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Toxic effects of copper sulfate and copper nanoparticles on minerals, enzymes, thyroid hormones and protein fractions of plasma and histopathology in common carp *Cyprinus carpio*

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ABSTRACT

Differences in toxicological effects of dissolved copper and copper nanoparticles were studied in common carp, *Cyprinus carpio*. The fish were exposed to 0.25 mg L^{-1} copper as copper sulfate (0.25Cu), 0.25 mg L^{-1} copper as copper oxide nanoparticles (0.25NCu) and 25 mg L^{-1} copper as copper oxide nanoparticles (25NCu) over 14 days. Plasma biochemical, enzymatic and hormonal characteristics, and liver and kidney histopathology were examined at the end of the experiment. The results showed that both forms of copper had no significant effects on plasma calcium levels, however, significantly increased plasma phosphorous levels, compared to control group (no added copper). Plasma alanine transaminase (ALT) activity increased in 0.25Cu and 25NCu treatments compared to the control and 0.25NCu treatments. Nanoparticle copper exposure significantly decreased plasma alkaline phosphatase (ALP) activity compared to the control and 0.25Cu treatments. Only copper sulfate exposure caused plasma aspartate transaminase (AST) elevation. Both copper forms increased plasma T_4 and free T_4 (FT_4); however, copper sulfate effect was higher than nanoparticle copper. Copper sulfate exposure increased plasma albumin fraction, whereas, 25 mg L^{-1} copper nanoparticle exposure increased plasma α_2-globulin fraction compared to the control. Both copper forms damaged the fish liver and kidney, however, copper sulfate caused more severe damages compared to nanoparticle copper. Overall, except for plasma ALP and α_2-globulin fraction, dissolved copper seems to be more toxic than nanoparticle copper in common carp.

1. Introduction

Nano metals have unique properties because of their particle size of less than 100 nm; therefore, they affect biological systems differently compared to soluble form of the same metals. Copper nanoparticles are insoluble form of copper with a wide range of applications (Shaw and Handy, 2011). Although insoluble in water, copper nanoparticles release copper ions into the surrounding water due to small particle size (Shaw and Handy, 2011), which may affect organisms' biological systems similar to soluble copper salts (e.g. copper sulfate). Copper nanoparticles have different effects on aquatic organisms compared to copper ions. In *Epinephelus coioides*, copper nanoparticles has been found to have more adverse effects on gut than gill and liver; whereas, copper sulfate had more adverse effects on gill and liver compared to gut (Wang et al., 2014; Wang et al., 2015). Ionic copper, at the concentration equal to dissolved copper in copper nanoparticles suspension, has less adverse effects than the copper nanoparticles suspension on *Danio rerio* embryos (Bai et al., 2010). Copper nanoparticles exposure decreased blood hematocrit percentage and plasma sodium and potassium levels more than equivalent ionic copper levels in *Oncorhynchus mykiss* (Shaw et al., 2012). Also, copper nanoparticles and ionic copper damage *O. mykiss* tissues similarly (Al-Bairuty et al., 2013). In *Cyprinus carpio*, copper nanoparticles caused lower plasma copper elevation and had less toxic effects on plasma lipids, iron and ceruloplasmin and blood

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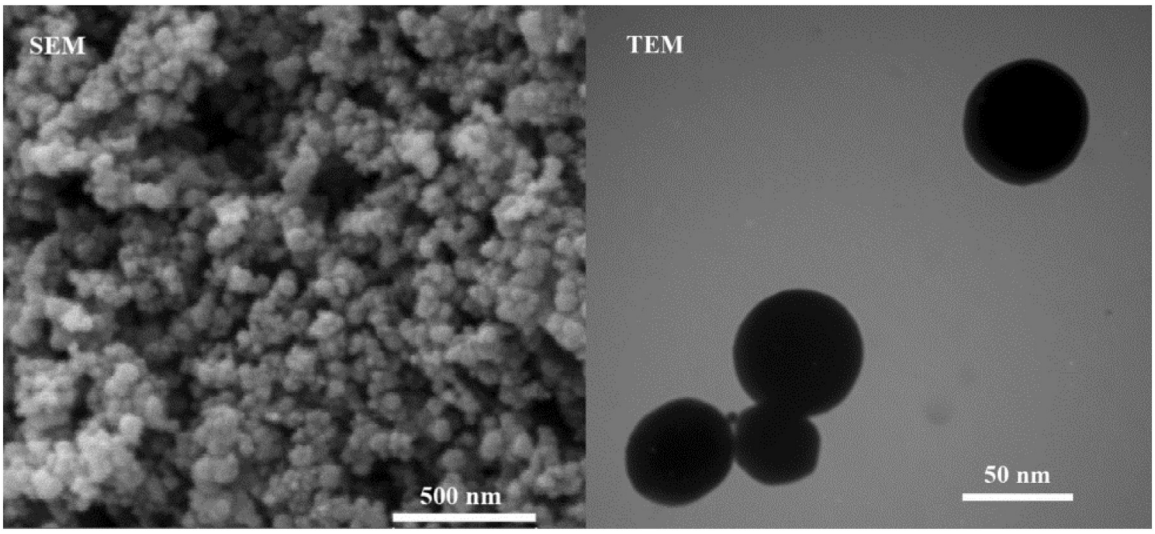

Fig. 1. The particle size of copper nanoparticles determined by transmission electron microscopy (TEM) and scanning electron microscope (SEM).

hematology compared to copper sulfate (Hedayati et al., 2016; Mazandarani and Hoseini, 2015).

Blood calcium and phosphorus levels are potential tools to monitor bone metabolism (Malekpouri et al., 2011). Copper exposure leads to calcium efflux in freshwater fish (Pelgrom et al., 1995; Sayer et al., 1991). On the other hand, copper exposure increases both calcium and phosphorus levels in marine fish (Cardeilhac et al., 1979). Also, fish kidney plays a role in calcium homeostasis (Lafeber et al., 1988) and copper exposure damages fish kidney (Al-Bairuty et al., 2013), which may lead to calcium homeostasis failure.

Copper exposure causes injury to fish liver (Al-Bairuty et al., 2013; Grosell et al., 1996; Handy et al., 1999). The effects of copper on fish liver histopathology are both direct and indirect [due to hypoxia (Al-Bairuty et al., 2013)]. Serum/plasma alanine transaminase (ALT) and aspartate transaminase (AST) are indicators of liver damage, which elevate during fish exposure to copper (Fırat et al., 2011; Hoseini et al., 2012; Öner et al., 2008).

Alkaline phosphatase (ALP) is an enzyme with a variety of functions that acts as transphosphorylase at alkaline pH and is important in the skeleton mineralization in aquatic animals and in membrane transport activities (Bernet et al., 2001). Fish serum/plasma ALP is sensitive to waterborne copper exposure showing either elevation or reduction depending on experimental conditions (Karan et al., 1998; Öner et al., 2008).

Copper toxicity alters thyroid hormones' level in fish. Exposure of *Anguilla anguilla* to waterborne copper for 7 days had no significant effects on TSH and T_4, but significantly decreased T_3 levels, suggesting alteration in conversion of T_4 to T_3 (Oliveira et al., 2008). However, these results were not in line with previous study on the same species, where, Teles et al. (2005) found no change in plasma T_3, but significant decrease in plasma T_4 in the copper-exposed fish. Another study showed that the effects of copper exposure on plasma thyroid hormones depend on fish species, exposure time and copper concentration (Eyckmans et al., 2010).

Fish serum proteins are suitable and sensitive markers for environmental stress and diseases (Manera and Britti, 2008; Sala-Rabanal et al., 2003). Data about the effects of waterborne copper on fish serum protein fractionation is scarce. It has been demonstrated that copper exposure has no significant effects on *Sparus aurata* serum protein fractionation, although alters several proteins, individually (Isani et al., 2011). Further studies on this topic seem necessary.

Although several studies investigated the difference between copper sulfate and nanoparticles effects on fish, no comparisons were made in the case of aforementioned plasma parameters to date. Therefore, the aim of the present study was to illustrate the difference between copper sulfate and copper nanoparticles effects on plasma calcium, phosphorous, hepatic enzymes, thyroid hormones and serum proteins along with liver and kidney injuries in common carp.

2. Materials and methods

2.1. Copper sources and preparation

Copper (II) sulfate pentahydrate was purchased from Sigma-Aldrich Corporation (98% < purity; St. Louis). Nano-Cu (99.9% purity, particle size of less than 40 nm, Fig. 1) was provided by Iranian Nanomaterials Pioneers Co. (Mashahd, Iran).

A stock solution of copper sulfate ($1\,g\,L^{-1}$) was prepared immediately before adding to the tanks' water. A stock solution of copper nanoparticles ($1\,g\,L^{-1}$) was prepared by dispersing certain amount of copper nanoparticles into distilled water followed by 20 min ultrasonication. The stock solution was added to the tank water immediately after sonication.

2.2. Subjects and maintenance conditions

A total number of 360 carps with average weight of 30.68 ± 2.65 g were randomly distributed into 12 fiberglass tanks (30 fish per tank). Each tank contained 144 L dechlorinated tap water and equipped with a biofilter (sponge filter). The tanks' water was replaced (75%) by freshwater every day. The fish were fed with commercial feed based on 2% of body weight daily and allowed to acclimatize to the experimental conditions for 14 days.

2.3. Copper exposure and blood sampling

After acclimation, the tanks were divided into four treatments (three tanks per treatment): control (measured copper content = $0.003 \pm 0.0006\,mg\,L^{-1}$), 0.25Cu ($0.248 \pm 0.015\,mg\,L^{-1}$ copper as copper sulfate), 0.25NCu ($0.25\,mg\,L^{-1}$ copper as copper oxide nanoparticles) and 25NCu ($25\,mg\,L^{-1}$ copper as copper oxide nanoparticles). $0.25\,mg\,L^{-1}$ copper has been used to investigate copper toxicity in common carp (Karan et al., 1998; Pelgrom et al.,