THE EFFECTS OF COPPER OXIDE NANOPARTICLES ON THE ALT AND AST ENZYMES IN COMMON CARP (CYPRINUS CARPIO)

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Abstract- As a result of the development of nanotechnology, there are increasing concerns about the potential dangers of chemical release containing nanoparticles on the environment. The aim of this study was to evaluate the effect of copper oxide nanoparticles (CuO NPs) on AST and ALT in the blood serum of common carp. Six groups of carp (Cyprinus carpio) were studied with a weight of 35±2 g involved in the control group and 5 groups exposed to various concentrations of copper nanoparticles (5, 10, 50, 100 and 150µg/l). Bleeding was performed from the caudal fish treatments at 24, 48, 72 and 96 h and prior exposure to nanoparticles (base). An auto-analyzer was used to measure the serum concentrations of the enzymes ALT and AST. Statistical analysis showed that fish exposed to nanoparticles had significantly increased blood serum ALT and AST compared with the control group. A comparison of different days showed significant differences with the basic group. The results showed that CuO nanoparticles increased the levels of ALT and AST enzymes in the serum, because it could be due to liver necrosis and leakage of these enzymes into the blood. Thus, these enzymes can be used as an indicator of copper oxide nanoparticle contamination.

Key words-

I. INTRODUCTION

As a result of the development of nanotechnology, there are increasing concerns about the potential dangers of chemical release containing nanoparticles on the environment [3, 5]. Nanoparticles such as metal oxides (ZnO, TiO_2 and CuO) are widely used in industrial production and trade. It is certain that there is release of nanoparticles into the aquatic environment. This study presents information about the toxicity of nanoparticles on aquatic organisms [2, 4, 10, 11, 17]. Industries that use copper oxide nanoparticles include the pharmaceutical, consumer products, pesticides, antibacterial textiles production and as anti-fouling paint boats [9, 10, 13]. In general, copper oxide nanoparticles are widely used. Marine life may obtain nanoparticles through the gills and external mucosal surfaces of the body. Nanoparticles diffuse more or form a complex than other soluble in water [11]. One of the nanoparticles produced is the copper oxide nanoparticle (CuONPs). Copper is an essential element for living organisms because it is a cofactor with different enzymes (cytochrome C oxidase, superoxide dismutase, ceruloplasmin and monooxygenases) and plays a central role in cell metabolism. However, high concentrations of copper could be due to their participation in oxidation and reduction reactions toxic to aquatic organisms in the environment [12]. Gradually, metallic nanoparticles through the decomposition of the particles, dissolved metal ions are released and will lead to the production of free ions toxic hidden. However, there is little information about the bioavailability and accumulation of secondary metal nanoparticles and effects on different organs of the fish [11, 17]. The aim of this study was to investigate the effects of copper oxide nanoparticles on the enzymes ALT and AST in common carp.

II. MATERIALS AND METHODS

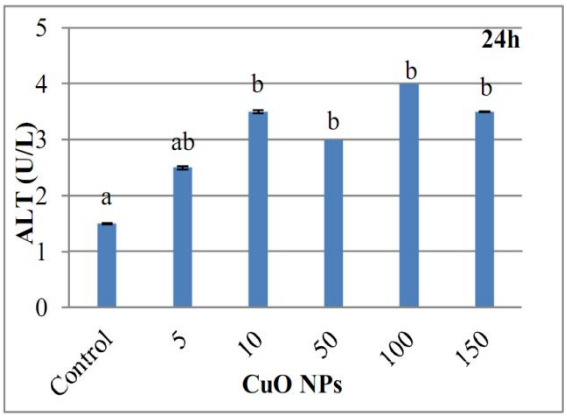
Stoke was prepared with a concentration of 100 µg/l of copper oxide nanoparticles with size of 40 nm. The experiment consisted of six (6) treatments (control, 5, 10, 50, 100 and 150 µg/l) and each group included 16 fish (weight of 35±2 g) in 18 aquariums (40 liters) with three replicates of each treatment. The experiment was conducted using static and standard method (OECD). Blood samples were collected from the caudal fin of four (4) fish per treatment after 24, 48, 72 and 96 h of exposure to copper nanoparticles and before exposure to the aquarium (base). Plasma was separated in order to measure alanine aminotransferase (ALT) and aspartate aminotransferase (AST), using centrifugation at 3000 rpm for 15 min [1]. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was conducted using a test kit. Statistical analysis was done using SPSS.16 software. After determining the normality of the data, using one-way ANOVA (One Way Anova) must be the difference between treatments and control, and between different time and the basis, if there are significant differences in the level ($P<0.05$) was used of Duncan test.

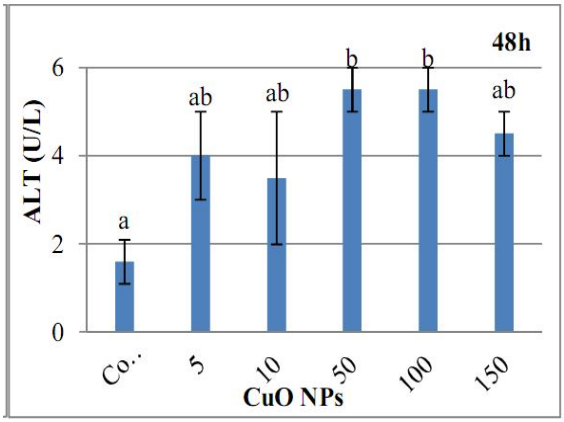
III. RESULTS

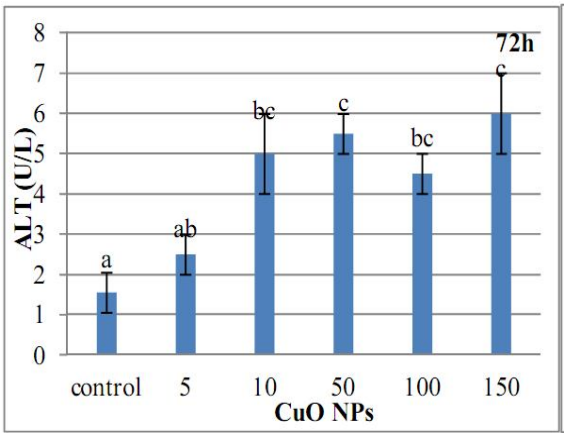
Mortality was not observed during the test period in any of the treatment and control groups. The results of measurements of serum enzymes in common carp exposed to different concentrations of copper

nanoparticles in different days are shown in Figures 1 to 4.

Comparing the average amount of the enzyme alanine aminotransferase (ALT) showed significant difference among the different treatments in the different concentrations with the control group ($P<0.05$). The least amount of ALT in different concentrations in the control group 1.5 (U/L) and the greatest amount of ALT in the time of 24, 48, 72 and 96 h, 4, 5/5 , 6 and 3.5 (U/L) respectively, between concentrations of 100 and 150 µg/l. Thus, according to the results increased ALT levels in fish exposed to copper nanoparticles was compared to the control group. But between different groups exposed to different concentrations of CuO, had no significant difference ($P>0.05$) (Figure 1).







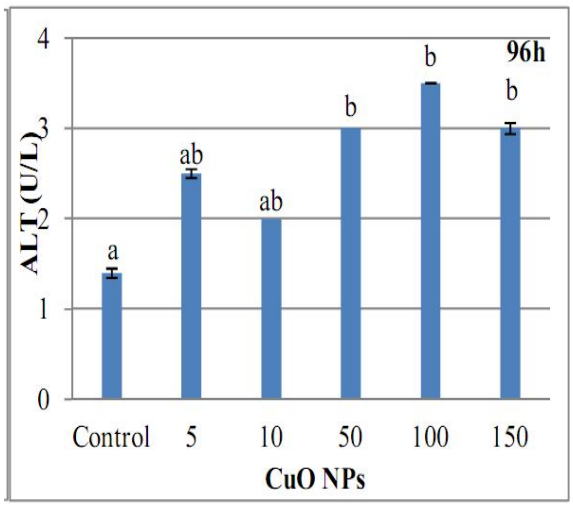


Figure 1. Compare ALT (U/ml) in different concentrations copper oxide nanoparticles (µg/l).

The statistical analysis showed that ALT levels are significant difference between different days and basis group ($P<0.05$). The lowest and highest amount of ALT was observed in the basic group 1.62 (U/L) and 4.7 (U/L) in 100 (µg/l) copper nanoparticles. Comparisons between different days of exposure to copper nanoparticles also showed significant differences ($P<0.05$). ALT levels after 24, 48 and 72 h had increased but ALT levels declined after 96 h (Figure 2).

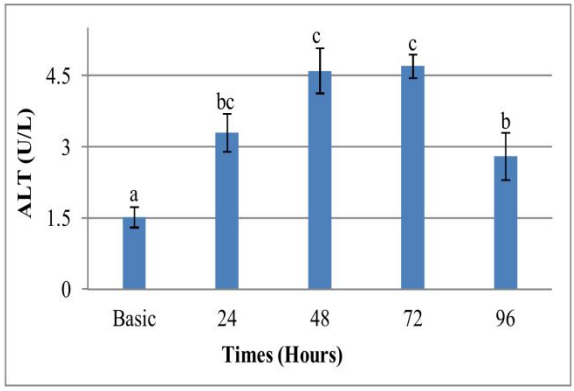


Figure 2. Compare ALT (U/ml) at different times

Comparing the statistical analysis of the enzymes aspartate amino transferase (AST) showed that different treatments had a significant difference ($P<0.05$) with the control group. According to the results obtained, the lowest AST was observed in the control 67.3 (U/L) and the highest AST was observed in different time of 24, 48, 72 and 96 h, respectively, 184, 277.5, 234 and 200 (U/L) at high concentrations of copper nanoparticles. Therefore, the enzymes AST increased in fish exposed to copper nanoparticles compared to the control group. The statistical analysis showed that AST had no significant difference between different treatments in different concentrations ($P>0.05$) but in the 72 h between the groups include concentrations of 10, 50, 100 and 150 (µg/l) compared to the group 5 (µg/l) concentration was significantly different ($P<0.05$) (Figure 3).

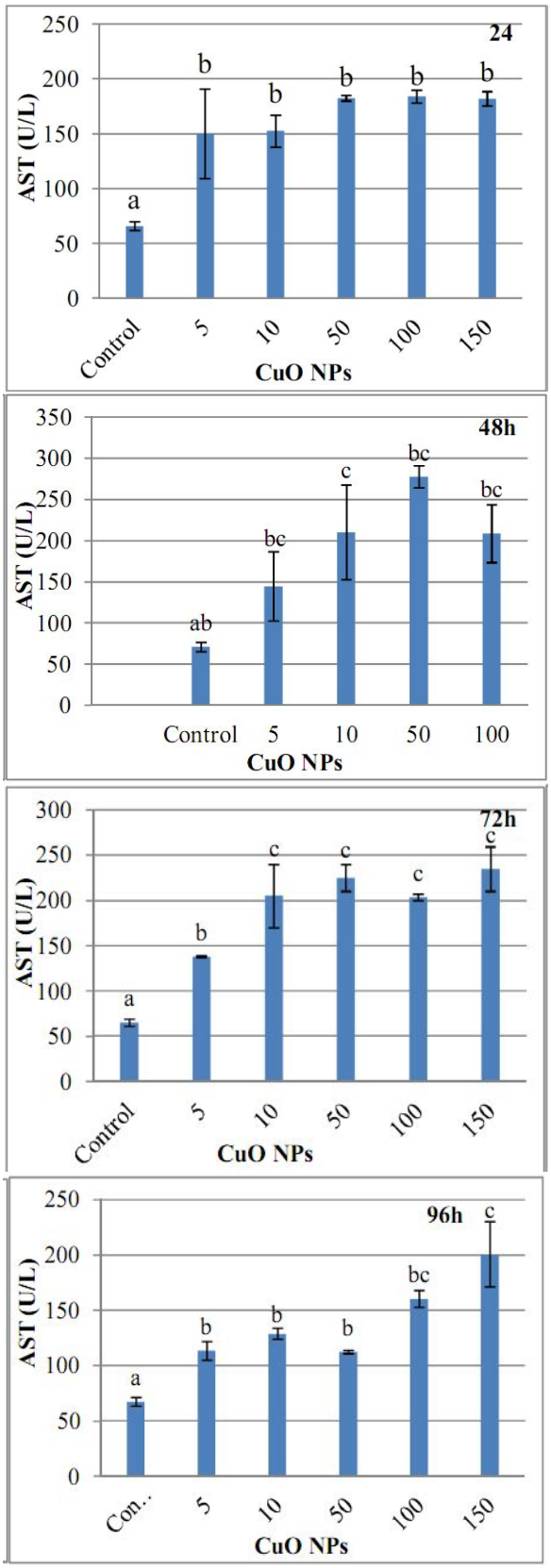


Figure 3. Compare AST (U/ml) in different concentrations copper oxide nanoparticles (µg/l).

The results of statistical analysis showed that AST enzyme levels showed significant differences between different days with the basic group ($P<0.05$). The lowest and highest amount of AST was observed respectively, in base 66 (U/L) and 48 h after exposure to copper nanoparticles 216.7 (U/L). However, AST increased after 24 and 48 h, but after 72 and 96 h the amount of the enzyme decreased to 143.2 (U/L) (Figure 4).

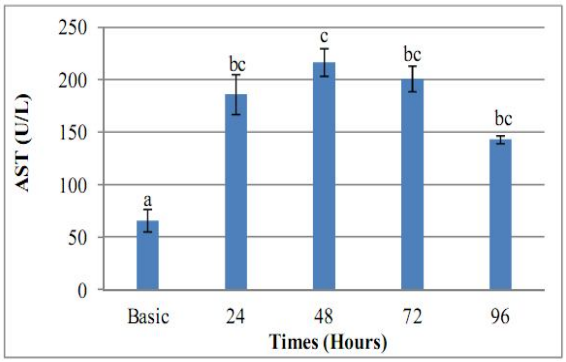


Figure 2. Compare ALT (U/ml) at different times

DISCUSSION

Many researchers have concluded that blood cells are a good indicator for assessing the environmental impact of toxic substances or other types of stress [14]. The activity of enzymes such as AST and ALT are used to diagnose fish diseases and the discovery of tissue damage caused by environmental pollution [8].

Increase enzymes of the liquid serum outside are the cell sensitivity and even minor damages [16] and also reflect the disruption of tissue under stress. In general, toxins lead to changes in liver cells and the tissue damage caused emission of cellular enzymes into the blood serum. Hence, it increases the serum cytosolic enzymes of the liver due to leak into the bloodstream and an indication of liver toxicity is caused by toxins [8].

In this study, the enzymes aspartate amino transferase (AST) and alanine aminotransferase (ALT) in the blood serum of fish exposed to copper oxide nanoparticles was increased compared to the control group and the statistical analysis showed that this increase can be compared with the control group, at a significant difference of ($P<0.05$). Hence, AST and ALT were increased in the blood of fish respectively from 67.3 and 1.5 (U/L) to the amount of 277.5 and 6 (U/L) in high concentrations of copper oxide nanoparticles. In normal circumstances, these enzymes remain within the liver cells, but these enzymes are released into the blood stream when liver cells are damaged. Studies show that free radicals caused by nanoparticles can attack liver cells and can cause the release of enzymes stored in the serum. Hence, it can be concluded that as a result of fish liver damage, enzyme release into the blood increased.

Vignesh et al. (2013) obtained similar results. In this study, enzymes ALT and AST increased in blood fish, respectively, 15 and 16 (U/L) in the control group to 30 and 21 (U/L) in 50 (µg/l) silver nanoparticles after exposure for twenty days. The

liver damage is due to increase of enzymes in the blood [18]. In another study (Sathya et al., 2012), acute effects of silver nanoparticles on blood common carp, blood parameters and electrolytes increased in the plasma [16]. Harvey et al. (1994) reported that the increased levels of AST and ALT in the blood may be due to increased liver cell damage [12]. Lee et al. (2014) studied the effect of ZnO nanoparticles on the enzymes AST and ALT present in common carp (Cyprinus carpio) for 12 weeks [15]. The results showed that increased levels of these enzymes in the blood serum was significant with the control group ($P<0.05$). According to the results, they said that these enzymes can be useful as an indicator of pollution related to zinc oxide nanoparticles. From the results of this study, it can also be concluded that exposure in fish with copper nanoparticles increases enzymes in ALT and AST of the blood, and this can be used as an indicator of pollution by nanoparticles.

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