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Bioaccumulation, cytotoxicity and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*

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ABSTRACT

Selenium (Se) is an essential trace-element that becomes toxic when present at high concentrations for aquatic organisms. The knowledge about the mechanism of Se toxicity in freshwater ecosystem is still poorly studied. Thus the aim of the present study was to assess the impact of environmentally relevant concentrations of Se toxicity: 5, 10, 25, 50 and 100 μg/L or water only (control) for periods of 96 hour (h) to test for Se accumulation (gill, liver and brain), its effects on enzymatic and non-enzymatic antioxidant defenses (gill and liver), oxidative stress effects on lipid, protein (gill and liver), DNA (liver) and inhibition of AchE (brain) activity were measured in Mozambique tilapia, *Oreochromis mossambicus*. Our result showed that Se accumulation was observed in the gill, liver and brain tissues of fish exposed to different concentrations and accumulation varied upon different tissues. Enzymatic (SOD, CAT, GPx and GST) and non-enzymatic (GSH and MT) antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) were significantly increased after 96 h exposure of higher concentrations Se in the gill and liver tissue with the exception of GST activity was significantly inhibited in liver after 96 h exposure of higher concentrations of Se. In contrast, catalase (CAT) activities were inhibited for both tissues of Se exposure at 96 h. Reduced glutathione (GSH) and Metallothionein (MT) levels were increased in the gill and liver tissues after exposure to Se for 96 h. We also observed that Se affected antioxidant defense, increasing oxidative stress indicator of lipid peroxidation (LPO) and protein carbonyl (PCO) in gill and liver tissues of fish exposed to Se for 96 h at the concentration dependent manner. Increased DNA damage scores observed in liver tissue of fish exposed to Se for concentrations dependent manner, indicating potential of Se on fish. We also observed inhibition of acetylcholine esterase (AchE) activity in brain tissue of fish exposed to Se for higher concentrations. The changes in these parameters can be used as suitable biomarkers for monitoring the toxicity of Se in the aquatic environment.

1. Introduction

Trace elements of metal represent one of the most widespread and serious form of environmental contamination (Cardwell et al., 2013; Torre et al., 2013; Velez et al., 2016; Coppola et al., 2017; Aliko et al., 2018; Capillo et al., 2018). Selenium (Se) is an essential trace element to animals but toxic at excessive level. The margin between essentiality and toxicity of selenium is very narrow (Lemly, 2002a). The Se is present in aquatic ecosystems originates from both natural and anthropogenic sources (Navarro-Alarcon and Cabrera-Vique, 2008). Selenium levels are generally in the range of 1–10 μg/L in natural water (Sohrin and Bruland, 2011; European Commission, 1998) though levels can exceed above 50–1000 μg/L in water receiving effluents from agricultural irrigation or coal mining (Schiavon et al., 2012; USEPA, 2009).

Selenium is an essential micronutrient in animals for normal growth and development, such as the elements of zinc or copper (Aliko et al., 2015; Pagano et al., 2017). The metabolic functions of the selenoenzyme particularly Se dependent antioxidant enzyme of glutathione peroxidase (GPx) are protect the cell membrane from oxidative stress, because it is a part of the mechanism responsible for the metabolism and detoxification of oxygen (Rotruck et al., 1973; Nève, 1991). However, the excessive amounts of Se can be very toxic to aquatic organisms (Lemly, 2002a), because it has a narrow range between

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nutritional requirements and toxic levels (Kobayashi et al., 2002). At levels slightly above homeostatic requirement, Se is toxic (Zhang et al., 2014) presenting carcinogenesis (prostate, liver), cytotoxicity (arrest cell cycle and inhibiting cell growth) and genotoxicity (affecting DNA) effects (Sun et al., 2014). The toxicity of Se is not only related to its chemical similarity to sulfur and to its ability to be substituted during the assembly of proteins, but also to the oxidative stress (Lavado et al., 2012). Selenium species, particularly the inorganic ones, react with thiols and generate oxygen free radicals that account to Se toxicity to cells (Mézes and Balogh, 2009; Spallholz and Hoffman, 2002).

Biochemical biomarkers are commonly used to measure the effects of contaminants on organisms in ecotoxicological studies (Savorelli et al., 2017; Faggio et al., 2016). Under normal metabolic processes, a balance between generation and neutralization of reactive oxygen species (ROS) include superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}HO$) is maintained by organisms. However, it has been proved that bioaccumulate for metal ions enhance the formation of reactive radicals in the cells which cause the cell damage (Valko et al., 2005). Painter (1941) reported that the selenite toxicity is related to the oxidation of endogenous thiol. More recently, it has been suggested that Se-mediated thiol oxidation cause reactive oxygen species (ROS) (Stewart et al., 1998; Chen et al., 2007; Plano et al., 2010), and oxidative stress can be a factor related to Se-induced toxicity (Kitahara et al., 1993; Shen et al., 2001; Kim et al., 2007; Xiang et al., 2009). Therefore, Se can be bioaccumulate in tissues and increase the production of ROS to damage the biological molecules such as lipid or protein or DNA. This oxidative damage can be assessed by measuring the lipid peroxidation (LPO), protein carbonylation (PCO) (Spallholz et al., 2004; Miller et al., 2007; Misra and Niyogi, 2009; Cruz et al., 2016). The genotoxicity of environmental contaminants can be monitored using a broad range of *invitro* and *invivo* biomarkers of DNA damage. The comet assay used as an excellent tool for monitoring environmental genotoxicity in fish (Selvi et al., 2013).

Aerobic organisms require mechanism that prevent or limit cellular damage which is caused by reactive oxygen species (ROS), and cells have evolved interdependent enzymatic and non-enzymatic antioxidant defense systems (Banaee et al., 2013; Alomar et al., 2017). Antioxidant enzymes of superoxide dismutase (SOD) decomposes superoxide anion to hydrogen peroxide, and catalase (CAT) decomposes H_2O_2 to molecular oxygen and water, and glutathione peroxidase (GPx) reduce both H_2O_2 and lipid hydroperoxide (Almeida et al., 2007; Monteiro et al., 2010). Glutathione S-transferase (GST) catalyzes the conjugation of pollutants to eliminate them from the cellular system (Matozzo et al., 2013). In addition, a non-enzymatic antioxidant enzymes of metallothionein (MT) and glutathione (GSH) that function as radical quenchers and reductant in conjugation with xenobiotics (Dondero et al., 2005; Livingstone, 2001). Besides the antioxidant enzymes, other environmental contamination biomarkers can be used as tool to evaluate the organism response to pollutants, such as acetylcholinesterase (AChE) activity for neurotoxic effect. Acetylcholinesterase plays an important role in the cholinergic system including nerve impulse transmission in synapses (Munari et al., 2014), and it cleaves acetylcholine into choline and acetate. This enzyme is responsible for the degradation of the neurotransmitter acetylcholine in the synaptic cleft. Its inhibition is strongly associated with exposure of metals (Romani et al., 2003; Senger et al., 2006; Pretto et al., 2010).

Fish are ideal sentinel for detecting and documenting aquatic pollutants and largely used as bio-indicator of environmental pollution (Van Der Oost et al., 2003; Fazio et al., 2012; Sehonova et al., 2018). Tilapia has an important place in the world of aquaculture breeding and is a good biological model for toxicological studies due to diverse characteristics, namely their high tolerance to a wide variety of environmental conditions (Fontainhas Fernandes, 1998). Se toxicity mechanisms are not well-understood in fish. Studies regarding Se toxicity in aquatic environment are still scarce, however, with most reports dealing with its protective effects against exposure to toxic elements such as mercury, lead, chromium and cadmium (Orun et al., 2008; Ralston and Raymond, 2010; Bjerregaard et al., 2011). To the best of our knowledge, there is limited reports are available on the effects of Se-toxicity in fish compared to other toxic metals, such as copper (Cu) or cadmium (Cd) (Pyle and Couture, 2012), which might be an important aspect of the mechanistic action of Se toxicity.

The present study was designed to investigate the acute toxicity effects of Se exposure on 96 h for inducing oxidative stress in Mozambique tilapia, *Oreochromis mossambicus*. However, the effect of Se on the induction of neurotoxicity, oxidative stress and the antioxidant defenses has not been thoroughly investigated in freshwater fish. Therefore, present study for assessing the accumulation of Se for induction of neurotoxicity (AchE) (brain), genotoxicity (DNA) (liver), oxidative stress effect (LPO and PCO), enzymatic (SOD, CAT, GPx and GST) and non- enzymatic (MT and GSH) antioxidant defenses were assessed in gill and liver tissue of *O. mossambicus*.

2. Materials and methods

2.1. Experimental animals

O. mossambicus (n = 180; 8.4 ± 0.6 g; 7.7 ± 0.3 cm; mean ± SD), were purchased from a local fish farm located in Karaikudi, Tamil Nadu (India). Fish were placed in 1000-L tank with aerated and filtered dechlorinated freshwater for acclimated period in the laboratory conditions for one week. During the acclimatization period, the physical and chemical characteristics of water parameters were, T: 29.4 ± 1.1 °C; pH: 7.0 ± 0.3; salinity: 0.25 ± 0.05 ppt; dissolved oxygen: 6.9 ± 0.4 mg O_2 L^{-1}; total ammonia: 0.09 ± 0.01 mg N-NH_4 L^{-1}; conductivity: 342.6 ± 16.2 µs/cm; alkalinity: 42.7 ± 6.2 mg $CaCO_3$/L; total hardness: 136.5 ± 9.6 mg $CaCO_3$/L, respectively and this parameter did not differ during the experimental period. The fish were fed with commercial fish food (Tairoun Feed Company, Taipei, Taiwan) for once a day and maintained under a 12:12 h light/dark photoperiod. Fish were fed with acclimation period (7 days), but were fasted for 24 h prior to experimental period to prevent extensive water contamination by excrements. Experiments were conducted in accordance with national and institutional guidelines for animal welfare. All procedures involving experimental design and fish handling were reviewed and approved by the Committee of Animal Care and Use, Faculty of Science, Alagappa University.

2.2. Experimental design

After acclimation, fish were subjected to static mode test for 96 h in 100-L glass aquaria with 80-L of freshwater. Ten fish were placed in each group of aquaria with different nominal concentrations of Se: 5 µg/L^{-1}, 10 µg/L^{-1}, 25 µg/L^{-1}, 50 µg/L^{-1} and 100 µg/L^{-1} of Se, in the form of sodium selenite (Na_2SeO_3) and exposed to these conditions for 96 h (no mortality occurred from these exposures). The lowest Se doses was choose on the tested was based on environmentally relevant concentrations of 5–10 µg/L (Sohrin and Bruland, 2011; European Commission, 1998) and the others doses were defined for the geometric progression of ratio 25 to a maximum concentration of 100 µg/L, which would be safe for *O. mossambicus*. Each exposure was performed in triplicate for a total of 30 fish per treatment. Two fish (n = 12 in each group) from each tank were used in all analysis. Survival of fish was monitored for daily and fish were not fed during the experimental period. Fish in the control group were maintained under the same holding condition, but without addition of sodium selenite to the water. During the experimental period the water was not changed over the 96 h to avoid stressing the animals. Levels of dissolved oxygen, temperature, salinity, pH, total ammonia, total hardness, alkalinity and conductivity were recorded every 24 h. After 96 h, the water samples are collected to determine the total and dissolved concentrations of Se in water samples for non-filtered and filtered through a 0.45 µm syringe