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Physiological and biochemical effects of nickel on rainbow trout (*Oncorhynchus mykiss*) tissues: Assessment of nuclear factor kappa B activation, oxidative stress and histopathological changes

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HIGHLIGHTS

- We investigated biochemical and physiological effects in the liver, gill and kidney tissues of fish exposed to nickel.
- NFkB immunopositivity was detected in all tissues exposed to nickel.
- Nickel exposure induces oxidative and histopathological damage in all tissues.
- Nickel may disturb the biochemical and physiological functions of fish.
- Our manuscript makes contribution for the toxicity assessment.

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ABSTRACT

We investigated changes in nuclear factor kappa B (NFkB) activity, antioxidant responses and histopathological effects in the liver, gill and kidney tissues of rainbow trout exposed to nickel chloride (Ni). Two different concentrations (1 mg/L and 2 mg/L) were administrated to fish for 21 days. Tissues were taken from all fish for NFkB activity, histopathological examination and determination of superoxide dismutase (SOD), catalase (CAT) enzyme activity and of lipid peroxidation (LPO), and glutathione (GSH) levels. The findings of this study indicated that Ni exposure led to a significant increase in LPO indicating peroxidative damage and antioxidant enzymes SOD and CAT activity in tissues ($p < 0.05$), but 2 mg/Ni concentration caused a significant decrease in CAT activity in kidney tissues ($p < 0.05$). One of mechanism in the antioxidant defense system seems to be GSH, which increased in gill and kidney tissues of fish exposed to Ni ($p < 0.05$). NFkB immunopositivity was detected in all tissues. Ni exposure caused lamellar thickening, cellular infiltration in gill tissues, hydropic degeneration of hepatocytes in liver tissues, hyalinous accumulation within the glomeruli and tubular degeneration in kidney tissues. Our results suggested that Ni toxicity may disturb the biochemical and physiological functions of fish by causing changes in NFkB activity and oxidative and histopathological damage in the tissues of rainbow trout. This study can provide useful information for understanding of Ni-induced toxicity.

1. Introduction

In recent centuries, agricultural, industrial and anthropogenic activities have led to the increasing release of heavy metal into aquatic areas (Nowicka et al., 2016), thus aquatic organisms, especially fish are exposed to a significant amount of these contaminants in aquatic environments (Heath, 1987; De Mora et al., 2004; Atli et al., 2006; Hwang et al., 2006). Therefore, heavy metal contamination is a worldwide problem and may cause adverse effects by affecting the ecological balance of the environment and the diversity of aquatic organisms (Okocha and Adedeji, 2011). Thus, it is necessary to evaluate the potential risks of heavy

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metals such as nickel to aquatic organisms especially fish. Of the heavy metals, nickel chloride (NiCl2) is released both through anthropogenic activity and through natural sources. The progress of industrialization has led to increased emission of pollutants into ecosystems (Cempel and Nikel, 2006). Ni is a ubiquitous, naturally occurring trace metal (0.0086% of the earth's crust), with increased concentrations in water bodies: water generally contains Ni at concentrations < 10 μg/L (Kienle et al., 2008; Cempel and Nikel, 2006). Ni concentrations in freshwater environments contaminated with Ni reach up to 2.5 mg/L (Eisler, 1998). Rivers have been shown to carry concentrations from 0.001 to 0.01 mg Ni/L up to 0.5 and 2 mg Ni/L (Chau and Kulikovsky-Cordeiro, 1995).

Nickel is one of the environmental pollutants and is known to be a potential toxicological hazard for living organisms because of its carcinogenic, teratogenic, genotoxic and immunotoxic effects (Costa et al., 2005; Cempel and Nikel, 2006; Vijayavel et al., 2009; Adjroud, 2013; Kubrak et al., 2013). The effects of Ni have been investigated in many organisms, but the effect of Ni on fish has scarcely been studied. A few studies indicated that Ni exposure caused histopathological damage in different fish species (Athikesavan et al., 2006; Scheil et al., 2010). A study by Athikesavan et al. (2006) showed that Ni induced histopathological damage in freshwater fish, (*Hypophthalmichthys molitrix*) exposed to 5.7 mg/L Ni. It has been found that Ni can lead generation of reactive oxygen metabolites (Kubrak et al., 2013), lipid peroxidation (Rodriguez et al., 1991) and thus Ni can cause oxidative stress by ROS generation (Klein et al., 1991). Histopathological and oxidative stress studies would give a potential toxicological assessment of heavy metals in aquatic organisms (Javed et al., 2016). ROS and oxidative damage induced by contaminants may be a mechanism of toxicity in aquatic organisms living in polluted areas (Monteiro et al., 2006). Therefore, antioxidants can be used as bioindicators of aquatic environmental health; they are sensitive to environmental pollutants (Li et al., 2011). Antioxidant enzymes found in all organisms are superoxide dismutase- (SOD), catalase- (CAT) and glutathione- (GSH) dependent enzymes (e.g., glutathione S-transferases (GST), glutathione reductase (GR), and glutathione peroxidase (GSH-Px)) (Xing et al., 2012a, b).

Oxidative stress occurs in the presence of reactive oxygen compounds in the cells of all aerobic organisms, as a consequence of an imbalance between antioxidant and prooxidant systems; this leads to cell damage causing oxidation in biomolecules such as proteins, nucleic acids, lipids and carbohydrates (Anjana Vaman et al., 2013; Ye et al., 2016; Hintsala et al., 2016). NFkB transcription factor complex is one of the cellular sensors which responds to oxidative stress (Helenius et al., 1996). Oxidative stress may activate the nuclear transcription factor (NFkB), causing damage to DNA (Ye et al., 2016). NFkB is induced by ROS and inhibited by antioxidants (Campo et al., 2008). It plays an important role in many physiological functions, such as apoptosis and cell proliferation, and regulates expression of many genes, such as nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), synthesis of cytokines, adhesion molecules and chemokines, in cells (Barnes and Karin, 1997; Han et al., 1998; Ozbek et al., 2009; da Silva et al., 2014). NFkB is found in the cytosol in an inactive form associated with regulatory inhibitory proteins called IkBs (Sangrador-Vegas et al., 2005). Stressful stimuli such as hypoxia and reactive oxygen species (ROS) induce NFkB activity which is involved in acute stress and immune response (Schreck et al., 1992; Barchowsky et al., 1996; Campo et al., 2008). NFkB can be induced by the treatment of the cells with toxins such as nickel (Schmitz, 1995).

The effects of Ni have been investigated in many organisms, but the effect of Ni on rainbow trout has scarcely been studied and there is no study associated with NFkB in fish exposed to environmental pollutants, especially heavy metals. Thus, we aimed to assess NFkB activity, histopathological damage, and changes in SOD and CAT activities, with LPO and GSH levels in liver, gill and kidney tissues of rainbow trout (*Oncorhynchus mykiss*) after 21 days of Ni exposure.

2. Materials and methods

2.1. Fish

Rainbow trout, *Oncorhynchus mykiss*, weighing 150 ± 5.82 g, 14.62 ± 0.61 cm were provided by Ataturk University (Erzurum, Turkey), the Faculty of Fisheries, and the Inland Water Fish Breeding and Research Center. During the acclimation period of 15 days, fish were held in fiberglass tanks containing 400 L of dechlorinated tap water, with 15 fish in each tank. Tanks have flow-through system (water flow rate: 0.5 L/min). The temperature was 10–12 °C, the pH 7.4, water hardness was 173.5 mg/L, with dissolved oxygen 7.1 mg/L. Fish were fed daily with commercial trout pellets at 2.5% body weight during the acclimation periods. The experiments were carried out in accordance with the approved ethical rules of Ataturk University (Erzurum, Turkey).

2.2. Chemical exposure

Nickel chloride ($NiCl_2$, CAS No: 7718-54-9, purity ≥ 98%) was purchased from Sigma-Aldrich Co and its stock solution was prepared by dissolution in distilled water. The nominal concentrations used for this study were 1 mg/L and 2 mg/L. These concentrations were chosen because they may be found in a polluted environment (Eisler, 1998). Two experimental groups and the control group each comprised 15 fish. Three replicates were performed for each group. A 21-day chronic toxicity test was performed in accordance with standard Organization for Economic Co-operation and Development (OECD) guideline number 204 (Fish, prolonged toxicity test) (OECD, 1984). Fish were exposed to 1 mg/L and 2 mg/L NiCl2 concentrations by adding to water every day for 21 days respectively under controlled laboratory conditions (OECD, 1984; Topal et al., 2015). Chemical treatment was renewed every 12 h. At the end of the exposure, fish were sacrificed by cervical section, and liver, gill and kidney tissues of both control and treated fish were immediately removed. A portion of these tissues was stored at −20 °C until the analysis of antioxidant enzyme activities. The other portion of the tissues was fixed in 10% neutral buffered formalin solution for NFkB activity and histopathological examination.

2.3. Lipid peroxidation (LPO) determination

The level of LPO in tissues was determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test (Ohkawa et al., 1979). The tissues were scraped, weighed and homogenised in 10 ml of 100 g/L KCl. The homogenate (0.5 ml) was added to a solution containing 0.2 ml of 80 g/L sodium laurylsulphate, 1.5 ml of 200 g/L acetic acid, 1.5 ml of 8 g/L 2-thiobarbiturate and 0.3 ml of distilled water. The mixture was incubated at 98 °C for 1 h. Upon cooling, 5 ml of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery rate was over 99%. The results were expressed as nmol MDA/g tissue.

2.4. Superoxide dismutase (SOD) activity

SOD activity was measured according to Sun et al. (1988). SOD estimation was based on the generation of superoxide radicals