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Biochemical Changes Induced by the Toxicity of Variable Sizes of Silver Nanoparticles

Qais Jarrar[1,2*], Abdelkader Battah[2], Fatima Obeidat[2] and Khairat Battah[2]

[1]Department of Biomedical Science, Faculty of Medicine and Health Sciences, University of Putra, Malaysia.
[2]Department of Pathology, Microbiology and Forensic Medicine, Faculty of Medicine, University of Jordan, Jordan.

Authors' contributions

This project was carried out in collaboration between all authors. Author QJ anchored the literature for the study, performed all experimental work of the project and wrote the first draft of the manuscript. Author AB managed all aspects of the project while authors FO and KB managed the statistical analysis and provided the background for the interpretation of the results. All authors read and approved the manuscript.

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ABSTRACT

Background: Silver nanoparticles (SNPs) rapid involvement in industry and nanomedicine increased human exposure to variable forms of these particles, with possible potential risk on human health.
Aims: The aim of this study is to investigate the biochemical changes induced by variable sizes of SNPs toxicity.
Place and Duration of Study: Faculty of Medicine, The University of Jordan and the

**Corresponding author: Email: jarrarq@yahoo.com;*

College of Applied Medical Sciences at Aljouf University, Saudi Arabia, between January 2013 and January 2014.
Study Design: Forty-two male mice were subjected to a daily single dose (1mg/kg body weight) of SNPs using five different sizes (10 nm, 20 nm, 40 nm, 60 nm and 100 nm) for 35 days.
Methodology: Biochemical changes of the following eleven biochemical tests were determined: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, total bilirubin, creatinine, total protein, albumin, urea, uric acid and total cholesterol.
Results: Silver nanoparticles significantly elevated aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, total bilirubin and creatinine, with no significant change in total protein level while albumin and total cholesterol levels were lowered.
Conclusion: The findings indicate that exposure to SNPs produced significant biochemical changes that might affect the functions of the vital organs. Moreover, these alterations were size-dependent with smaller particles (10 nm and 20 nm) induced more alterations than the larger ones.

Keywords: Silver nanoparticles; AST; ALT; ALP; nanotoxicity.

1. INTRODUCTION

Exposure to silver nanoparticles (SNPs) is becoming more reality in our lives owing to their unique antibacterial, antiviral and antifungal activities which allow wide use of these particles in medicine and industry and increase human exposure to variable forms of these particles, with possible high potential risk on human health [1-3]. In addition, these fine particles are invested in medical cloth, footwear, sunscreen, athletic shirts, wound dressing, burns, skin ulcers, medical catheter, masks, cosmetics, bone cement, tooth paste and deodorants [4,5]. Also, SNPs are used in delivering pneumonia medications and proved to induce antiviral activity against HIV-1 at noncytotoxic concentration by inhibiting binding of the virus to the host cells [6,7].

Silver nanoparticles accumulate mainly in the liver and considered to be toxic to other organs including lungs, spleen, endometrium and brain [3,8,9]. The smaller nature of these particles together with their high surface area to volume ratio enables them to enter and traverse the tissue components as biological molecules do [10]. Smaller nanoparticles have easier clearance from the site of injection, maximum interaction with tissue components and lower likehood aggregation than the larger ones. Also, small size nanoparticles (NPs) have longer circulating residue and slower passage to the interstitial spaces from blood stream than the large size ones [11,12]. Toxicological studies provided evidences between SNPs size and their toxicity and concluded that smaller SNPs have more oxidative dissolution and release more silver ions that bind or interact with the tissue and cell component leading to depletion of dissolved oxygen and protons [13-16].

In vitro toxicological studies showed that SNPs produce reactive oxidative species that could damage plasma membrane and cell organelles [17]. On the other hand, Asharani et al. [13] concluded that SNPs could induce DNA damage, increased chromosomal aberrations, G2/M cell cycle arrest and reduction in the metabolic activity and ATP production. In addition, other studies have demonstrated size-dependent genotoxic and cytotoxic consequences of SNPs [18,19]. Some studies reported that SNPs oral administration to mice for 28 days increased significantly levels of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine

transaminase (ALT) with no changes in the total protein, albumin, creatinine and blood urea nitrogen [8,20]. On the other hand, no change was found on ALP, AST and ALT plasma levels of Sprague-Dawley rats exposed orally to various SNPs (0-100 mg/kg) for two weeks [21].

Justified concerns are being raised over the unwanted or unexpected potentially adverse effects of SNPs applications on human health with a need to explore their potential risks before their practical application. Limited studies have been carried out on the biochemical alterations induced by SNPs exposure. With this objective, the present toxicological study was designed to find out biochemical alterations induced by variable sizes of SNPs.

2. MATERIALS AND METHODS

2.1 Experimental Animals Treatment

A total of 42 adult healthy male mice (BALB/C, age 9-11 weeks) were used throughout the present study. All mice were randomly divided into 6 groups (assigned to control group and five test groups) of 7 animals each and housed at room temperature (24±1℃) and 12 hr light-12 hr dark cycle and kept in Faculty of Medicine animal facility of The University of Jordan. The mice were provided with commercial pellets and tap water *ad. libitum*. Following a period of stabilization (7 days), members of the treated groups were exposed to intraperitoneal (i.p.) injection with a daily single dose (1mg/kg body weight) of SNPs (10, 20, 40, 60 and 100 nm; Sigma-Aldrich, USA) for 5 days per week for 7 weeks.

2.2 Biochemical Analysis

After 35 days of treatment, blood samples were drawn from the orbital sinus and cardiac puncture of each mouse from all groups and collected to heparinized vacutainers tubes. Plasma samples were separated by centrifugation for 6 minutes at 3200 round/minute and subjected for the following biochemical tests: AST, ALT, ALP, total proteins, albumin, creatinine, urea, uric acid, total bilirubin, triglyceride and total cholesterol. These tests were carried out by using Auto-chemistry Analyzer-BS 300- 2772121272 belongs to College of Applied Medical Sciences at Aljouf University, Saudi Arabia. Mindray Multifera calibrator (lot SM 311) was used to calibrate the Auto-Chemistry Analyzer for the tested parameters.

2.3 Statistical Analysis

The amount of change on each biochemical parameters of mice subjected to different sizes of SNPs for 35 days ± Standard deviation (S.D) for each group (n=7) after treatment with different sizes of SNPs was calculated. The significant differences between SNPs treated groups and the control one was tested by student *t*-test where *P* values < 0.05 were considered statistically significant.

3. RESULTS

3.1 Effect on Liver Function Enzymes

The biochemical changes as seen in Table 1, showed significant (p value < 0.001) elevation of AST in the blood of all SNPs treated mice except those received 40 nm (p value < 0.05).

On the other hand, ALT levels were raised only in the blood of mice exposed to 10, 20 and 40 nm but not in the those received 60 and 100 nm particles.

3.2 Effect on Alkaline Phosphatase

Table 1 shows that alkaline phosphatase blood level was significantly (p value < 0.05-0.001) increased in groups of mice exposed to 20, 40, 60 and 100 nm SNPs while no significant elevation was noticed in those treated with 10 nm particles.

3.3 Effect on Total Protein and Albumin

Total protein levels were lowered slightly in all mice exposed to SNPs with more decrease in the level of albumin in the blood of mice treated with 10, 20, 40 and 60 nm SNPs but not those received 100 nm particles where high level of albumin was observed. However, the decrease in albumin levels failed to reach the statistical deference in all treated mice.

3.4 Effect on Triglyceride

Triglycerides blood levels showed significant (p value <0.05) elevation in mice subjected to 20, 40, 60 and 100 nm SNPs while those exposed to 10 nm particles exhibited less alteration in triglycerides concentration in comparison to control mice.

3.5 Effect on Total Cholesterol

As representative in Table 1, total cholesterol levels were decreased significantly (p value <0.01) in mice exposed to 10, 20 and 40 nm while those received 60 and 100 nm where less affected.

3.6 Effect on Total Bilirubin

Blood total bilirubin levels was higher (p-value<0.01-0.001) in mice exposed to 10 and 20 nm and 40 nm SNPs in comparison with the control ones while less elevation was seen in the blood of mice received 60 nm with almost no alterations in those treated with 100 nm particles.

3.7 Effect on Creatinine

Creatinine blood levels were raised significantly (p-value<0.01-0.001) in all mice treated with SNPs in comparison with control ones.

3.8 Effect on Urea and Uric Acid

Urea blood level was raised insignificantly in mice received 20 nm SNPs but not in the other groups while non significant elevation of uric acid level was seen only in the blood of mice received 10 nm particles.

Table 1. Biochemical analysis results after exposure to different sizes of SNPs for 35 days

Parameter	AST (U/L)	ALT (U/L)	ALP (mg/dl)	Total bilirubin (mg/dl)	Total proteins (g/l)	Albumin (mg/dl)	Creatinine (mg/dl)	Urea mmol/l	Uric acid (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Control n=7	211.97±41.03	49.83±5.35	58.43±17.94	20.14±2.53	54.86±5.30	166.29±23.11	1.41±0.27	9.29±0.76	6.86±2.19	201.71±29.12	125.57±13.99
10 nm n=6	348.84±71.01***	117.17±25.5***	69.17±29.42	30.83±4.26***	52.67±7.84	149.17±13.23	2.02±0.39**	7.83±2.23	10.33±2.73	210.83±42.83	98.67±12.85**
20 nm n=6	435.31 ±163.13***	135.67±33.43***	119.83±22.76***	30.00±7.25**	48.50±2.17	139.33±23.19	2.817±0.37***	14.17±7.41	5.17±1.17	264±19.72*	100.00±6.78**
40 nm n=4	237.19±96.57*	126.75±12.61***	99.67±39.33*	40±11.69***	51.00±3.17	146.75±40.53	2.88±0.56***	6.50±0.58	7.00±1.01	253.7 5±24.67*	97.75±18.57**
60nm n=5	333.38 ±61.67***	53.60±11.88697	137.80±32.30***	26.75±2.97**	47.40±4.98	133.40±30.83	2.42±0.43***	9.40±1.82	5.20±0.84	243.6±22.71*	113.8±5.07
100 nm n=5	448.36 ±157.05***	59.80±22.82	125.40±22.37***	17.00±8.22	51.60±2.61	198.20±40.34	2.86±0.36***	9.60±1.82	8.20±0.84	253.40±56.96*	114.4±15.47

T-student test was used as a statistical tool for comparison of different groups with the control one.
"" Indicates p value<0.05, "**" p value <0.01 and "***" for p value < 0.001.*

4. DISCUSSION

The liver is an important organ performs more than 500 vital metabolic functions and is the site of detoxification and homeostasis [22]. This organ plays a central role in the metabolism and excretion of drugs and xenobiotics making it highly susceptible to their adverse and toxic effects. The concentration of the liver enzymes ALT, AST and others in the blood is used as an indicator for liver functions and in detecting hepatocytes injury [22]. While ALT is primarily found in the hepatocytes and is released during liver damage and hepatocellular necrosis, AST is found in several organs beside the liver and is a biomarker of hepatic tissues together with red blood cells, cardiac and skeletal muscle and is therefore not specific to the liver [22].

The finding of the present work showed significant elevation of AST in the blood of all SNPs treated mice while ALT levels were raised only in the blood of mice exposed to 10, 20 and 40 nm with nonsignificant changes in those received 60 and 100 nm particles. These findings might indicate that liver was affected by SNPs exposure while the AST elevation could also indicate other vital organs were affected beside the liver by the toxicity of these particles. The current result is online with that of Monfored and Soltani [23] who reported that SNPs induced significant increase in AST and ALT levels in the blood of rainbow trout (*Oncorhynchus mykiss*).

The results of the present work showed significant elevation of ALP due to SNPs toxicity. Alkaline phosphatase rises in hepatocellular damage, bile duct obstruction, intrahepatic cholestasis, hepatobiliary diseases and associated with drug and chemicals induced hepatotoxicity [22,24]. The elevation of ALP due to SNPs exposure with cholestasis in 20 nm SNPs intoxicated mice might indicate the sensitivity of the bile duct lining by these particles. Significant change was observed in ALP activity by 56 nm SNPs over a period of 90 days, while oral administration of one mg/kg of 42 nm SNPs to mice for 28 days increased significantly levels of ALP, AST and ALT [8,20]. Some studies reported that different sizes of NPs showed variable kinetics where those particles larger than 10 nm are cleared via biliary clearance while smaller ones are cleared via renal route [25]. The epithelial lining of the bile duct is the source of ALP that flows of through the bile to maintain the proper level of this enzyme in the blood. These together may explain the absence of effect of 10 nm particles on the activity of ALP in comparison with the larger ones. On the other hand, no significant change was found on the plasma levels of ALP, AST and ALT due to variable sizes of SNPs (0-100 mg/kg) administered orally for two weeks to Sprague-Dawley rats (21).

Albumin is a protein made specifically by the liver and is one of its markers for biosynthetic activity and dysfunction. Blood total proteins concentration is often reduced slightly during hepatocellular damage with more decline is seen in the concentration of albumin in comparison with the reduction in other types of proteins produced by the liver such as globulins [22,26]. The present study showed that total proteins levels were lowered slightly while more decrease was seen in albumin blood level of mice exposed to SNPs indicating an impact in the liver function. These findings are inconsistent with those of Monfored and Soltani [23] who reported that SNPs induced significant decrease in the total proteins level in the blood of rainbow trout (*Oncorhynchus mykiss*) but in agreement with the results of other investigators [8,20].

The findings of the present work showed a decrease in total plasma cholesterol in mice treated with 10, 20 and 40 nm while those received 60 and 100 nm SNPs were less affected. Moreover, exposure to SNPs increased significantly triglycerides blood level.

Decreased levels of cholesterol are seen in hepatocellular necrosis and indicate hepatocytes injury while triglycerides are part of the major forms of lipids found in plasma and are elevated in some hepatic and renal diseases [22,27].

Bilirubin is a breakdown product of hemoglobin in red blood cells and cleared by the liver where it is taken up into hepatocytes, conjugated and secreted into the bile, which is excreted into the intestine [22]. Hepatocytes damage makes these cells unable to excrete bilirubin in the normal way, thus causing bilirubin building up in the blood and extracellular fluids. The results of the present work showed an elevation in bilirubin blood level due to 10, 20, 40 and 60 nm SNPs exposure but not to that of 100 nm. This might indicate insufficient ability of the liver to remove the wastes including bilirubin and can signal a number of problems among of which deficiencies in bilirubin metabolism and obstruction of the bile ducts.

The elevation of creatinine together with urea and uric acid due to SNPs treatment as seen in the results of the present study might indicate disorder in the kidney function of mice exposed to these ultrafine particles. The significant increase of creatinine due to SNPs exposure might indicate a deficiency in the filtering function of the kidney.

The biochemical changes as seen in the present work reveal that smaller SNPs are more toxic than the larger ones. Smaller NPs have much greater surface area to volume and have longer circulating residue than the larger ones with more potential particle size toxicity that could be related to dissolution rate and bioavailability. Several toxicological reports indicated that SNPs oxidative dissolution is size related where smaller particle release more silver cations due to their greater surface area to mass ratio [16]. In addition, oxidative dissolution of smaller particles produces more hydrogen peroxide and depletes dissolved oxygen and protons leading to cellular oxidative stress [14].

Some studies indicated that the liver is the potential target and the major accumulation site of SNPs [8,9]. This is in line with the indication of the present study that the liver is more affected by SNPs toxicity than the kidney, which might be related to the liver as being the site of detoxification with high blood flow exposure that make the hepatic tissues more susceptible to toxic effects than the renal ones. In addition, the smaller particles have long circulation and more bioavailability. These together may indicate that the accumulation of these particles and their impact on the liver are size dependent.

5. CONCLUSION

One might conclude from the significant results of the present study that exposure to SNPs produces biochemical changes might affect the vital organs with smaller SNPs are more toxic than the larger ones of the same composition where 10 and 20 nm are more toxic than the larger ones with probable variable toxicokinetics. The present findings might recommend a need for more studies on the toxicokinetic of SNPs concerning shape and structure of these particles in relation to body pathophysiology.

CONSENT

Not applicable.

ETHICAL APPROVAL

The experimental protocol was approved by the graduate studies committees at the Faculty of Medicine and the Faculty of Graduate Studies, The University of Jordan, while the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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