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ORIGINAL ARTICLE

Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*

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Abstract This study was carried out to determine the median lethal concentrations (LC_{50}) of Zinc nanoparticles (ZnNPs) on *Oreochromis niloticus* and *Tilapia zillii*. The biochemical and molecular potential effects of ZnNPs (500 and 2000 μg L^{-1}) on the antioxidant system in the brain tissue of *O. niloticus* and *T. zillii* were investigated. Four hundred fish were used for acute and sub-acute studies. ZnNP LC_{50} concentrations were investigated in *O. niloticus* and *T. zillii*. The effect of 500 and 2000 μg L^{-1} ZnNPs on brain antioxidants of *O. niloticus* and *T. zillii* was investigated. The result indicated that 69 h LC_{50} was 5.5 ± 0.6 and 5.6 ± 0.4 for *O. nilotica* and *T. zillii*, respectively. Fish exposed to 500 μg L^{-1} ZnNPs showed a significant increase in reduced glutathione (GSH), total glutathione (tGSH) levels, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activity and gene expression. On the contrary, malondialdehyde (MDA) levels significantly decreased. Meanwhile, fish exposed to 2000 μg L^{-1} ZnNPs showed a significant decrease of GSH, tGSH levels, SOD, CAT, GR, GPx and GST activity and gene expression. On the contrary, MDA levels significantly increased. It was concluded that, the 96 h LC_{50} of ZnNPs was 5.5 ± 0.6 and 5.6 ± 0.4 for *O. nilotica* and *T. zillii*, respectively. ZnNPs in exposure concentrations of 2000 μg/L induced a deleterious effect on the brain antioxidant system of *O. nilotica* and *T. zillii*. In contrast, ZnNPs in exposure concentrations of 500 μg L^{-1} produced an inductive effect on the brain antioxidant system of *O. nilotica* and *T. zillii*.

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1. Introduction

The development of nanotechnology produces many nanoparticles (NPs) that are important in medicine, agriculture and industry (Grażyna et al., 2014). Nowadays, nanoparticles of

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metals are widely used in many sectors such as medicine, agriculture and industry; however, few studies were done on their environmental impact and fate (Paresh et al., 2009). Metal NPs may leak into natural bodies of water in their life cycles (production, storage, transportation, consumption, disposal, or reproduction). There is lack ofinformation regarding the magnitude of NPs released into the aquatic system and their impact on living organisms. Therefore, there is an urgent need for information on the ecological risks of metal NPs (Alkaladi et al., 2015). Recently, researchers are interested to investigate the toxicity of NPs. Some studies have proved the toxicity of NPs, such as metal oxides to bacteria, human cells, rodents and aquatics (Lin and Xing, 2007). NP toxicity mechanisms are complicated (Warheit et al., 2006). It may stimulate the reactive oxidative species (ROS) generation through disruption of intracellular metabolism (Long et al., 2006) or damage the antioxidant defense system (Brown et al., 2004), resulting in protein, lipids, DNA and carbohydrate damage (Kelly et al., 1998). Brain is the most liable organ in the body for the adverse effects of oxidative damage because it contains a high level of unsaturated fatty acids, which are easily peroxidizable, its disproportional large consumption of oxygen per unit weight and its not particularly generous antioxidant defense (Nikolaos et al., 2006; Afifi et al., 2010). Transcript level alterations are the earliest sensitive bio-indicators for biological responses to stress. Thus, genes with expression levels, that are altered in response to environmental stresses can be used for diagnosis and quantify of the effects of these stresses on the organisms (Dondero et al., 2006). The ecotoxicological data on ZnNPs are just emerging and scanty. Our previous work proved the toxic effects of Zinc oxide nanoparticles (ZnONPs) on the liver and gills of freshwater fish *Oreochromis niloticus* at low exposure level for $LC50_{96h}$ was 3.1 ± 0.4 mg L^{-1}. Also, we indicated that the toxicity of ZnONPs occurred through the induction of lipid peroxide (LPO) synthesis (Alkaladi et al., 2014, 2015). The toxicity of ZONPs in juvenile carp was documented and manifested by the inhibition of superoxide dismutase (SOD), catalase (CAT), and GPx activity and reduced GSH content as well as increase in the level of LPO (Linhua and Lei, 2012). Oberdörster (2004) reported that, uncoated fullerenes produced an oxidative stress and lipid peroxidation in fish brain tissue, this proved the bad impact of NPs on aquatic health.

No studies have investigated the toxic effects of ZnNPs on expressions of oxidative stress-related genes in the brain of *O. niloticus* and *Tilapia zillii*. In the current study, we aimed to assess the changes of gene expression and activity of antioxidant enzymes in the brain tissue of both *O. niloticus* and *T. zillii* exposed to different levels of ZnNPs.

2. Materials and methods

2.1. Nanoparticles

Zn nanoparticle was purchased from Sigma–Aldrich Co. LLC. GmbH, Steinheim, Germany. Zn nanoparticle was in the form of nanopowder, 35 nm avg. part. Size, ⩾99% trace metals basis. Zn nanoparticle surface area was determined using the multi-point Brunauere Emmette Teller (BET) method. The measured surface areas were 40 m^2/g that did not differ from the manufacturer's values.

2.2. Preparation of Zn nanoparticle suspension

The Zn nanopowder was suspended directly in deionized water at concentrations of 500 and 2000 μg L^{-1}. ZnNPs were dispersed using ultrasonic vibration (40 kHz) for 30 min to prevent NP aggregation. ZnNP suspension was daily prepared. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine Zn concentrations in the exposed water at zero, 12 and 24 h of exposure to verify that the exposure concentrations are the same as the prepared concentrations as indicated in Table 1. Zn shape and size were determined using the transmission electron microscope (TEM) (JEM-1011, JEOL, Japan). ZnNP nanoparticle was nearly spherical and very fit with the nano-scale, and the measured particle size was close to the manufacturer information as indicated in Fig. 1.

2.3. Fish preparation

The Ethics Committee of King Abdualaziz University approved the procedures of the current experiment. Four hundred males of both *O. niloticus* and *T. zillii*, weighting 90 ± 5 g and 15 ± 3 cm in length were used in this study. Fish were kept in 40 aquaria (n = 10 fish/aquarium). The aquarium water was changed daily. An aeration system (Eheim Liberty 150 Bio-Espumador cartridges) was used for water aeration. The temperature was maintained at 28 ± 2 °C and dissolved oxygen, at 7.0 ± 0.5 mg L^{-1}. Fish were fed with a commercial fish diet. The daily feed amount was 10% of body weight and the fish were fed 3 times daily. Fish were acclimatized for 15 days before the beginning of the experiments.

2.4. Acute toxicity

A graded series of ZnNP suspension of 0, 1, 3, 5, 7 and 14 mg L^{-1} was used in triplicate for the lethal toxicity study. Ten fish each of *O. niloticus* and *T. zillii* were exposed to each

Table 1 The actual ZnNP concentrations (mg L^{-1}) in the exposed water.

Concentrations	Time (h)				
	Zero	12		24	
	M ± SD	M ± SD	% of change	M ± SD	% of change
Control	nd	nd	nd	nd	nd
500 μg L^{-1}	500 ± 30	470 ± 23	−6	450 ± 33	−10
2000 μg L^{-1}	2000 ± 125	1920 ± 104	−4	1890 ± 106	−5.5

nd = not detected.