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ORIGINAL PAPER

Histopathological effects of waterborne silver nanoparticles and silver salt on the gills and liver of goldfish *Carassius auratus*

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Abstract This study aimed to compare histopathological effect of waterborne silver nanoparticles and silver salt ($AgNO_3$) on the gills and liver of *Carassius auratus*. Therefore, one hundred and five live specimens of goldfish were obtained and treated in five aquariums with 0, 0.01, 0.025, 0.05, and 0.1 ppm of $AgNO_3$ and 0, 0.1, 0.5, 1, and 5 ppm of Ag nanoparticles (mean particle size of 5 nm). Fish were sampled after 14 days of exposure. Results showed that the kinds of pathologies observed with Ag NPs were broadly of the same type as $AgNO_3$ including hyperplasia, edema and lifting of the gill epithelium, and lamellar fusion of the gills, and hemosiderosis, hemorrhage, hydropic swelling, and pyknotic nuclei of the liver. Overall, the data showed that although Ag nanoparticles and $AgNO_3$ pathology were similar, but Ag nanoparticles caused less injury than $AgNO_3$ in the gills and liver of goldfish. Therefore, it is more proper to use nanoform of Ag in industrials.

Keywords Silver nanoparticles · Gill pathology · Liver injury · Silver toxicity · Fish

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Introduction

Ecotoxicology is the study of the impact of environmental contaminant on ecosystems. Understanding the effect of toxicants on fish supports the larger ecotoxicological goal of comprehending the action of ecotoxicans on fish population (Bols et al. 2001). Ecotoxicans are a diverse group of substrate that have two general properties: they are depleted into the environment, and they have the potential to impact on ecosystem and animals at relatively low concentration (Connell et al. 1999). Heavy metals are the major chemical substrates that contaminate the ecosystems (Bols et al. 2001). Silver, as ionic Ag^+, is one of the most toxic metals known to aquatic organisms in laboratory testing and is of concern in various aquatic ecosystems because of the severity of silver contamination in the water column, sediments, and biota (Eisler 1996). Silver was used as halide in the manufacture of photographic imaging materials, jewelry, coins, indelible inks, eating utensils and used as silver salt in caustics, germicides, antiseptics, and astringents (Klaassen et al. 1986). In addition, relatively recently, a new form of Ag metal has been engineered comprising of Ag nanoparticles (Ag NPs) that can have novel and size-related physicochemical properties differing significantly from those from larger particles (Fabrega et al. 2011). Ag NPs are widely used in medicine, cosmetics, environmental remediation or electronic devices (Fabrega et al. 2011) and have distinctive physicochemical properties, including surface-enhanced Raman scattering, high electrical and thermal conductivity, chemical stability, catalytic activity, and nonlinear optical behavior (Capek 2004; Frattini et al. 2005).

Silver toxicity and the pathological effects of waterborne silver are well known in freshwater fish species (Janes and Playle 1995; Wood et al. 1996; Zhou et al. 2005). The gill is considered the main route for waterborne Ag uptake and destruction of tissue by reaching the branchial epithelial cells via the Na^+ channel and coupling to the proton ATPase in the apical membrane of the gills, and blocking the Na^+ K^+ ATPase which affects ionoregulation of Na^+ Cl^- ions across the gills (Bury and Wood 1999). Ag can also cause tissue damage and accumulate in the liver tissue affecting the ability of fish to cope with low oxygen levels and inducing oxidative stress (Bilberg et al. 2010a, b; Scown et al. 2010).

Many researchers studied the ecotoxicity of nanomaterials to aquatic ecosystems (Moore 2006; Handy et al. 2008; Klaine et al. 2008; Kahru and Savolainen 2010; Handy et al. 2011, Khabbazi et al. 2014a, b); however, the environmental impacts of Ag NPs are, as yet, unknown (Fabrega et al. 2011), and data on internal organ pathologies from Ag NPs in *Carassius auratus* (goldfish) are generally lacking. In addition, the relative hazard of pathology from nanoforms of Ag compared to traditional metal salts is unknown. Therefore, the aim of this study was to determine the effects of dissolved Ag and Ag NPs on the gills and liver of goldfish following waterborne exposure to these materials. Another goal of current study was to compare and contrast the effects of Ag metal with Ag NPs, to identify any nano-specific pathologies. This research was done in Aquaculture laboratory of Gorgan University of Agricultural Sciences and Natural Resources in the autumn of 2013.

Materials and methods

One hundred and five live specimens of goldfish were obtained. Samples weighted 56.33 ± 12.05 g. They were acclimatized randomly in 100-L aquariums for 1 week. Ag nanocolloid was prepared from Nonaka Company, Iran (Antimicrobial Product 2 brand, 4000 ppm nanosilver concentration, mean particle size of 5 nm). In addition, silver nitrate ($AgNO_3$) with 5000 ppm concentration was purchased from Merck Company (Merck Company, Frankfurter, Germany).

Five aquariums were treated with 0.01, 0.025, 0.05, 0.1 ppm of $AgNO_3$ with one control group (no Ag NPs) and 0, 0.1, 0.5, 1, 5 ppm of Ag NPs. No feeding occurred during the test to avoid confounding the exposure with potential food particles in the water. There were no significant differences between aquariums in water quality, and the following were constant: pH: 7.56 ± 0.45 (TS1); temperature: 19 ± 1 °C; hardness: 293 ± 2.35 ppm; and dissolved oxygen: 8.80 ± 0.06 mg L^{-1} (DO-5510). 80 % of water was changed every 12 h with re-dosing after each change, and the photoperiod was 12-h light and 12-h dark.

Fish were sampled from each of the triplicate tanks from each treatment after 14 days of exposure for histopathological studies. Histological examinations were performed as described in Bucke (1982). Fish were anaesthetized with 200 ppm eugenol concentration in 5-L tanks, and tissues were collected in the following order: the second gill arch was taken from gills, and the hind part of liver was taken by abdominal dissection. Collected tissues were fixed in formalin solution 1–10, and dehydration with ethanol 96 %, clearing with xylenol,

Table 1 *C. auratus* biometric results in $AgNO_3$ test

Factors	Control	0.1 ppm concentration	0.25 ppm concentration	0.5 ppm concentration	1 ppm concentration
Total length (cm)	16.33 ± 0.57[a]	15.00 ± 1.32[a]	14.67 ± 2.75[a]	13.83 ± 1.60[a]	14.83 ± 0.28[a]
Total weight (g)	61.66 ± 14.57[b]	54.33 ± 13.31[b]	44.67 ± 16.16[b]	45.33 ± 12.89[b]	41.00 ± 5.29[b]

Data were represented by mean ± standard deviation. Identical letters indicate no significant difference (ANOVA, $P < 0.05$)

Table 2 *C. auratus* biometric results in nanosilver test

Factors	Control	5 ppm concentration	10 ppm concentration	20 ppm concentration	30 ppm concentration
Total length (cm)	16.33 ± 0.57[a]	14.90 ± 1.93[a]	15.83 ± 0.76[a]	15.66 ± 0.28[a]	16.16 ± 0.76[a]
Total weight (g)	61.66 ± 14.57[b]	45.66 ± 14.15[b]	56 ± 14.73[b]	56.66 ± 10.01[b]	62.66 ± 3.51[b]

Data were represented by mean ± standard deviation. Identical letters indicate no significant difference (ANOVA, $P < 0.05$)

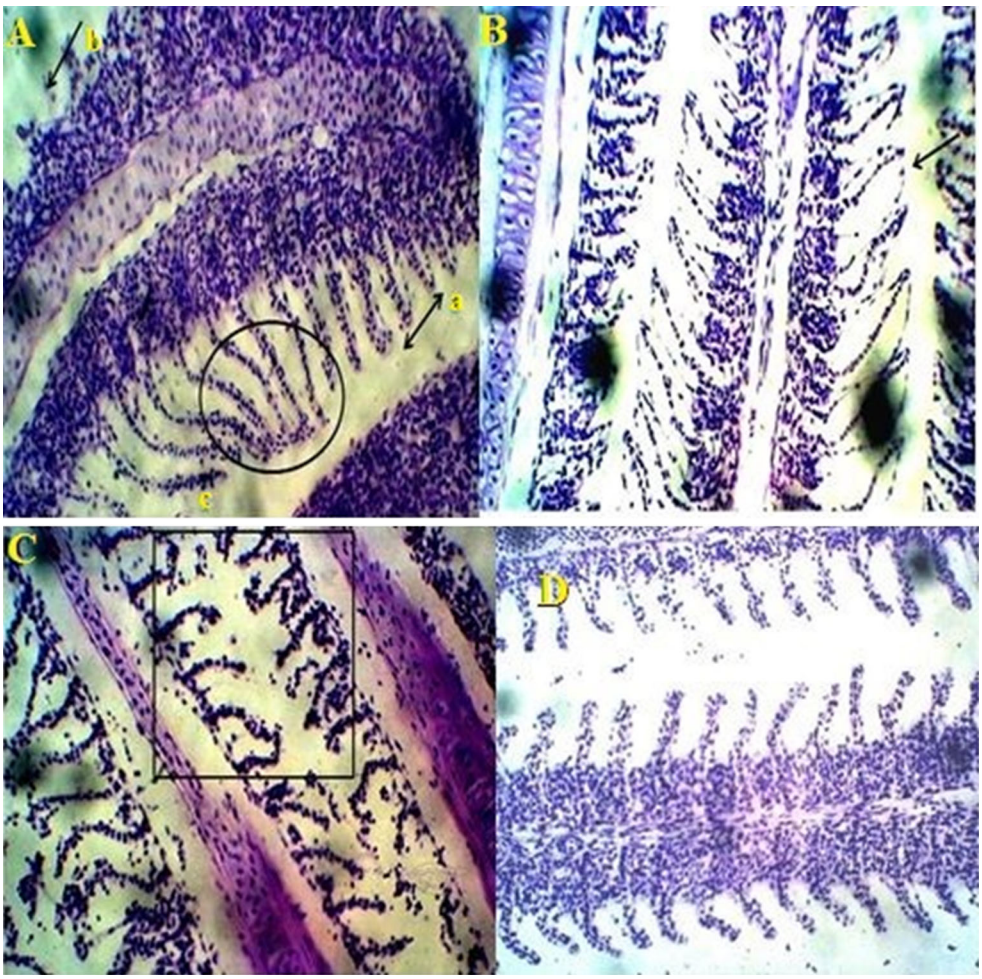

Fig. 1 Microphotographs of gill histopathological changes by $AgNO_3$ or Ag NPs in *C. auratus*. **A** secondary lamellae shrinking (*a*), secondary lamellae destruction (*b*), and lamellar fusion (c), **B** edema, **C** secondary lamellae Clubbing, **D** normal gill. All pictures are magnified ×40

Table 3 Index and scores for the *C. auratus* gills exposed to Ag NPs and $AgNO_3$ sublethal concentrations

Treatment	Concentration	Lesions				Hyperplasia
		Secondary lamellae shrinking	Secondary lamellae destruction	Lamellar fusion	Epithelial lifting	
$AgNO_3$	0	−	−	−	−	−
	0.01	−	+	+	+	−
	0.025	+	++	++	+++	++
	0.05	++	++	+++	++	++++
	0.1	++++	++++	++++	+++	++++
Ag NPs	0	−	−	−	−	−
	0.1	+	+	−	++	−
	0.5	−	+	++	++	++
	1	++	++	+	+++	++++
	5	+	++	++	+++	+++

(−) no observed lesions, (+) 1–3 observed lesions, (++) 3–5 observed lesions, (+++) 5–11 observed lesions, (++++) 11 and more observed lesions

impregnation with paraffin, embedding, sectioning, mounting, and staining with H&E were performed, respectively (Khabbazi et al. 2014b). All of these steps were conducted by tissue processor under defined program (Tissue processor, Triangle biomedical sciences USA). Histopathological changes induced by treatments in the tissues were photographed using Nikon photomicroscope. Quantitative histological measurements were taken in several tissues of gills and livers.

Data were analyzed by using SPSS 20 one-way analysis of variance (ANOVA). The least squares difference (LSD) post hoc test ($P < 0.05$) was used to identify

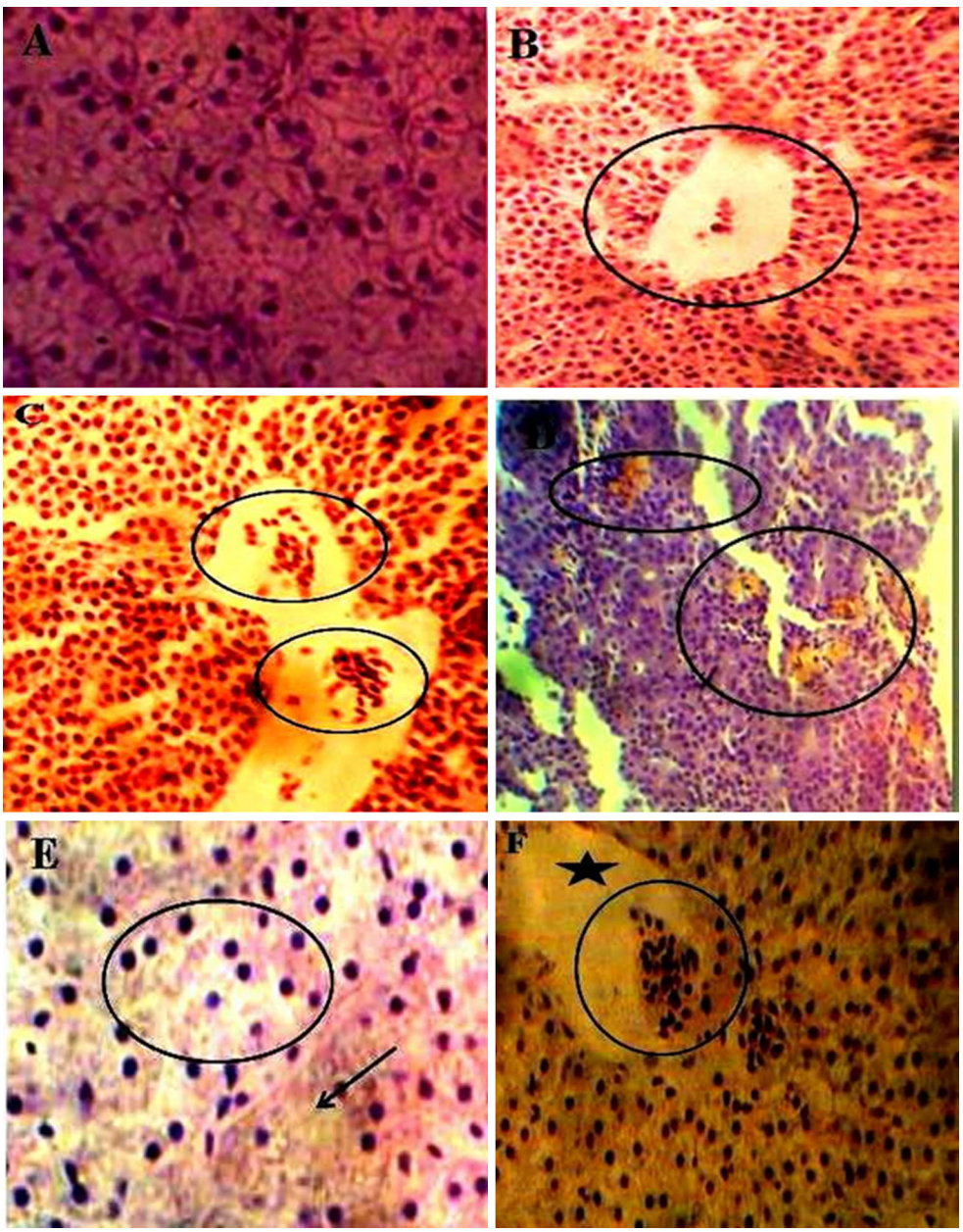

Fig. 2 Microphotographs of liver histopathological changes by $AgNO_3$ or Ag NPs in *C. auratus*. **A** Normal liver, **B** hydropic swelling, **C** hemorrhage, **D** hemosiderin, **E** necrosis, and **F** pyknotic nuclei and hydropic swelling. All pictures are magnified ×40

treatment effects at the end of the experiment (day 14). To illustrate the intensity of tissue damage by following the procedure, scoring system was applied as described analogously in Mitchell et al. (2012): (−) was used for no observed injuries, (+) for 1–3, (++) for 3–5, (+++) for 5–11, and (++++) for 11 and more observed injuries in samples.

Results and discussion

Tables 1 and 2 show mean total weight and length of fishes in treatments after experiment dubitation. No significant differences were observed in total length and weight of fish in all treatments with control group ($P > 0.05$).

Histological observation on the gill

Gill morphology of goldfish was normal in all the unexposed control groups. Exposure to waterborne Ag nitrate and Ag NPs caused various gill injuries after 14 days. Histological examination of gills showed areas of hyperplasia, edema and lifting of the gill epithelium, and lamellar fusion (Fig. 1). Exposure to Ag NP treatment produced similar gill pathologies to those observed with $AgNO_3$ (Fig. 1), but the extent of these injuries was less severe in the fish exposed to Ag NPs in comparison with $AgNO_3$ (Table 3).

Histological observation on the liver

No injuries were observed in control group. Histopathological examination showed different types of lesions such as hemosiderosis, hemorrhage, hydropic swelling, and pyknotic nuclei (Fig. 2). Results showed that Ag NPs caused similar injuries in comparison with $AgNO_3$, but the severities of injuries were less in Ag NP groups (Table 4).

This study showed the effects of dissolved Ag compared to Ag NPs on the liver and gills of goldfish. Overall, the results indicated that dissolved Ag and Ag NPs cause similar types of injuries in these two organs. After 14 days, these injuries were greater with $AgNO_3$ than Ag NPs. Results showed that the $AgNO_3$ concentration had greater injuries than Ag NPs in goldfish gills and livers especially in higher concentrations. Bioaccumulation is the major factor in metal toxicities. There is some evidence that waterborne exposure to metal NPs may result in particle accumulation in or on the epithelial cells (e.g., Ti NPs, Moger et al. 2008), but NP accumulation is less than metal salt and the metal salt is more bioavailable and/or bioreactive than the nanoform (e.g., $CuSO_4$, Al-Bairuty et al. 2013).

Gills are the first organ that encountered to toxicants. Mallatt (1985) stated that gill lesions can be divided into two groups, one that reflects the direct effect of toxicants and another corresponding to defense responses of fishes.

Table 4 Index and scores for the *C. auratus* gills exposed to Ag NPs and $AgNO_3$ sublethal concentrations

Treatment	Concentration	Lesions				Liver cell destruction
		Hydropic swelling	Hemorrhage	Hemosiderin	Necrosis	
$AgNO_3$	0	−	−	−	−	−
	0.01	+	+	−	+	+
	0.025	++	+	−	+	++
	0.05	++	++	+	+++	++++
	0.1	+++	+++	++	++++	++++
Ag NPs	0	−	−	−	−	−
	0.1	−	+	−	−	−
	0.5	−	+	−	+	+
	1	+	++++	−	++	+
	5	+	++	−	+++	++

(−) no observed lesions, (+) 1–3 observed lesions, (++) 3–5 observed lesions, (+++) 5–11 observed lesions, (++++) 11 and more observed lesions

Several studies have been demonstrated on laboratory experiments, to determine the toxicity of heavy metals, organochlorine pesticides, and petroleum hydrocarbon products to fish gill (Al-Attar 2007; Garcia-Santos et al. 2007; Patnaik et al. 2011; Santos et al. 2011; Hesni et al. 2011; Moitra et al. 2012; Ullah and Zorriehzahra 2015). Hyperplasia and lamellar fusion known to be induced by many gill tissue irritants; however, focal points of cellular hypertrophy and necrosis followed by epithelial rupture reflect the direct deleterious effects of heavy metals in fish gills (Mazon et al. 2002). In addition, similar gill injuries by titanium (TiO_2) in *Cyprinus carpio* (Hao et al. 2009) and Cu (Al-Bairuty et al. 2013) were reported. Further, the types of gill injuries by silver evaluated in this study also have been reported by other researchers (Griffitt et al. 2009; Bilberg et al. 2010a, b).

Ag accumulates in large amounts in the kidney and liver and acts as a very potent inducer of metallothionein synthesis (Coleman and Cearlry 1974; Wood et al. 1999). The rate of accumulation of heavy metals is positively correlated to their concentrations (Portman 1972). Results showed that level of injuries increased with Ag concentrations. This suggests that higher metal concentrations increase the rate of accumulation of Ag in liver. The types of injuries reported here (Fig. 2) for $AgNO_3$ and Ag NPs were coincided with other reports about histological changes in the hepatic tissue of fish (Lee et al. 2007; Yeo and Kang 2008; Mishra and Mohanty 2009). Some studies also revealed a link between hepatic lesions and the concentration of hemosiderin (Khan 1998 and 1999). Hemosiderin was not observed in Ag NP treatment and observed only in higher $AgNO_3$ concentrations (Table 4). This suggests that Ag NPs accumulate less than Ag salt in liver.

Conclusion

In conclusion, this study has demonstrated that Ag NPs and $AgNO_3$ cause similar lesions in gills and liver of goldfish, but the severity of Ag NPs is less than $AgNO_3$. It is well known that organ pathology is not necessary for determining the adverse effect of Ag, and accumulation in all of the internal organs should be considered. For this purpose, further studies should be focused on heavy metal accumulation in fish organs.

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