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Effect of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia, *Oreochromis mossambicus*

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ABSTRACT

The aim of the present study was to assess the oxidative stress, antioxidant response and histopathological changes of nickel nanoparticles (Ni NPs) exposure (14 days) in Mozambique tilapia, *Oreochromis mossambicus*. Ni NPs were synthesized by metal salt reduction method and characterized by X-ray diffraction (XRD) and Transmission electron microscopy (TEM). The XRD peaks at 44°, 51° and 76° were indexed to the (1 1 1), (2 0 0) and (2 2 0) Bragg's reflections of cubic structure of Nickel, respectively. The crystallite sizes were calculated using Scherrer's formula applied to the major intense peaks and found to be the size of 56 nm. TEM images showed that the synthesized Ni NPs are spherical in shape. Biochemical analysis indicated that the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity was significantly affected by Ni NPs treated *O. mossambicus*. Reduced antioxidant enzymes and the contents of antioxidants were lowered in the liver and gills of fishes treated with Ni NPs. After 14 days of exposure, a significant accumulation of Ni in the Ni NPs in experimental group was observed in the gill and skin tissues, with the highest levels found in the liver. Ni NPs exposed fish showed nuclear hypertrophy (NH), nuclear degeneration (ND), necrosis (NC) and irregular-shaped nuclei were observed in liver tissue. The hyperplasia of the gill epithelium (GE), lamellar fusion of secondary lamellae (LF), dilated marginal channel (MC), epithelial lifting (EL) and epithelial rupture were observed in gill tissue. Degeneration in muscle bundles (DM), focal area of necrosis (NC) vacuolar degeneration in muscle bundles (VD), edema between muscle bundles (ED) and splitting of muscle fibers were noticed in skin tissue. Further ecotoxicological evaluation will be made concerning the risk of Ni NPs on aquatic environment.

1. Introduction

Nickel (Ni) is a metal, its unique physical and chemical properties, its alloys are highly used in the manufacturing of jewelry, medical implants, stainless steel, Ni–Cd batteries and in the nickel-plating industry. Widespread extraction and use has resulted in increased levels of Ni in biogeochemical cycles and, as a result, increased human exposure through environmental contamination and occupational exposure (Munoz and Costa, 2012). Nickel nanoparticle (Ni NPs) is a product with many new characteristics, which include a high level of surface energy, high magnetism, low melting point, high surface area and low burning point and it can be widely used in modern industries such as catalysts, sensors and electronic applications (Zhang et al., 2003; Sivulka, 2005). These broad applications, however, increase human and environmental exposure and thus the potential risk related to their toxicity. There are some significant studies evaluating the toxic potential of Ni NPs. Ni NPs caused cytotoxicity and apoptosis in mouse epidermal JB6 cells (Zhao et al., 2009), cytotoxic effect in leukemia cancer cells (Guo et al., 2008) and toxicity and developmental defects reported in zebra fish (Ispas et al., 2009). Ni NPs were identified in lung macrophages and high levels of Ni measured in urine and kidneys showed evidence of acute tubular necrosis (Ahamed, 2011). As water resources are particularly vulnerable to direct and indirect contamination by nanomaterials, their potential toxicity to aquatic biota should be evaluated (Gong et al., 2011).

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The production, processing, and recycling of Ni products have resulted in a high level of pollution such that Ni contamination now occurs in water, soil and air (Lippmann et al., 2006). A wide range of environmental pollutants, as heavy metals, are known to induce oxidative stress in aquatic animals including fish. The generation of reactive oxygen species (ROS) induced by heavy metals is commonly associated with cellular injures due to alterations in DNA, proteins and membranes (Leonard et al., 2004). To counteract the adverse effect of ROS, living organisms have a complex and effective antioxidant defense system comprising enzymatic and non-enzymatic mechanisms such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbate, reduced glutathione (GSH) and Vitamin E (Kelly et al., 1998). Variations in these antioxidant defenses are very sensitive in revealing a pro-oxidant condition and have been used as biomarkers of oxidative stress in fish (Ahmad et al., 2006a, 2006b; Oliveira et al., 2008). Recently Rajakumar and Rahuman (2012) demonstrated that *O. mossambicus* adult fish used as a high-through put, highly efficient and cost effective to investigate the toxicity of Ag NPs. It can be concluded that analytical approaches to NPs in the aquatic environment are still in an initial phase of development.

The aim of the present study is to evaluate the effects of Ni NPs, on adult tilapia involving 14 day exposure, the metal burdens in fish tissues were measured, and toxicological endpoints such as oxidative stress, and histopathological changes were determined.

2. Experimental procedure

2.1. Synthesis of Ni NPs

The synthesis of Ni NPs was prepared by using poly vinyl pyrrolidone (PVP). 3.3 mM solution of $NiCl_2 \cdot 6H_2O$ (Nickel chloride) was added with 60 mL of pure ethanol in 125 mL round bottom flask at room temperature. An amount of 0.025 g PVP was added to the solution and the mixture was stirred until the total dissolution of PVP. A volume of 8 mL hydrazine hydrate followed by 1.7 mL of 1 M NaOH solution were added under strong magnetic stirring. The mixed solution was heated up to 60 °C with constant shaking and within 5 min the solution turned black indicating the formation of Ni NPs. The solution was then allowed to cool in ambient conditions at room temperature. After cooling, acetone was added in excess in order to precipitate out the NPs. The resulting solution was ultra-centrifuged at 20,000g and particles were collected and washed with chloroform: methanol (1:1) solution (Singh et al., 2011).

2.2. Characterization of Ni NPs

XRD measurements of the synthesized Ni NPs were carried out on films of the respective solutions drop-coated onto glass substrates using Phillips PW 1830 instrument operating at 40 kV voltage with a current of 30 mA with Cu$K\alpha$1 radiation. The diffracted intensities were recorded at 2θ angles from 20° to 80°. The size of the nanoparticles was confirmed by using Transmission electron microscopy analysis (Hitachi H-7100 using an accelerating voltage of 120 kV and methanol as solvent). The elemental analysis was carried out under energy dispersive X-ray spectroscopy (INCAPENTA-FET). Dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) used for the characterization of hydrodynamic size and zeta potential (ζ) of Ni NPs in solution were performed on a Malvern Instruments Zetasizer Nano-ZS instrument as described by Murdock et al. (2008).

2.3. Particle suspension preparation

A stock solution of 10 g/L Ni NPs was prepared by dispersing the nanoparticles (dry powders) in double distilled water with sonication for 6 h in a bath-type sonicator (100 W, 40 kHz) at different concentrations (0.1, 1.0 and 10 mg/L) of the exposure solutions were obtained by diluting the stock solution with aerated tap water and a further 30 min sonication immediately prior to dosing each day.

The concentration of Ni NPs in the exposure solution was quantified by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500). Ni NPs samples (1 mL) with the combination of 3 mL of concentrated nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) were added into each of polytetrafluoroethylene (PTFE) digestion tubes. After 15 min, the vessels were sealed to avoid any acid leakage and put into the microwave. Then the samples were digested using a three-stage digestion protocol (5 min at 150 °C, 180 °C and 200 °C) and cooled down. Subsequently, Ni concentration in digested samples was determined by ICP-MS. The instrumental parameters were included the RF power 1.0 kW, Nebulizer pressure 1.0 kPa, carrier gas (Ar) flow rate 15.0 L/min, peristaltic pump rate 15 rpm/min, Integration time High WL Range 5 s, Low WL Range 30 s, and wavelength 336.12 nm. The pretreatment method could recover (95 ± 5) percent Ni concentration in the tested samples (Hao and Chen, 2012).

2.4. Experimental fish

Mozambique tilapia (*O. mossambicus*) is a fresh water fish abundant in India, was chosen as the test organism. The fish was obtained from a local aquatic breeding base, (Ram Ragu fisheries) Walajapet, Tamil Nadu, India. The same batches of *O. mossambicus* with mean values of 4.0 ± 1.0 cm in length and 2.0 ± 1.0 g in weight were acclimatized to laboratory conditions for 2 weeks prior to the experiments. In the toxicity study, they were maintained in 10 L aerated glass aquarium containing dechlorinated tap water. The water quality parameters were as follows: pH 7.1–7.6, temperature 27 ± 2 °C, dissolved oxygen concentration 6.5–7.8 mg/L, hardness (as $CaCO_3$) 82 mg/L, photoperiod, 12 h light/12 h dark.

2.5. Experimental design

In the sub-acute toxicity test, adult fish (10 per group) were maintained in 10 L glass aquaria, and exposed at different concentrations (0.1, 1.0 and 10 mg/L) of Ni NPs for 14 day. Three replicates were performed for each concentration. To maintain a relative stable aqueous phase concentration of Ni NPs, the exposure solution was completely refreshed daily. The control group was provided with fresh aerated tap water without any NPs under the same conditions. Fish were not fed during the experiment period in order to minimize the risk of Ni NPs absorbing to food or fecal material and help to maintain water quality. The study was conducted in accordance with the permission and approval of Government of India, Ministry of Environment and Forest, New Delhi, India (Committee for the purpose of control and supervision of experiments on animals; Reg. No.: 1011/c/CPCSEA).

2.6. Oxidative stress analysis

All tests of biomarkers were conducted within 2 days after the preparation of samples. The tissue samples were extracted from the experimental fishes in random and were performed in triplicate. The frozen organs (about 0.5 g) were homogenized in five-fold chilled 100 mmol/L, pH 7.4 sodium phosphate buffer solution containing 20 percent (v/v) glycerol, 1 mmol/L EDTA, and 1.4 mmol/L dithioerythritol (DTE) by hand on ice using a glass tissue homogenizer. The homogenates were centrifuged at 10,000*g* for 10 min at 4 °C and the resultant supernatant was stored in 0.5 mL aliquots to assess possible effects on oxidative stress and antioxidant defense. All biochemical assays were performed in triplicates.

SOD activity was determined based on the ability of the enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) (Crouch et al., 1981). Briefly, the assay mixture consisted of 50 mM phosphate buffer (pH 7.8) with 5 mM EDTA, 50 mM xanthine, 1 mM NBT, 5.4 U/mL xanthine oxidase and 50 mL aliquot of tissue homogenate. The reaction was initiated by adding xanthine oxidase. The reduction of NBT by superoxide anion to blue formazan was measured at 560 nm. The rate of NBT reduction in the absence of tissue was used as the reference rate. One unit of SOD was defined as the amount of protein needed to decrease the reference rate to 50 percent of maximum inhibition. The SOD activity was expressed in units/mg protein.

CAT activity was determined by the method of Aebi (1983) by measuring the decrease of H_2O_2 concentration at 240 nm for 2 min in 50 mM sodium phosphate buffer (pH 7.0) containing 15 mM H_2O_2 and the enzyme extract. Briefly, the assay mixture consisted of 1.95 mL phosphate buffer, 1 mL H_2O_2 and 0.05 mL tissue homogenate in a final volume of 3 mL. The CAT activity was calculated in terms of nmol H_2O_2 consumed/min/mg protein using a molar extinction coefficient of 43.5 $(M\ cm)^{-1}$.

The POD activity was assayed using guaiacol as a hydrogen donor by measuring the change at 470 nm over 1 min. Enzyme activity was defined as unit (one activity unit defined as absorbance at 470 nm changes 0.01 per min) per gram fresh weight of tissue (Chance and Maehly, 1955).

2.7. Tissue Ni content analysis

For tissue Ni content analysis, liver, gill, and skin were dissected from Ni NPs exposed *O. mossambicus* and the wet weights were immediately recorded. Tissue samples were digested with a combination of 2.0 mL concentrated HNO_3 and 2.0 mL hydrogen peroxide at 60 °C for 3 h in a loft dryer. The temperature was subsequently increased to 160 °C for 1 h for further digestion. The samples were cooled and diluted in a total volume of 10 g 2 percent HNO_3. Nickel contents were quantified via ICP-MS (Wu and Zhou, 2013).