

Effect of CuO nanoparticles on some hematological indices of rainbow trout *oncorhynchus mykiss* and their potential toxicity

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Abstract

Objective(s): This study aimed to determine the possible toxicity of CuO nanoparticles (NPs) on *Oncorhynchus mykiss* by evaluating hematological parameters.

Materials and Methods: Fish were sampled and treated in 4 aquariums containing the concentration ranges of 1, 5, 20 and 100 ppm of CuO NPs. There was one control group (no CuO NPs) and three replicates. The physicochemical properties of water were as follows: the temperature was 22 ± 2 C°, oxygen saturation was $90.9\pm 0.2\%$, pH was at 7 ± 0.004 and the concentration of CaCO₃ was 270.

Results: No mortality was observed after 96 hours of exposure. The analysis of hematological parameters showed that CuO NPs affected the counts of white blood cells, lymphocytes, eosinophils, neutrophils, hematocrits, MCH, MCHC and MCV and did not have any effects on monocytes and hemoglobins.

Conclusion: The data showed that the overall hardness (270 ppm) neutralized the lethal effect of copper on *O. mykiss* and no mortality was recorded.

Keywords: Copper nanoparticles, Fish, Hematological parameters, Lethality

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Introduction

Although copper (Cu) in low amounts is essential to animals and higher plants (e.g., cytochrome c oxidase and superoxide dismutase) (1), the hazards and pathological effects of excess amounts of copper are reasonably well known in fish (2; 3). Cu can have adverse effects on fish by decreasing glutathione levels (4), catalyzing the formation of reactive oxygen species (ROS) via a Fenton-like reaction (5) and interacting with antioxidant enzymes (6). Histopathological studies showed that copper nanoparticles and copper sulphate caused organ injuries including aneurisms, hyperplasia, and necrosis in the secondary lamellae of the gills; necrosis in the mucosa layer and vacuole formation in the gut; swelling of goblet cells; hepatitis-like injuries and cells with pyknotic nuclei in the liver; damage to the epithelium of some renal tubules and increased Bowman's space in the kidney; some mild changes in the brain; alteration in the thickness of the mesencephalon layers and enlargement of blood vessel on the ventral surface of the cerebellum in *Oncorhynchus mykiss* (7).

Nanotechnology has become a rapidly growing industry with a vast number of potential applications such as in cosmetics, electronics, paints, medical devices, food packaging, catalysts, antimicrobial fabrics, water treatment membranes, etc. (8; 9; 10; 11; 12). CuO nanoparticles (NPs) are increasingly used in medicine, industry, or as pesticides (13) and can be simply entered into aquatic resources. In aquatic animals, NPs can enter organisms by various routes such as direct passage across the gills and other external epithelial surfaces (14). Fish is considered a valuable source of protein in the human diet and it is full of polyunsaturated fatty acids which help preventing human cardiovascular diseases (15).

The rainbow trout is an economically important species commercially farmed in many countries throughout the world. It has a rapid growth, and can easily adapt to environmental conditions. Many studies have been conducted on the ecotoxicity of nanomaterials (16; 17; 18; 19), but data on the effects of CuO NPs on hematological parameters of *O. mykiss* are scarce.

NPs are very reactive and able to pass through cell membranes in organisms. Furthermore, their interactions with biological systems are relatively unknown. Therefore, the aim of this study was to determine the CuO NPs potential toxicity in *O. mykiss* and its impact on hematological parameters. These data can be useful in aquatic toxicity management and environmental safety.

Materials and Methods

One hundred and fifty-five live specimens of *O. mykiss* were obtained. Samples weighted 18 ± 3 g. They were acclimatized randomly in 15 cm aquariums for one week. Four aquariums were treated with 25, 30, 50, and 100 ppm of CuO NPs with one control group (no CuO NPs).

No feeding occurred during the test (during 96 hours). There were no significant differences between aquariums in water quality and the following parameters were constant: pH: 7 ± 0.004 ; temperature: 22 ± 2 °C; hardness: 270 ± 0.05 ppm and oxygen saturation: $90.9 \pm 0.2\%$. The photoperiod was 12 h light and 12 h dark. CuO NPs were prepared in water-in-oil microemulsions as described in Capek (20). Blood samples were collected and transferred to glass tubes and hematometric parameters were determined. Hematological parameters were estimated according to routine clinical methods (21). The acid-hematin method of Sahli in hemometer was used to analyze hemoglobin percentage and

Table 1. Hematological parameters of *O. mykiss* exposed to CuO NPs at 4 doses.

Hematological parameters	Control	1 ppm	5 ppm	20 ppm	100 ppm
RBC* (10^6 mm^3)	0.64±0.01	0.65±0.01	0.63±0.01	0.63±0.02	0.6±0.005
WBC* (10^3 mm^3)	6716.66±47.25	5530.96±115.32	6163.33±40.41	5860.96±714.3	5040±52.91
MCH* (10^{-3} pg)	94.70±0.6	65.06±0.92	98.16±1.23	93.46±1.01	101.80±3.35
MCHC* (%)	19.56±0.64	18.63±0.56	17±0.72	18.43±0.35	21.23±0.90
MCV* (10^{-4} mm^3)	502.33±6.80	526.33±3.51	529±5.29	499.33±6.65	495±4.58
HB (g/100ml)	6.06±0.05	6.16±0.15	6.23±0.15	6.06±0.05	5.96±0.11
HCT* (%)	31.33±0.57	34.33±0.57	34.66±0.57	32.66±0.57	29.66±0.57
Lymphocyte* (%)	86.33±2.08	79.66±1.52	77.66±1.52	78.33±2.08	74±3
Monocyte (%)	2±0	1.66±0.57	2.33±2.3	3.66±2.08	5±4
Eosinophil* (%)	1.66±0.57	2.66±0.57	4.66±1.15	7.33±1.15	6±1.73
Neutrophil* (%)	10.26±0.7	16.36±0.65	15.66±0.61	12.6±0.55	15.36±1.65

Each value is a means ± standard error. * shows statistically significant difference (P<0.05)

Naeubaur's double hemocytometer was used to enumerate the erythrocytes (22). Mean cell hemoglobin (MCH), mean corpuscular volume (MCV), and mean cell hemoglobin concentration (MCHC) were calculated based on Decie and Lewis (23). Mortality rates were recorded after 24, 48, 72 and 92 hours and the dead fish were quickly removed from the aquarium. In order to determine the nominal concentration of toxins causing mortality, LC1, LC10, LC30, LC50, LC70, LC90 and LC99 were recorded within 24, 48, 72 and 96 hours. After 96 hours, blood samples were taken. One-way analyses of variance (ANOVA) were used to analyze hematological parameters. Differences between means were determined using Duncan's multiple range test at 5% probability level.

Results and Discussion

No mortality was observed during the acclimation and the test. This is to say that within 96 h no mortality was recorded in treatment aquariums. It is noteworthy that no mortality was observed even after 2 weeks. The analysis of hematological parameters showed that CuO NPs stimulated white blood cells, lymphocytes, eosinophils, neutrophil, hematocrit, MCH, MCHC and MCV, and did not have any effects on monocytes and hemoglobins (Table 1).

Changes in the quantitative and qualitative characteristics of blood cells occur when anomalies in blood components interfere with normal functions (24).

Hematological data showed that CuO NPs exerted a certain influence on some of the blood indices in this study. Cu is regularly used in the form of CuSO_4 in aquaculture to control algal blooms and as a therapeutic chemical for ectoparasitic and bacterial infections (25; 14); it is also imported into aquatic resources. pH and water hardness are two major factors modifying the toxicity of copper (26; 27; 28; 29).

Howarth and Sprague (28) demonstrated that high hardness decreased the toxicity of copper at any pH. This phenomenon can be explained by the influence of biological membrane permeability on toxic metals due to the change in hardness, resulting in an increase of the passive flux of metal ions across the membrane as the calcium concentration decreases (29; 30). The uptake of calcium and magnesium ions by the cell membrane causes it to stabilize, which reduces its permeability to metal ions (31) and as a result toxicity of heavy metals is governed by the water hardness. In this study, total hardness (270 ppm) neutralized the lethal effect of copper on *O. mykiss* and no mortality was recorded.

CuO nanoparticles toxicity

The comparative lethal toxicity of copper affirms the lethality of copper to *O. mykiss* and other aquatic animals. Richey and Roseboom (32) reported the acute toxicity of copper to some types of fish. They indicated that the LC₅₀ 48h of copper to rainbow trout in size of a yearling and alkalinity of 250, is 0.75 mg/l. Furthermore, in size of 12-16 centimeters (4.42 grams) and alkalinity of 250 LC₅₀ 48h is 0.27 mg/l, whereas, LC₅₀ 24h with low alkalinity is 0.43 mg/l. In addition, MATC/60 days Brook trout at the size of 0-60 days within alkalinity of 178; LC₅₀ 96 h 3 years old Atlantic salmon within alkalinity 4; LC₅₀ 72h yearling Silver salmon within alkalinity 78 and LC₅₀ 96h yearling Coho salmon within alkalinity 74 was 0.006, 0.125, 0.19 and 0.067 mg/l, respectively (32).

Surely CuO NPs caused no mortality on *O. mykiss* with the size of 18 g and the hardness of 270 mg/l in this study. However, the effect of CuO NPs on hematological parameters was evident.

Statistical analysis showed that numbers of RBCs were statically different in the treatment and control group. Thomas and Egee (33) stated that the transport of oxygen (O₂) and carbon dioxide (CO₂) within the blood is intricately related to the electrolytes and the acid-base status of the red blood cells (RBCs). This suggests that CuO NPs caused respiratory restrictions which led to changes in the number of RBCs. In addition, other results indicate that monocytes have a relatively short respiratory burst response following activation (34; 35). Al-Bairuty et al. (7) reported that CuO NPs caused some kinds of gill injuries such as hyperplasia, oedema, lamellar fusion and clubbed tips. Studies that compare the effect of CuO NPs on erythrocyte count and hemoglobin content are scarce. MCV is the index most often used. It measures the average volume of red blood cells by dividing the hematocrit by RBC. Alteration in the values of MCV, MCH and MCHC in *Cyprinus carpio* exposed to pesticides in

60 and 120 µg/L concentrations was reported by Svoboda et al., (36) and Banaee et al., (37).

The number of white blood cells may increase or decrease significantly in certain diseases (37). There was a significant difference ($p < 0.05$) between three types of white cells (lymphocytes, neutrophile and eosinophile). Changes in white blood cell count suggest dysfunction in hematological tissues (spleen and kidney) or certain infectious diseases. Al-Bairuty et al., (7) reported some damage to the epithelial cells of the renal tubules, changes in the Bowman's space, and an increase in the foci of melanomacrophage deposits in *O. mykiss* exposed to CuO NPs. Kosai et al., (39) stated that 7 days of 46 mg/l Cu exposure caused tubular swelling, atrophy of the glomerulus, and necrosis of the renal epithelium in *Oreochromis niloticus*. In addition, one study in mice showed damage in pathological observation in the kidney after 72 h of oral gavage of 108–1080 mg/kg Cu-NPs (38). Lower than normal levels of lymphocytes (lymphopenia) can be an indicator of immune system deficiency and poisonous substance treatments can also deplete the body's supply of lymphocytes (37). Many researchers reported deletion in lymphocytes in fish exposed to pesticides such as *Heteropneustes fossilis* (40) and *Cyprinus carpio* (41; 36; 37). Banaee et al., (37) stated that most infections cause neutrophilia. The degree of elevation often indicates the severity of the infection. Tissue damage from other causes raises the neutrophile for similar reasons. Poisonings, and severe disease, like kidney failure all cause neutrophilia (42). Ghosh and Banerjee (43) reported that after *Heteropneustes fossilis* exposed to dimethoate, neutrophile and eosinophile increased in blood parameters.

Conclusion

This study has demonstrated from the viewpoint of hematology that CuO NPs

stimulated the immune system of *O. mykiss*, but this effect did not have any lethality on this species at pH: 7 ± 0.004 ; temperature, 22 ± 2 °C; hardness, 270 ± 0.05 ppm and oxygen saturation, $90.9 \pm 0.2\%$. This study showed that the Sublethal effects of CuO nanoparticles are much less than Cu. Overall, CuO NPs have less mortality; therefore, it is beneficial to use NPs instead of heavy metals in industry because they have fewer detrimental effects on water resources, fisheries, the environment, and humans.

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