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Sublethal effect of nanosilver on the structure of gill of Caspian roach (*Rutilus rutilus caspicus*) fingerlings

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Abstract: Widespread use of nanosilver can be led the contamination of aquatic environment and impact on living organisms such as fishes. We investigated histopathological changes in the gills tissue of Caspian Roach fingerlings after two weeks exposure to sublethal concentrations of nanosilver. Following one and two weeks exposure, necrosis, shortening of secondary lamellae, edema, destruction of epithelial lamella, shortening of secondary lamellae, epithelial lifting and curling of secondary lamellae were observed in fingerlings' gill tissues. This observation showed that exposure to sub-lethal concentrations of nanosilver is caused damages in the gill tissues of Caspian roach. The results demonstrated direct correlation of gill tissue damage and toxin exposure i.e. increasing nanosilver concentration is caused more tissue damage. Hence, histopathological changes of gill can considered as a proper indicator for nanosilver contamination of aquatic environments.

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Introduction

Development of science and technology has been led to increase the production of substances that can cause welfare in some aspects of human life. Unfortunately, the improper use of these chemicals can have adverse effect on humans and other organisms. The use of nanoparticles (NPs) has been dramatically developed in many fields. Nanosilver is one of important NPs because of its bactericide effect (Farkas et al., 2011). In addition, nanosilver is applied in odor resistant textiles, food packaging, cosmetics, household appliances and medical devices. Like other chemicals, overuse of this substance may contaminate the environment and release Ag particles (NPs or aggregates) via sewage discharge into the aquatic environment (Benn and Westerhoff, 2008). Also, waste nanosilver can contaminate groundwater through drainage and finally influence on non-target aquatic organisms such as fishes and crab. Fishes are very susceptible to environmental pollution which may cause significant damage on its vital organ such as gill. (Banaee et al., 2011; John, 2007).

Despite of widespread use of this NPs i.e. nanosilver, there is little information is available about its potential detrimental effects on the environments particularly on fishes (Farkas et al., 2011; Handy and Shaw, 2007). Few studies has discussed on the toxicity of nanosilver on human health particularly on respiratory exposure or from in vitro assays using mammalian cells (Lovern et al., 2007).

The evaluation of ecotoxicological risks resulted by pesticides was done to demonstrate the influence of toxicants on non-target organisms such as fish. The present study is a contribution to the evaluation of toxicity and effects of a nanosilver based toxic to fish gill tissue. Histological changes in animal tissues

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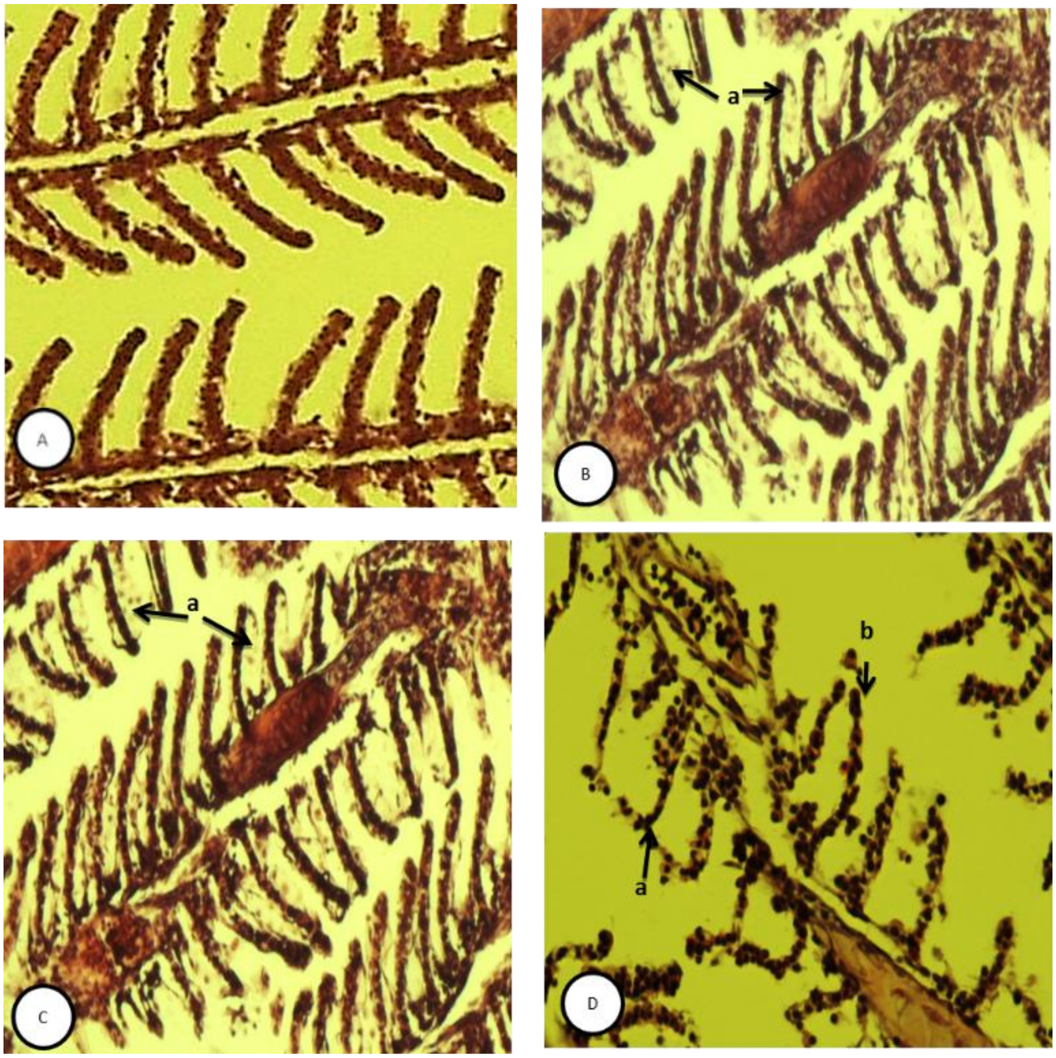

Figure 1. (A) Control gill: gill filament or primary lamella, secondary lamellae. H&E, X400, (B) Gill tissue exposed to 11.6 mg L^{-1} nanosilver for one week: a, epithelial lifting. H&E, X100, (C) Gill tissue exposed to 23.2 mg L^{-1} nanosilver for one week: a, shortening of secondary lamellae; b, haemorrhage at primary lamella; c, epithelial lifting and d, odema. H&E, X100 and (D) Gill tissue exposed to 34.8 mg L^{-1} nanosilver for two weeks: a, curling; b, necrosis. H&E, X100.

provide a suitable and easy method to discern of chronic effect of contaminate, in different tissues and organs (Bernet et al., 1999). Also, histopathological studies of exposed fish to pollutants have been revealed that fish organs can be considered as an adequate indicators of water quality (Velmurugan et al., 2007; 2009).

The gill is vital organs in fish respiration, osmoregulation, acid base balance and ammonia excretion (Heath, 1995). Fish gill are also sensitive to water pollution because of their great surface area and external position. For this reason fish gills are considered to be most suitable indicators of water contamination levels.

The Caspian roach (*Rutilus rutilus Caspicus*), an anadromous species, is an economically valuable (Coad, 1980; Soleimani et al., 2011) and a main food sources for sturgeon species in the Caspian Sea (Keyvanshokooh and Kalbassi, 2006). This fish enter the rivers of the Caspian Sea that are in close proximity to municipal waste for reproduction, so there is a possibility of exposure to this substance. Hence, this study was aimed to determine the histopathological effects of nanosilver on gill tissue of Caspian roach fingerlings.

Material and methods

Experimental design: Caspian roach fingerlings (with average weight of 1.66 ± 0.05 g; mean ± S.D.)

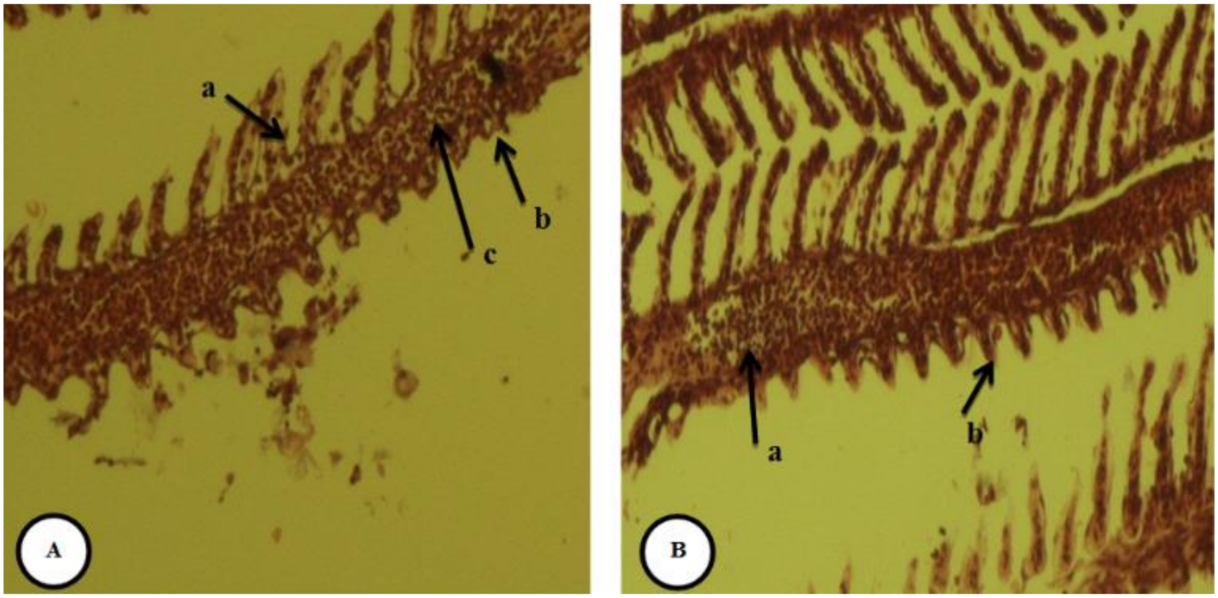

Figure 2. (A) Gill tissue exposed to 46.4 mg L^{-1} nanosilver for two weeks: a, epithelial lifting; b, haemorrhage at primary lamella and c, collapsed of secondary lamellae. H&E, X100 and (B) Gill tissue exposed to 46.4 mg L^{-1} nanosilver for two weeks: a, haemorrhage at primary lamella, (b) shortening of secondary lamellae. H&E, X100.

of this experiment were obtained from the Sijwal Fish Reproduction Center (Golestan Province, Iran). Their lengths were in the range 5-7 cm. The specimens were acclimated to the laboratory conditions for two weeks prior to experiment in a 20l glass aquarium filled by dechlorinated tape water. During two week acclimatization period, fish were fed twice a day whit a commercial food (Sari animal feed and aquatic factories).

Fish were randomly divided into fifteen tanks at a density of seven fish per tank. Fish were exposed to 0 (control), 11.6, 23.2, 34.8 and 46.4 mg L^{-1} of nanosilver with three replicates provided from commercially available nanosilvere, (Partonar Co). This concentrations were determined based on 0, 25, 50, 75 and 100% of h LC50 value for Caspian roach fingerling that reported by Shaluei et al., (2012), (46.4 mg L^{-1}). During experiment, water temperature, dissolved oxygen, pH and salinity were 27.3 °C, 7.07 mg L^{-1}, 7.3 and 1.2 ppt, respectively. Also, photoperiod regime was adjusted to 13L: 11D during experiment. Tanks were aerated continuously and 10% of the water was replaced daily with water containing the experiment concentration of nanosilvere.

Preparation of tissue: At the end of one week (3 specimens per tank) and two weeks (3 specimens per tank), fish removed from tanks and anesthetized using clove oil (150ppm). Fish gill dissected and fixed in 10% buffered formalin (Roberts, 1989). Tissues sections of 4 μm were prepared and stained with haematoxylin-eosin based on Cruz and Pitogo (1989). The mounted slides were observed and photographed using a Nikon E 200 eclipse microscope. Degree of tissue changes have been shown in table 1 as mild (+), moderate (++), severe (+++) and none (-).

Results

No alterations were observed in the control gill tissue. The structural details of the control gill is shown in figure 1a. The histological changes affected by nanosilver on gill of treatments are shown in Table 1. Results revealed the epithelial lifting of fish gill in 11.6 mg L^{-1} treatment (Fig. 1b). Curling and Necrosis was observed after two weeks of exposure to 34.8 mg L^{-1} nanosilver (Fig. 1d). Two weeks of exposure to 46.4 mg L^{-1} nanosilver was caused epithelial lifting, haemorrhage at primary lamella, collapsing of secondary lamellae and

Table 1. Summarized histopathological effects in the gills of *R. rutilus caspicus* exposed to nanosilver and control fish.

Concentration (mg L⁻¹)	Terms (Week)	Shortening of secondary lamellae	necrosis of secondary lamella	Haemorrhage at primary lamella	Epithelial lifting	Collapsed of secondary lamellae	Adhesion of secondary lamellae	Curling of secondary lamellae
Control		-	-	-	-	-	-	-
11.6	1	-	-	+	++	-	-	-
	2	++	-	+	++	+	+	++
23.2	1	+	-	+	++	+	-	+
	2	++	+	++	++	++	+	+
34.8	1	++	+	++	+++	++	++	++
	2	+++	++	++	+++	++	++	++
46.4	1	++	+++	+	+++	+	+	++
	2	+++	+++	+++	+++	++	++	++

shortening of secondary lamellae in the gills of experimental fish (Fig. 2a, b).

Discussion

In most aquatic animals such as fish, gills are vital organs for their respiratory, osmoregulatory and secretory functions. In present study, the histopathological changes of gill tissues in Caspian roach during one and two weeks exposure to nanosilver include necrosis, shortening of secondary lamellae, edema, destruction of epithelial lamella, shortening of secondary lamellae, epithelial lifting and curling of secondary lamellae.

Several other studies have shown similar effects of contaminates on gills of difference fish species (Yildirim et al., 2006; Velmurugan et al., 2007; Xing et al., 2012). Hyperemia, fusion of secondary lamellae and telangiectasis were histopathological alterations of gill in Nile Tilapia (*Oreochromis niloticus* L.) exposed to deltamethrin (Yildirim et al. 2006). Khoshnood et al. (2011) have reported the lamellar epithelia and lamellae fusion after 48 h exposure to mercuric chloride in the gill of Persian sturgeon (*Acipenser persicus*). Epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, edema and lamellar fusion were observed in Merigal (*Cirrhinus mrigala*) exposed to different sublethal concentrations of lambda-cyhalothrin (Velmurugan et al., 2007). Histopathological changes in the gill tissues of Labeo fish (*Labeo rohita*) exposed to atrazine were epithelial hyperplasia, curling of secondary lamellae, changes in chloride cells and degradation of epithelial and pillar cells (Jayachandran and Pugazhendy, 2009). In a study that Scown et al (2010) conducted, it was found exposure of rainbow trout to NPs can have highly negative effect on different tissues such as gill, including the presence of high concentrations of NPs in the gills.

Gill tissues injuries can be divided into two groups: direct and indirect (Richmonds and Dutta, 1989). For example, the observed epithelial damage of the gill is a direct responses induced by the action of pesticide. The defense responses are lifting up of the epithelium and lamellar fusion. Former pathological change increases the distance to avoid the toxicant to reach the blood circulation and later one i.e. lamellar fusion a rejoinder that reduces the amount of sensitivity of gill surface area. Also, gill hyperplasia may use as a defensive mechanism leading to diminish in the respiratory area and an enhancement in the toxicant-blood diffusion distance (Cengiz, 2006). Costs of defense response occur at the gills and the results are a respiratory disorder that itself caused is destruction of gills.

All these damages can cause reduction of up taking oxygen via gill resulting decrease of fish activity particularly in larvae and juveniles. Destruction of gill tissue may cause severe physiological problems and eventually leading to the loss of fish. The histopathological observation of this study showed that exposure to sub-lethal concentrations of nanosilver is caused damages in the gill tissues of Caspian roach. As a conclusion, this study demonstrated direct correlation of gill tissue damage and toxin exposure i.e. increasing nanosilver concentration is caused more tissue damage.

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