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Effects of silver nanoparticles administration on the liver of rainbow trout (*Oncorhynchus mykiss*): histological and biochemical studies

Ali Louei Monfared[1] and Salman Soltani[2]

[1]*Department of Anatomy, Faculty of Para-Veterinary Medicine, University of Ilam, Ilam, Iran*
[2]*Faculty of Veterinary Medicine, University of Shahid Chamran Ahwaz, Ahwaz, Iran*

ABSTRACT

Despite increasing application of silver nanoparticles (NPs) in industry and consumer products, there is still little known about their potential toxicity, particularly to organisms in aquatic environments. Regarding fast development of the nanotechnology and its diverse applications, is very important having enough data on the probably its side effects on the aquatic body organs. Therefore, present investigations were taken on the effects of nanosilver administration on the liver's histology and biochemistry in rainbow trout. For this study, the silver nanoparticles were synthesized in a one-step reduction process in an aqueous solution. 60 O. mykiss were obtained from a local commercial hatchery. Fish were divided randomly into four groups. Experimental fish were exposed to concentration of 3, 300 and 1000 mg/L of nanosilver solution for eight weeks. At the end of experiments, blood samples were collected and biochemical analyses of sera were performed. Tissue samples were also taken and histological alterations of the liver were examined. In the hepatic structure of the treated fish with nanosilver; local congestion in the liver parenchyma, decreasing in the size and diameter of the hepatocytes and massive destination in the hepatic sinusoids sizes were seen In addition, the serum levels of total protein was decreased significantly ($P<0.05$) by elevation in the nanosilver concentration. On the other hands, AST and ALT levels in sera showed a significant increase in the nanosilver treated fish ($P<0.05$) when compared to control group. It is concluded that nanosilver treating could induces hepatic side effects and its administrating in the aquatic systems should be reconsidered.

Key words: Liver, Histology, Biochemistry, Nanosilver, Rainbow trout.

INTRODUCTION

Silver (Ag) nanoparticles are used as antimicrobial adjuvant in various products such as clothes and medical devices where the release of nano-Ag could contaminate the environment and harm wildlife. The purpose of this study was to examine the sublethal effects of nano-Ag and dissolved Ag on *Oncorhynchus mykiss* rainbow trout [1]. Current growth in the nanotechnology industry and the increasing numbers of products making use of the unusual properties of engineered nanoparticles (NPs) is becoming extremely important in the global economy. Increased production and use of nanoproducts will inevitably lead to increased levels of discharge of nanomaterials into the environment through their intentional and accidental releases or via weathering of products that contain them. The aquatic environment is particularly vulnerable as it is likely to act as a sink for many of these particles, as it does for many chemical discharges. The fate of NPs in the aquatic environment, their interactions with biotic and abiotic components, and their potential to cause harm are all still poorly understood, and these uncertainties are driving concerns on the risks they may pose to human and environmental health. [2]. Due to the wide application of nanomaterials in industry, agriculture, business, medicine and public health; nanotechnology has gained a great deal of public interest [3].

Silver found in the body of mammals (including humans) has no known biological purpose and is suspected of being a contaminant [4]. Silver, as ionic Ag+, is one of the most toxic metals known to aquatic organisms in laboratory testing, although large industrial losses to the aquatic environment are probably infrequent because of its economic value as a recoverable resource [5]. Silver, however, is of concern in various aquatic ecosystems because of the severity of silver contamination in the water column, sediments, and biota.

Long-term industrial or medical exposure to silver and its compounds may increase blood concentrations of silver to levels which can have toxic effects, such as induction of sarcomas, anemia, and enlargement of the heart [6]. It has been reported the toxicity of silver nanoparticles in zebrafish models. Their results suggest that silver nanoparticles induce a dose-dependent toxicity in embryos, which hinders normal development [7]. In fish and amphibian toxicity tests with 22 metals and metalloids, silver was the most toxic tested element as judged by acute LC50 values [8].

It is very important having enough data on the probably its side effects on the aquatic body organs, regarding fast development of the nanotechnology and its diverse applications. Therefore, these studies investigate the effects of nanosilver administration on histology and biochemistry of the liver in rainbow trout.

MATERIALS AND METHODS

The silver nanoparticles (NPs) were synthesized in a one-step reduction process in an aqueous solution. In a typical preparation, a 400-μL aliquot of a 0.1-M AgNO3 aqueous solution was added into 100 mL of an aqueous solution containing 0.10 wt. % of the soluble starch and vigorously stirred for 1 h. The pH of the resulting solution was adjusted to 8.0 by adding 0.1 M NaOH solution. Under this experimental condition, the initial reaction mixture was colorless, and the growth of the AgNPs was monitored at different intervals using UV-vis absorption spectroscopy. After about 1 h, the solution turned light yellow, which indicated the initial formation of the AgNPs. The mixture was maintained at 50°C for 24 h, and the color of the reaction solution became yellow.

60 *O. mykiss* (weight=130±6.9 g; Total length=11.52±2.5 cm) were obtained from a local commercial hatchery (Ilam, Iran). Fish were transported in well aerated condition to the laboratory of freshwater fish research station in Ilam University. They were kept for a week in 200 L aquariums to acclimatize to the laboratory environment. During this period, they were fed five times a day (at 08.00, 11.00, 13.00, 15.00 and 18.00 h) by commercial pellets (33% protein). After acclimatization, they were divided randomly into four groups. Control group was kept in dechlorinated tap water without any add-on material, while experimental groups were exposed to concentration of 3, 300 and 1000 mg/L of nanosilver solution, respectively for eight weeks. Each treatment was done in two replicates. The water was renewed every 12 h as 30% of nanosilver is lost by volatilization. At the end of the experiment, the fish were weighted, sacrificed and blood samples were collected by cardiac puncture. In order to histological study the liver was removed from body and weighed. Then hepatosomatic index (HSI) was according to following standard formula:

$$HSI = \text{liver weight (g)/body weight (g)} \times 100. \text{ [9]}$$

For histological evaluation, hepatic specimens were taken and washed with saline. The samples fixed in buffered formalin (10%), processed for sectioning (5-6 μm) and stained with H&E. The sections were examined with an Olympus BX60 microscope and visualized through the Color-View Camera (Olympus, Tokyo, Japan).

For biochemical assaying, the blood samples were centrifuged (5 min at 5000g, Hettich D7200) immediately at room temperature and plasma were separated and stored at −20°C until analysis. Total protein was assayed by biuret method, separately (ZiestChemie, Iran). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by kinetic enzyme assays (ZiestChemie, Iran).

All data are presented as mean ± SD. Data were analyzed by one-way ANOVA followed by Duncan's multiple comparisons test. Multiple comparisons tests were only applied when a significant difference was determined in the ANOVA analysis, $P < 0.05$. The SPSS 13.0 (Chicago, USA) was used for analyzing the results.

RESULTS

In the control group, the liver exhibited a normal architecture and pathological abnormalities were not seen. Fish exposed to different concentration of nanosilver for 8 weeks showed significant reduction ($P<0.05$) in the liver weight and HIS index compared to control group (Table1).

The liver tissue from nanosilver exposed fish is illustrated in Figures 1 to 6. In the liver tissue of the exposed fish to nanosilver; local congestion in the hepatic parenchyma (Figure 1), decreasing in the size and diameter of the hepatocytes (Figure 2, Table2) and massive destination in the hepatic sinusoids sizes (Figure 3) were seen.

In addition, the serum levels of total protein was decreased significantly ($P<0.05$) by elevation in the nanosilver concentration. On the other hands, AST and ALT levels in sera showed a significant increase in the nanosilver treated fish ($P<0.05$) when compared to control group.

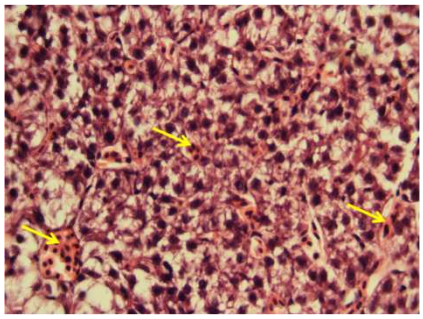

Fig. 1) Transverse sections through the liver of the 3 mg/L of silver nanosilver treated fish. The figure shows the local congestion in the hepatic parenchyma (arrow). (Haematoxylin and Eosine stain) (× 400).

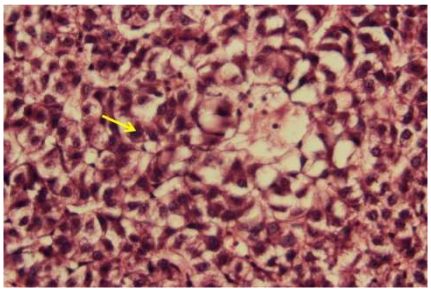

Fig. 2) Transverse sections through the liver of the 300 mg/L silver nanosilver treated fish. The figure shows decreasing in the size and diameter of the hepatocytes (arrow). (Haematoxylin and Eosine stain) (× 400).

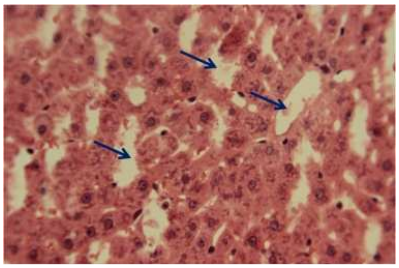

Fig. 3) Transverse sections through the liver of the 1000 mg/L silver nanosilver treated fish. The figure shows massive destination in the hepatic sinusoids sizes (arrow). (Haematoxylin and Eosine stain) (× 400).

TABLEI: Some morphometric measurments (means ± SE) in control and treated rainbow trout (*O. mykiss*) with different concentrations of nanosilver particles

Parameters/Groups	Control	3 mg/L of nanosilver particles	300 mg/L of nanosilver particles	1000 mg/L of nanosilver particles
Liver weight(g)	6.13 ± 0.34^{a}	2.36 ± 0.46^{b}	4.53 ± 0.42^{c}	3.97 ± 0.15^{c}
Hepatosomatic index (HSI)	2.35 ± 0.78^{a}	1.05 ± 0.97^{b}	0.99 ± 0.275^{c}	1.93 ± 0.64^{c}

** Means in the same row with different letters are significantly different (ANOVA, P<0.05).*

TABLEII: Some histometric indices (means ± SE) in control and treated rainbow trout (*O. mykiss*) with different concentrations of nanosilver particles

Parameters/Groups	Control	3 mg/L of nanosilver particles	300 mg/L of nanosilver particles	1000 mg/L of nanosilver particles
Diameters of hepatocytes (µm)	8.9±0.05 [a]	4.2 ±0.05 [b]	3.3 ±0.07 [c]	3.1 ±0.02 [a]c*
Hepatocyte nuclear diameter (µm)	4.96±0.07 [a]	2.1 ±0.03 [b]	1.3 ±0.02 [b]	1.7 ±0.8 [b]
Sinusoids size (µm)	19.3±0.04[a]	27.7 ±0.23 [b]	38.3 ±0.76 [b]	37.7 ±1.05 [b]

**Means in the same row with different letters are significantly different (ANOVA, P<0.05).*

TABLEIII: Some serum biochemical parameters (means ± SE) in control and treated rainbow trout (*O. mykiss*) with different concentrations of nanosilver particles

Parameters/Groups	Control	3 mg/L of phenol	300 mg/L of phenol	1000 mg/L of phenol
Total protein (g/dl)	4.66 ± 0.47 [a]	2.58 ± 0.21 [b]	2.14 ± 0.34 [b]	3.03 ± 0.67 [c]
AST (U/L)	25.6 ± 4.6 [a]	39.5 ± 5.6 [b]	49.5 ± 6.3 [b]	53.6 ± 7.8 [b]
ALT(U/L)	9.9 ± 1.7 [a]	14.6 ± 7.1 [b]	17.8 ± 2.08 [b]	17.2 ± 7.8 [b]

**Means in the same row with different letters are significantly different (ANOVA, P<0.05).*

DISCUSSION

Despite increasing application of silver nanoparticles in industry and consumer products, there is still little known about their potential toxicity, particularly to organisms in aquatic environments. Similarly, there is little in the literature regarding the uptake and biodistribution of silver NPs into internal organs or the potential toxicity of silver NP in intact free-swimming fish [10-11].

Very few studies to date have investigated the effects of exposure to silver NPs in fish, and these have been focused on zebrafish embryos where mortality, notochord, and heart abnormalities have all been described [6, 10, 11]. In the present study, significant alterations in the liver weight, HIS index and also the liver structures as well as blood biochemistry were observed following the exposure to nanosilver administration.

There are several studies which have shown similar histological changes in the hepatic tissue of fish, resulting from exposure to different toxic chemicals include nanoparticles [10-15].

The findings of this study showed local congestion in the hepatic parenchyma, decreasing in the size and diameter of the hepatocytes and massive destination in the hepatic sinusoids sizes in the experimental fish when compared with control animals. These alterations could be driven from the fish excessive activity to metabolism and also get rid of the toxicant from its body during the process of detoxification [12]. Biochemical results of the present experiments showed a significant decrease in the serum levels of total protein and an increase in the values of AST and ALT in the nanosilver exposed fish. The decreased levels of total protein in fish exposed to nanosilver suggest that the protein might be used as an alternative source of energy, due to high energy demand that induced by nanosilver intoxication as it shown in *Brycon cephalus* [16].

In this study exposure to nanosilver resulted in a significant increase in the activities of plasma AST and ALT compared to control group. This is in accordance with finding of Nemcsók and Benedeczky in common carp [17]. AST and ALT are plasma non-functional enzymes which are normally localized within the cells of many organs including liver. They are also considered as an important indicator in assessing kidney and liver status [18- 19] and tissue injury or organ dysfunction [19]. The increase in AST, and ALT after nanosilver exposure was in accordance with histopathological findings. Therefore, the increase of these enzymes is an indicator of liver damage and thus otherwise alterations in the hepatic function.

The known toxicity of silver ions has led to the proposal in a number of studies that release of silver ions (Ag^+) from silver NPs could be in part responsible for toxic responses seen in exposures to silver NPs [20-21]. Although there are not a obsolete mechanism of action for toxic histological changes after nanosilver treatment; but previous study hypotheses that silver nanoparticles can disrupts the Na^+ ,Cl^- and H^+ exchanges at the gills, which initiates a complex chain of events culminating in cardiovascular collapse [20]. Other researchers proposed that exposure to nano-Ag involved genes in inflammation and dissolved Ag involved oxidative stress and protein stability [1].

This study showed that nanosilver induces hepatic damage and alterations in the serum biochemical parameters of *O. mykiss* juveniles in different concentrations. So, its administrating in the aquatic systems should be reconsidered.

REFERENCES

[1] Gagné F et al, *Chemosphere*, **2012**; 89(5):615-22.
[2] Tessa M et al, *Toxicol Sci*, **2010;** 115(2): 521-534.
[3] Ju-Nam Y, Lead JR., *Sci Total Environment*, **2008**; 400(1-3): 396–414.
[4] Smith IC and Carson BL., *An Arbor Science Publishers, Ann Arbor, Mich*. **1977**; 469.
[5] Nebeker AV, McAuliffe CK, Mshar R, Stevens DG., *Environment Toxicolo Chem*, **1983**; 2: 95-104.
[6] Aoki T, Nihonyanagi K, Oba T., *Experientia*, **1993**; 49: 792-794.
[7] Asharani PV, Wu YL, Gong Z, Valiyaveettil S., *Nanotechnol*, **2008**; 19(25): 255102.
[8] Birge WJ and Zuiderveen JA., *Abstracts of 3rd international conference*, **1995**; 6-9.
[9] White A and Fletcher TC. *J Fish Biol*, **1985;** 26: 755–764.
[10] Lee KJ et al, *ACS Nano*, **2007;** 1:133–143.
[11] Yeo MK and Kang M. *Bull Korean Chem Soc*, **2008;** 29, 1179–1184.
[12] Patel JM, and Bahadur A., *American-Eurasian J Toxicol Sci*, **2011**; 4(1): 01-05.
[13] Figueiredo-Fernandes A et al, *Pesquisa Veterinária Brasileira*, **2007**; 27(3):103-109.
[14] Kumar Das B, and Mukherjee SC., *Veterin Arch*, **2000**; 70(4): 169-180.
[15] Mishra AK, and Mohanty B., *Sci Total Environ*, **2009;** 407: 5031–5038.
[16] Hori TSF et al, *Comp Biochem Physiol*, **2006**; 143(1): 67-72.
[17] Verma SR, Rani S, and Delela, RC. *Toxicol Letters*, **1981**; 8: 67-71.
[18] Wells, RM et al, *Comp Biochem Physiol*, **1986**; 64: 565- 571.
[19] Salah El-Deen M, and Rogeps WA., *J Aqua Anim Health*, **1993**; 5:280-286.
[20] Navarro E et al, *Environ Sci Technol*, **2008;** 42: 8959–8964.
[21] Choi JE et al, *Aqua Toxicol*, **2010**; 100(2): 151-159.