Zinc nanoparticles induced brain lesions and behavioral changes in *Tilapia nilotica* and *Tilapia. zillii*

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**ABSTRACT**

In this study, the induced behavioral changes and neuropathology of prolonged exposure to high doses of Zinc nanoparticles (Zn-NPs) in two species of tilapia, *T. nilotica* and *T. zilli*, was investigated. Fish that were treated with 2000 µg Zn-NPs/L showed severe degenerative changes and vacuolation in the neuropil, particularly of the optic tectum, with congestion of the blood vessels of both the cerebral cortex and the meninx primitive. Loss of the Purkinje cell layer of the cerebellum was noticed as well. Fish showed behavioral changes that included loss of equilibrium, slower movement and swimming sideways. No significant differences (P < 0.05) were observed between *T. nilotica* and *T. zilli* in behavioral or pathological changes. The results highlighted the Zn-NPs neurotoxicity and its accompanied neuropathology and related behavioral changes in fish. More caution is needed during the usage of Zn-NPs to avoid possible deleterious impacts on human and animal health.

**Key words:** Behavioral alteration, Nanoparticles, Neuropathology, Tilapia.

**INTRODUCTION**

The potential toxicity of the engineered nanoparticles (NPs) has been demonstrated in numerous *in-vitro* studies to both neuronal and glial cells (Feng et al., 2015). Such NPs induced neurotoxicity and neurodegenerative changes are triggered through increased production of reactive oxygen species (ROS) initiating oxidative stress, besides induction of apoptosis and disruption of the cell cycle (Zhang et al., 2011; Hussain et al., 2006). Recently, neurodegenerative diseases such as Alzheimer’s disease have been linked to NPs as an important risk factor (Feng et al., 2015). How such nanoparticles gain accesses to the body is a matter of controversy; however, various routes have been suggested including inhalation, injection, dermal penetration, and ingestion followed by dispersing through the systemic circulation to various tissues (Shi et al., 2013). Brain is a possible location for the NPs by either crossing the blood brain barrier (BBB) (Sharma and Sharma, 2007) or migrating along the olfactory epithelium via the olfactory nerves (Win-Shwe et al., 2006). Engineered Zn-NPs are toxic to both gram negative and gram positive bacteria (Reddy et al., 2007). Adams et al. (2014) reported significant inhibition of the Escherichia coli growth by Zinc oxide (ZnO) NPs. Other researchers reported its toxic effect against many fungal species, marine algae and protozoa (Lin and Xing, 2007). Additionally, Hanely et al. (2008) demonstrated the ZnO NPs toxicity to cancerous cells via ROS production. Such properties of ZnO NPs with its easy production encourage its use in the agricultural sector, including fish farms as an anti-algae agent (Beyth et al., 2015). However, few reports described the lesions induced by ZnO NPs toxicity in fish. Lesions were mainly hydropic and vacuolar degeneration of the hepatic and renal tissues, with hyperplasia and vacuolation of the gill arches (Al-Bairuty et al., 2013). Researchers described the accumulation of ZnO NPs in the brain tissues of various species of fish, particularly largemouth bass, carp and Japanese Medaka (Hao et al., 2013). The NPs of ZnO reported to induce neurotoxicity of neuroblastoma cell culture (Valdiglesias et al., 2013) and neural stem cell apoptosis in experimentally treated mice (Deng et al., 2009). In this study, we investigated the induced behavioral changes and neuropathology of prolonged exposure to high doses of Zn-NPs in two species of tilapia: *T. nilotica* and *T. zilli* was investigated.

**MATERIALS AND METHODS**

Zinc nanoparticles (Zn-NPs) were purchased from Sigma-Aldrich Co. LLC. GmbH, Steinheim, Germany. Zn-NPs were in the form of nanopowder, 35 nm avg. part. Size, ≥99% trace metals basis. Zn-NPs surface area was determined using the multi-point Brunauer Emmette Teller (BET) method (Saddick et al., 2015). The measured surface areas were 40 m²/g that confirmed the manufacturer’s values. The Zn-NPs were suspended directly in deionized water in a concentration of 500 and 2000 µg/L and dispersed by ultrasonic vibration at 40 kHz for 30 min. Zn-NPs suspension was prepared daily. The concentration of Zn in the exposure...
solution was quantified by inductively coupled plasma mass spectrometry (ICP-MS) at zero, 12 and 24 h of exposure to verify the exposure concentrations were the same as the prepared concentrations. The Zn-NPs shape and size were determined using the transmission electron microscope (TEM) (JEM-1011, JEOL, Japan). Zn-NPs were nearly spherical and very fit with the nano-scale, and the measured particle size (35±5 nm) was close to the manufacturer information (Saddick et al., 2015).

**Fish preparation:** The experiment had the approval of the Ethics Committee of the University of King Abdulaziz. Four hundred males of both *T. nilotica* and *T. zillii*, weighing 90 ± 5 g for a length of 15 ± 3 cm were used in this study. Fish were maintained in thirty glass aquaria (n = 10 individuals/aquarium) containing 100 L of water (pH 7.16 ± 0.3, 0.52 mM Ca, and 0.24 mM Mg) that was changed daily, equipped with a continuous system of water aeration (Eheim Liberty 150 Bio-Espumador cartridges). The temperature was maintained at 28 ± 2 °C and dissolved oxygen, at 7.0 ± 0.5 mg/L. Fish were fed with commercial fish food (TFDNO35) Shandong Litong Biotechnology Co., Ltd, China. Fish were acclimatized for 15 days before the beginning of the experiments.

**Experimental design:** Fish were randomly divided into six groups, 30 fish in each group (3 replicates). The first and the fourth groups were left as a control for *T. nilotica* and *T. zillii* respectively; the 2nd and 3rd, groups were *T. nilotica* and were exposed to 500 and 2000 µg Zn-NPs/L respectively. The 5th and 6th groups were *T. zillii* and were exposed to 500 and 2000 µg Zn-NPs/L respectively. The fish were not fed during the experimental period in order to minimize the risk of Zn-NPs adsorbing to food or fecal material and help maintain water quality. Behavioral changes were monitored carefully twice a day (from the hour of 7 to 8 and 19 to 20) throughout the experiment. Fish of each group were anesthetized on ice and killed by transaction of the spinal cord 30 days post exposure (Gutiérrez-Praena et al., 2012)

**Pathology:** Brain was quickly removed and fixed in 10% neutral buffered formalin. Samples were routinely processed for histopathological evaluation, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes (Bancroft and Gamble, 2008) and examined microscopically.

**Statistical analysis:** The statistical package for social science (SPSS Inc., Chicago, IL, version 20, USA) was used for processing of the data. The result was shown as mean ± SD. Student’s t-test was used to calculate the differences between groups.

**RESULTS AND DISCUSSION**

Fish of both tilapia species in the control groups appeared and behaved in a normal manner. That were normal swimming behavior and natural colour. Groups that were treated with 2000 µg Zn-NPs/L showed loss of equilibrium and slower movements when compared to control groups that started at the 15th day post exposure. At the end of the experiment, approximately 60% of the fish in groups 3 and 6 were swimming sideways while 2% fish showed motionlessness. Behavioral changes in groups that were treated with 500 µg Zn-NPs/L were not significant (P < 0.05).

Histopathological examinations of the brain tissue revealed marked degenerative changes in the groups treated with 2000 µg Zn-NPs/L rather than groups that were treated with 500 µg Zn-NPs/L. Control groups revealed normal histological features of the brain and cerebellum (Fig. 1 & 2). Groups of *T. nilotica* and *T. zillii* that were treated with 500 µg Zn-NPs/L showed congested cerebral blood vessels with slight vacuolation of the neuropil when compared to fish of the control group (Fig. 3). Tilapia fish treated with 2000 µg Zn-NPs/L showed marked vacuolation of the neuropil with microglial cells infiltration around the degenerated neurons in the examined brains (Fig. 4). The cerebellum revealed vascular thrombosis with the presence of either apoptotic or complete absence of the Purkinje cell layer (Fig. 5).
Moreover, severe degenerative changes and vacuolation in the stratum album centrale and stratum fibrosum of the optic tectum were seen (Fig. 6). Moderate congestion of the blood vessels of the meninx primitive was noticed in approximately 50% of the examined brains (Fig. 7). There were no significant difference in the exhibited lesions between T. nilotica and T. zilli species.

Nanoparticles are a revolutionary novel material with potential applications in the fields of biotechnology, medicine and agriculture sectors. However, NPs from the industrial production and wastes may end up in the watercourses and present an ecotoxicological risk to the aquatic environment (Moore, 2006). Therefore, their fate and their impact on the environment and human health need to be thoroughly investigated. In this study, we noticed explicit behavioral changes in the fish that were treated with a 2000 µg Zn-NPs /L which might be linked to the degenerative changes that were seen in their brain and cerebellum. Many reports mentioned the ability of NPs to overcome the physical blood brain barrier and gain access to the brain, or through the nerve endings of the olfactory bulb (Koziara et al., 2006). Neurotoxin or progressive neurological pathologies have been linked directly to behavioral changes. The lesions in this study were mainly confined to the optic tectum and the cerebellum. The optic tectum of tilapia species is composed of two optic lobes that join along the brain sagittal plane at the dorsal segment of the mesencephalon and represent the visual center of the
fish. Six different layers were observed, named from the outer layer as: stratum marginale, stratum opticum, stratum fibrosum et griseum superficiale, stratum album centrale, stratum griseum centrale and stratum periventriculare (Groman, 1982). The tectum has different neuron concentrations and afferent fiber connections that are vital for the sensation and rapid decisions required for survival behavioral reactions. The results consisted of severe degenerative changes and vacuolation in the stratum album centrale and stratum fibrosum particularly in fish that were treated with a 2000 µg Zn-NPs /L that may thus have affected the visual response and reflex of the fish. The behavioral changes that were recorded consisted of slower movements and swimming sideways might be attributed to the neurodegenerative changes that were reported in the optic tectum which in turn affect the functioning of motor coordination of the fish’s body (Kermer et al., 2004). Mishra and Devi (2014) described similar lesions in the optic tectum following the administration of a sublethal dose of organophosphate pesticide Chlorpyryrifos. Described lesions were mainly consisted of spongiosis, congestion, degeneration and necrosis of the different layers of the optic tectum. The loss of equilibrium in the fish that were treated by 2000 µg Zn-NPs /L is evidently related to the alterations that were recorded in the cerebellum and appeared as vascular thrombosis and apoptosis of cells of the Purkinje cell layer. Many reports recorded the accumulation of Zn-NPs in the brain tissue of various fish species (Hao et al., 2013), however, we could not find any records for NPs induced neuropathology in aquatic fauna. The induced lesions may be attributed to the oxidative stress due to ROS production as described by Nel et al. (2006) who reported significant lipid peroxidation by nanomaterial in the brains of Juvenile largemouth bass. Release of ROS over a long period can evoke inflammation, apoptotic, and cell cycling pathways. Deng et al. (2009) reported neural stem cell apoptosis induced by Zn-NPs, which support our obtained the neurodegenerative lesions in fish treated with Zn-NPs.

CONCLUSIONS

Prolonged exposure of fish to high doses of Zn-NPs could induce neurotoxicity followed by neurodegenerative and apoptotic lesions in brain that probably responsible for the recorded behavioral changes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES


