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Survey of silver nanoparticles (Ag-NPs) effects on blood indices of Rainbow trout (*Oncorhynchus mykiss*) juveniles

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Abstract: Silver nanoparticles (Ag-NPs) are potentially toxicant substances whose widespread use has raised considerations regarding environmental risks caused by the discharge of those nanoparticles (NPs) into aquatic ecosystems. The aim of this study was to analyze the impact of Ag-NPs on the hematologic parameters Rainbow trout, *Oncorhynchus mykiss* juvenile. Hematological toxicities of Ag-NPs to rainbow trout juveniles were assessed in four treatment groups: Control (without Ag-NPs), 0.1gr/L Ag-NPs solution (T1), 0.25gr/l Ag-NPs (T2) and 0.5gr/l Ag-NPs (T3). Blood samples were collected from fish after 5 and 10 days of exposure. Analysis of blood parameters in the 5th and 10th days of the experiment showed that the values white blood cells (WBCs) were higher in all treatments than those in the control group ($P<0.05$) and also the red blood cells (RBCs) reduced in the high concentration of Ag-NPs in the 10th sampling day. Although the Hematocrit (Hct) and hemoglobin (Hb) concentrations were higher within the T1 and T2 groups than those in the control group both within the initial and second blood sampling, a significant decrease compared to the control group was determined in values of them both in the first and second sampling for the T3 group ($P<0.05$). The MCV and MCH significantly attenuated within the treatment groups within the first sampling. In contrast, the MCHC values not showed significant changes in trend because the concentration of Ag-NPs enhanced. Our results incontestable that silver nanoparticles induce important changes in hematological parameters of rainbow trout juveniles.

Keywords: Silver nanoparticles, Hematological indices, *Oncorhynchus mykiss*, juvenile.

1. Introduction:

Silver nanoparticles are particles smaller than 10 nm in diameter, which was first used in 1889 to prevent the growth of bacteria (Massarsky et al. 2014b; Nowack et al. 2011). Silver (Ag) nanoparticles are used as the antimicrobial adjuvant in various products such as clothes and medical devices where the release of nano-silver could contaminate the environment and harm wildlife. The purpose of this study was to examine the effects of nano-silver particles on O. mykiss rainbow trout juvenile (Gagné et al. 2009). Over time, the emergence of antibiotics and unknown-remaining mechanisms of silver toxicity gradually led to a decline in the application of this substance (Choi et al. 2009). The background knowledge of silver antibacterial effects and unsatisfactory performance of antibiotics on killing the strains of infectious microorganisms have consequently lead to

reverting to the utilization of silver but in form of synthetically nano-sized particles (1–100 nm) (Emam and Ahmed 2016; Varner et al. 2010). Therefore, mainly due to their anti-microbial properties, Ag-NPs are the fastest increasing manufactured nanomaterials. As such, the worldwide annual application of Ag-NPs is more than 320 t (Lourtioz et al. 2016); however, the increasing utilization, unfortunately, enhances their entrance likelihood into aquatic ecosystems (Šiller et al. 2013).

Aside from interests in the potential applications of Ag-NPs, little information is available regarding their potentially harmful effects on the environment and fish. Some studies showed that Ag-NPs can affect the physiology of different aquatic organisms such as fish, polychaete, and freshwater alga. (Farmen et al. 2012; Bilberg et al. 2010; Bilberg et al. 2011; Farkas et al. 2011; Cong et al. 2011; Navarro et al. 2008).However; the toxicological effects of Ag-NPs on fish blood remain not to be investigated (Shaw and Handy 2011).

Hematological parameters are progressively used in fish as indicators of the physiological or sublethal stress response to endogenous or exogenous alterations and are more quickly reflected in the poor condition of fish than in other commonly measured (Cataldi et al. 1998).

Accordingly, the present study used rainbow trout, as a well-known ecological receptor in aquatic toxicology and most cultured fish species in the aquaculture industry. We investigated the probable effects of Ag-NPs on hematologic parameters of rainbow trout juvenile in four treatment groups: Control (without Ag-NPs), 0.1gr/L Ag-NPs solution (T1), 0.25gr/L Ag-NPs (T2) and 0.5gr/L Ag-NPs (T3).

2. MATERIALS AND METHODS

2-1 Experimental Animals

One batch of freshwater rainbow trout was used for all the experiments (n = 80, Weight = 35±3.2g, Length = 2.04±1.2cm) were purchased from Coldwater Fishes Research Center (CFRC) (Tonekabon, Mazandaran, Iran). Fish were transferred to Fisheries Laboratory (Tonekabon Branch, Islamic Azad University, Mazandaran, Iran) and acclimated to the laboratory conditions for 2 weeks. Fishes were parceled in 8 fiberglass tanks (500 L). The average values for aerated and dechlorinated tap water used during both acclimation period and experiments were pH 7.79 ± 0.50, dissolved oxygen 7.93 ± 0.25 mg/L, the temperature of 20.50 ±1°C and total hardness 298±2.35 mg/L as $CaCO_3$. Water was renewed daily, and the water quality parameters mentioned above were measured twice a week during the toxicity test. Throughout the experiment periods, fish were held under a natural photoperiod condition (11:13 light-dark).

2-2 Materials and particle characterization

In this study, water-soluble form of colloidal, black Ag-NPs with the commercial name of Silver nanopowder (Ag, 99.99%, 20nm, w/~0.2% PVP; Stock# 1037; CAS# 7440-22-4) was used. It was concentrated at 4000 ppm (Ag-NPs). This was a product by US Research Nanomaterials Company, Huston, TX, the USA for antimicrobial purposes. The particle form in exposure study was photographed by Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD) in the US Research Nanomaterials Company, Huston, TX, USA (Fig. 1). The stock solution was added to the test water to achieve the target nominal Ag-NPs concentrations specific for each dose. Test solutions were prepared daily from the stock solution.

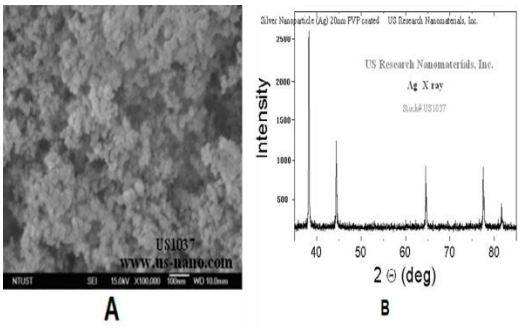


Fig.1. Ag-NPs characteristics: (A) Scanning Electron Microscope (SEM) and (B) XRD (Peaks).

2-3 Toxicity test

Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. The fish were first separated into 2 equal groups (n = 40) for 5 and 10 days. Every group divided into 4 subgroups containing 10 fish was exposed to test solutions in 200L-aquariums with the following concentrations of Ag-NPs: 0.0 (control), 0.1 (T1) and 0.25 (T2) and 0.5 g/L (T3), respectively. The test water in the aquariums was renewed daily and a freshly

prepared solution was added to maintain the concentration of Ag-NPs at a constant level.

2-4 Hematological analyses

At 5th and 10th days of the experiment, 6 fish per treatment were sampled randomly and deeply anesthetized with Clove powder (40 mg/L), and then, their blood was collected from the caudal vein into sterile syringes and immediately transferred into heparinized Eppendorf tubes.

For the hematology tests, the blood samples were placed into tubes containing 1% ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. The erythrocyte and leukocyte counts were determined by an improved Neubauer hemocytometer, with Hayem and Turck diluting fluids, and hemoglobin (Hb) measurement was determined by the cyanmethemoglobin method (Blackball and Daisley 1973). Capillary tubes containing blood were centrifuged for 3 min at 1000g and the hematocrit (Hct) was determined as the percentage of packed cell volume. The corpuscular indices, including the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were calculated using standard formulas (Svobodova et al. 1991) and values were also determined for Hct, blood Hb percentage, and red blood cell (RBC) count.

2-5 Statistical analyses

All results are expressed as mean ± SEM. Statistical analyses were carried out using the GraphPad Prism (Demo version). To evaluate the effect of exposure to Ag-NPs on hematological indices, all data were subjected to one-way ANOVA followed by Tukey's test at a 95% significance level ($p<0.05$), and also by t-test ($P<0.05$) to compare the hematological parameters in each of the control and treatment groups between the two sampling days (days 5 and 10).

3.Results

The number of WBCs was higher in all treatments than the control group ($P<0.05$). The highest values of WBCs were recorded for the T3 group both in the first and second blood sampling ($P<0.05$). Although Hct and Hb were higher in the T1 and T2 treatments than the control group ($P<0.05$), these parameters showed lower values than the control groups both in the first and second blood sampling for the T3 groups. The Ag-NPs increased the number of RBCs in all experimental treatments in the first blood sampling ($P<0.05$); however, the number of RBCs in the T3 group was lower than that of the control, T1 and T2 groups ($P<0.05$). The MCV and MCH in the treatment groups decreased as the concentration of Ag-NPs increased in the first blood sampling ($P<0.05$); but increased in the second blood sampling. In this respect, the lowest values of MCV and MCH were found in the T3 group in the first blood sampling. Also, there were no significant differences in MCHC values between the control group and treatment groups ($P>0.05$) (Fig.2).

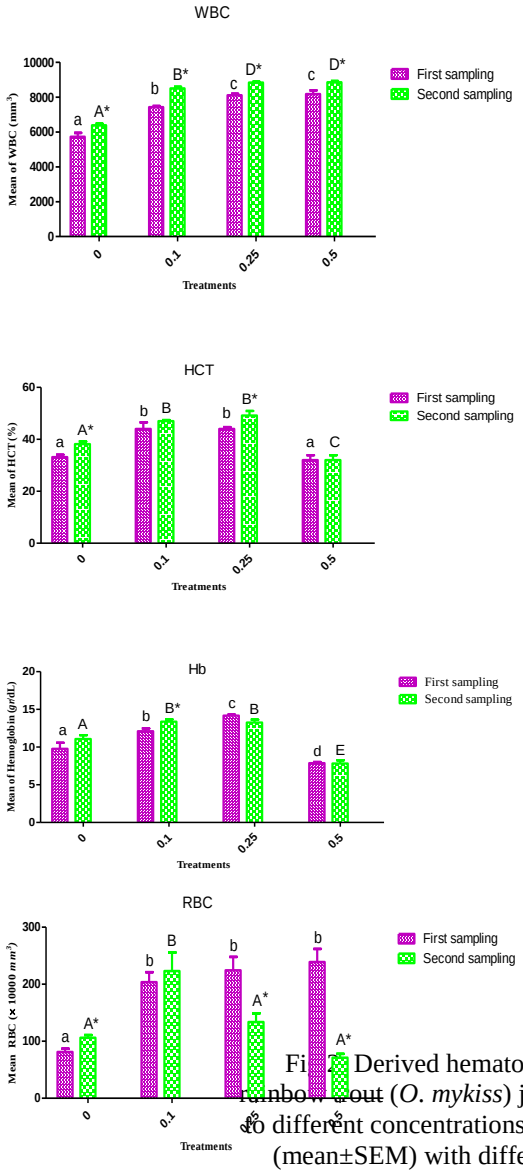
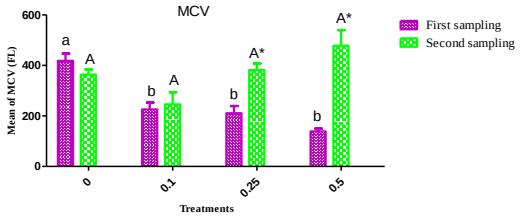
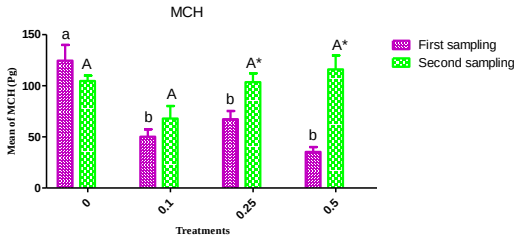


Fig.2. Derived hematological indices in rainbow trout (*O. mykiss*) juveniles, exposed to different concentrations of Ag-NPs. Bars (mean±SEM) with different letters are significantly different ($P<0.05$). Asterisks (*) indicate a significant difference ($p < 0.05$) between first sampling (5th day) and second sampling (10th day) in all groups ($p<0.05$) and small and capital letters indicate a significant difference between the control and nanoparticle-exposed animals ($p<0.05$) in the first and second blood sampling, respectively.





5- Discussion and conclusion

Despite the growth of the use of silver nanoparticles in the industry and consumer products, there is still very little data and information on the potential toxicity of these particles, especially in aquatic organisms. Ag-NPs may be released into aquatic environments from factory waste discharges, through leaks or spills during transportation and via materials containing Ag-NPs.

Hematology is a great tool for monitoring health standing, detection sickness, and following the progress of illness and response to therapy. In the present study, we examined Ag-NPs impacts on hematological parameters of juvenile rainbow trout, a most cultured fish species in aquaculture. In the present study, the number of WBCs was higher in all treatments than that in the control group with a maximum value in 0.5-g/L Ag-NPs treatment. Increases in WBCs are usually an immune reaction to foreign agents such as pathogens, pollutants, and toxins.

Elevated levels of WBCs in response to Ag-NPs may be due to their immunogenic impacts on the rainbow trout immune system. The values of Hct and Hb increased after exposure to 0.1, 0.25 g/L Ag-NPs; however, these values decreased in fish treated with 0.5g/l Ag-NPs. According to toxicology studies, some factors may be responsible for increasing of RBCs, Hct, and Hb after exposure to pollutants and heavy metals, including (a) Compensation of decreased oxygen-carrying capacity of Hb and RBCs by increasing the number of RBCs (Atamanalp et al. 2011) and (b) dilution of blood due to damages of gill epithelium, thus decreasing the Hct, RBCs, and Hb in volume unit (Joshi et al. 2002). In maximum concentration of Ag-NPs, i.e., 0.5 g/L, the Hct, RBCs, and Hb showed lower values than the control group. This demonstrates that a high concentration of Ag-NPs has adverse effects on the oxygen-carrying component of rainbow trout blood. These adverse effects may be the degradation of Hb and RBCs (Ololade and Oginni 2010), destruction of hematopoietic tissues (Ololade and Oginni 2010), and suppression of aerobic glycolysis and thus lack of enough energy for Hb synthesis (Joshi et al. 2002). In our study, the MCV and MCH in the treatment groups decreased as the concentration of Ag-NPs increased in the first blood sampling day. This may be due to the decreases in the size of RBCs (Benarjee et al. 2010).

The results obtained in this study are very similar to the findings of Imani and colleagues on adult rainbow trout (Imani et al. 2015). As a result, our results showed that Ag-NPs can significantly changes on the blood parameters of rainbow trout juveniles.

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