3 mRNA

As presented in Figure 2, the administration of Ag-NPs upregulated caspase-3 mRNA in a dose-dependent manner. In the groups treated with 30, 125, 300, or 700 mg/kg of Ag-NPs, the caspase-3 expression increased 6.33-, 7.41-, 7.94-, and 15.00-fold, respectively, compared to the control group ($P < 0.05$). However, our analysis showed that caspase-3 expression was not significantly different between the Ag-NP-treated groups ($P > 0.05$). The DNA product was confirmed by DNA sequencing (data not shown).

5. Discussion

The common use of nanosilver leads to its release into the environment and consequently to increased human exposure (21). Its potential for toxicity is a controversial research area and there is limited information on the subject (22). In vitro studies support cell toxicity for Ag-NPs (19), which can induce oxidative stress in human hepatoma

Figure 2. Expression of Caspase-3 mRNA in the Liver Tissue of Rats Treated With Ag-NPs for 28 Days and the Untreated Controls



Ag-NPs upregulated caspase-3 mRNA in a dose-dependent manner. The levels of caspase-3 mRNA are expressed relative to β-actin and presented as $2^{-\Delta\Delta ct}$ (Δct = Ct target gene-Ct housekeeping gene, and $2^{-\Delta\Delta ct}$ indicates the fold change in gene expression relative to the control) (*$P < 0.05$).

cells (3), DNA damage in testicular cells (23), reduced cell viability in alveolar macrophages and lung epithelial cells (24), and apoptosis in HeLa cells (25). The cytotoxic effects of Ag-NPs have been reported in various cancer cell lines (17, 26).

In vivo studies have been used for different routes of exposure to Ag-NPs, including intravenous, oral, inhalation, and intraperitoneal administration (17). Since a large number of silver nanoproducts are currently available in the food and beverage category (27), we used the oral route in our experiment. Most orally administered nanoparticulate silver has been described to be deposited in the liver, the major organ of detoxification (28).

In this study, we conducted an in vivo assessment in rats to investigate the effects of Ag-NPs (290 nm) on blood biochemistry parameters and caspase-3 expression in the liver. To determine the doses of nanoparticles, we used the study done by Kim et al. (29). At the end of the treatment, our results showed that TP, total bilirubin, Alb, ALP, ALT, and AST were not affected. Under normal conditions, these enzymes remain in the liver cells, and following cell damage, they are released into the serum (30). These findings suggest that Ag-NPs at these concentrations and durations of exposure did not cause any significant dysfunction in rat liver cells. Consistent with our study, Kulthong reported that oral administration of 180-nm Ag-NPs at doses of 50, 100, 250, 500, and 1000 mg/kg/day for two weeks did not significantly alter the serum ALT or AST levels (27). Elevated ALP and plasma cholesterol, dilatation of the central vein and bile duct, and hyperplasia were found in rats after oral administration of 60-nm Ag-NPs at more than 300 mg/kg/day for 28 days (29). Park et al. in a 28-day oral toxicity study using 42-nm Ag-NPs with concentrations of 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg in mice, showed that the

Table 2. Blood Biochemical Parameters in Rats Following Oral Administration of Ag-NPs for 28 Days[a,b]

	TP, mg/dL	T Bil, mg/dL	Alb, mg/dL	AST, IU/I	ALT, IU/I	ALP, IU/I
Control	4.57 ± 0.20	0.34 ± 0.02	3.47 ± 0.05	124.75 ± 18.55	29.66 ± 1.52	625.25 ± 234.60
30 mg/kg	5.30 ± 0.14	0.31 ± 0.02	3.62 ± 0.05	146.50 ± 20.04	37.00 ± 6.16	516.50 ± 51.53
125 mg/kg	4.95 ± 0.34	0.29 ± 0.03	3.22 ± 0.17	134.00 ± 32.62	33.00 ± 11.54	599.75 ± 227.39
300 mg/kg	4.95 ± 0.61	0.34 ± 0.02	3.25 ± 0.36	155.75 ± 21.25	20.75 ± 3.86	543.00 ± 75.49
700 mg/kg	4.60 ± 0.35	0.34 ± 0.01	3.17 ± 0.37	197.00 ± 84.22	28.00 ± 16.47	704.50 ± 144.54

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T Bil, total bilirubin; TP, total protein.
[a] Changes in biochemical factors were not significant.
[b] Values are expressed as mean ± SD.

serum levels of ALP and AST were considerably increased (17). These discrepancies in toxicology studies are due to variations in the size of Ag-NPs that are used (31) and the duration of exposure (27). In the experiment carried out in mice with oral administration of 1 mg/kg of Ag-NPs for 14 days, it was observed that Ag-NPs at sizes of < 100 nm were spread throughout the brain, lung, liver, kidney, and testis, while large-sized Ag-NPs (323 nm) were not detected in those tissues (17).

We observed that administration of Ag-NPs induced upregulation of caspase-3. Caspase-3, as an effector caspase, deals with the intrinsic pathway of apoptosis (18). Eckle et al. have shown that expression of caspase-3 represents a reliable marker of apoptosis in the rat liver (13).

According to the findings, this difference between the biochemistry parameters and expression of caspase-3 may be because activation of caspases happens only transiently in the early apoptotic stages (27), without yet affecting liver function.

This study revealed that oral administration of Ag-NPs of ~290 nm in diameter for 28 days had no effect on rat liver function, but likely led to early apoptotic stages. More detailed studies using smaller-sized Ag-NPs and longer administration periods are necessary to evaluate the in vivo effects of each dose.

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Footnotes

Authors' Contribution: Study concept and design: Mahsa Pourhamzeh, Zahra Gholami Mahmoudian, Zohreh Alizadeh, Massoud Saidijam, and Mohamad Javad Asari; analysis and interpretation of data: Zohreh Alizadeh and Massoud Saidijam; drafting of the manuscript: Mahsa Pourhamzeh and Zohreh Alizadeh; statistical analysis: Zohreh Alizadeh.

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