Japanese Journal of Veterinary Research 64(Supplement 2): S161-166, 2016

Zinc nanoparticles induced brain lesions and behavioral changes in two Tilapia species

Mohamed Afifi[1,2,4,*)], Osama A. Abu Zinada[1)], Haytham Ali[3)] and Michel Couderchet[5)]

[1)] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

[2)] Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Egypt.

[3)] Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt.

[4)] Department of Biological Sciences, Faculty of Science, University of Jeddah, Saudi Arabia.

[5)] Unité de Recherche Vigne et Vin de Champagne – EA 4067, University of Reims Champagne-Ardenne, France.

*Corresponding author Email: mama200100@gmail.com

Abstract

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*. 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 were observed between *T. nilotica* and *T. zilli* in behavioral or pathological changes. Our results highlight 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.

Keywords: Nanoparticles, Neuropathology, Tilapia, Behavioral alteration

Introduction

The potential toxicity of the engineered nanoparticles (NPs) has been demonstrated in numerous in vitro studies to both neuronal and glial cells[6)]. 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[22,9)]. Recently, neurodegenerative diseases such as Alzheimer's disease have been linked to NPs as an important risk factor[6)]. 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[19)]. Brain is a possible location for the NPs by either crossing the blood brain barrier (BBB)[18)] or migrating along the olfactory epithelium via the olfactory nerves[21)]. Engineered Zn-NPs are toxic to both gram negative and gram positive bacteria[16)]. Adams *et al.*[1)] 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[12]. Additionally, Hanely *et al.*[7] demonstrated the ZnO NPs toxicity to cancerous cells via ROS production. Such properties of ZnONPs with its easy production encourage its use in the agricultural sector, including fish farms as an anti-algae agent. 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[2]. Researchers described the accumulation of ZnO NPs in the brain tissues of various species of fish, particularly largemouth bass, carp and Japanese Medaka[7]. The NPs of ZnO reported to induce neurotoxicity of neuroblastoma cell culture[20] and neural stem cell apoptosis in experimentally treated mice[4]. 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*.

Materials and Methods

ZnPs was purchased from Sigma-Aldrich Co. LLC. GmbH, Steinheim, Germany. ZnPs 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[17]. The measured surface areas were 40 m^2/g that confirmed the manufacturer's values. The Zn nanopowder was suspended directly in deionized water in a concentration of 500 and 2000 μ/L and dispersed by ultrasonic vibration at 40 kHz for 30 min. ZnPs 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 24h of exposure to verify the exposure concentrations were the same as the prepared concentrations. The ZnPs shape and size were determined using the transmission electron microscope (TEM) (JEM- 1011, JEOL, Japan). ZnNPs was nearly spherical and very fit with the nano-scale, and the measured particle size (35±5nm) was close to the manufacturer information[17].

Fish preparation: The experiment had the approval of the Ethics Committee ofthe University of King Abdualaziz. 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-1. 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 throughout the experiment. Fish of each group were anesthetized on ice and killed by transection of the spinal cord 30 days post exposure

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 [3] , and examined microscopically.

Results

Behavioral changes: 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

Pathology: 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. zilli* 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

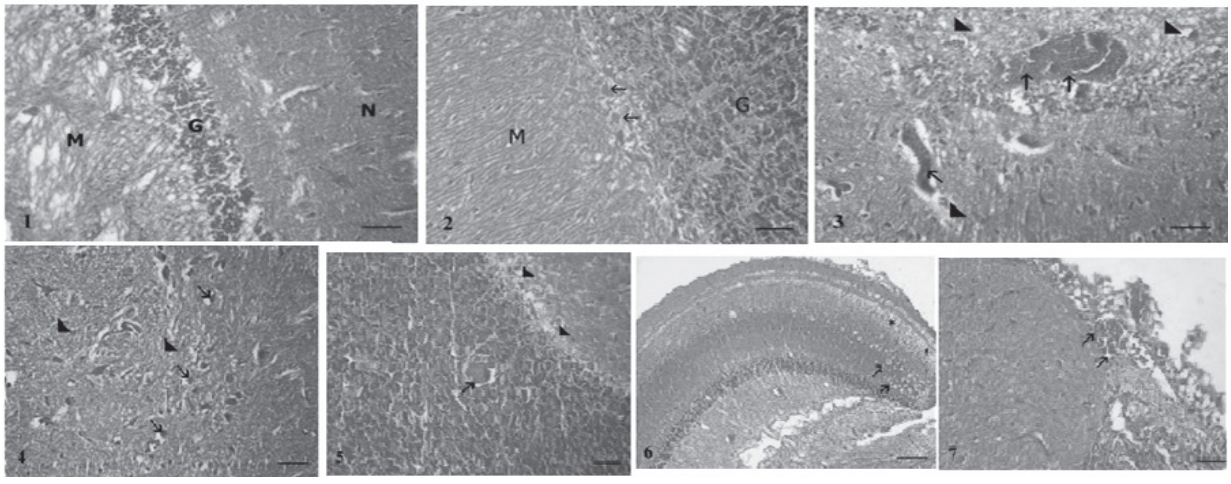

Fig. 1 Brain of control group of *T. nilotica*, showing normal histological structures of the optic tectum; molecular layer (M), granular layer (G) and neuropil (N). Stain: H&E. Bar= 50μm. **Fig. 2** Cerebellum of control group of *T. zilli*, showing normal histological structures; molecular layer (M), granular layer (G) and Purkinje cells (arrows). Stain: H&E. Bar= 50μm. **Fig. 3** Brain of Zn-NP-treated (500 μg/L) *T. nilotica*, showing congested cerebral blood vessels (arrows) with moderate vacuolation in the neuropil (arrowheads). Stain: H&E. Bar= 50μm. **Fig. 4** Brain of Zn-NP-treated (2000 μg/L) *T. nilotica*, showing sever degenerative changes and vacuolation in the neuropil (arrowheads) with microglial cells infiltration around the degenerated neurons (arrows). Stain: H&E. Bar= 50μm. **Fig. 5** Cerebellum of Zn-NP-treated (2000 μg/L) T. nilotica, showing vascular thrombosis in the granular layer (arrow) with degeneration and loss of the Purkinje cell layer (arrowheads). Stain: H&E. Bar= 50μm. **Fig. 6** Brain of Zn-NP-treated (2000 μg/L) *T. zilli*, showing severe degenerative changes and vacuolation in the stratum album centrale (arrows) and stratum fibrosum (asterisks) of the optic tectum. Stain: H&E. Bar= 100μm. **Fig. 7** Brain of Zn-NP-treated (2000 μg/L) *T. zilli*, showing moderately congested blood vessel of the meninx primitiva (arrows) with normal histological appearance of the neuropil. Stain: H&E. Bar= 50μm.

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*.

Discussion

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[14]. 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[11]. 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 grisium superficiale, stratum album centrale, stratum griseum centrale and stratum periventriculare*[5]. The *tectum* has different neuron concentrations and afferent fiber connections that are vital for the sensation and rapid decisions required for survival behavioral reactions. Our 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[10]. Mishra and Devi[13] described similar lesions in the optic tectum following the administration of a sublethal dose of organophosphate pesticide Chlorpyrifos. 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[9], 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.*[15] 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.*[4] 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.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under Grant no. (28-130-36-RG). The authors, therefore, would like to acknowledge and thank the DSR for their technical and financial support.

Conflicts of interest

The authors declare no conflict of interest.

References

1) Adams, C. P., Walker, K. A., Obare, S.O. and Docherty, K. M. 2014. Size-dependent antimicrobial effects of novel palladium nanoparticles. *PLoS One,* **9**: e85981.
2) Al-Bairuty, G. A., Shaw, B. J., Handy, R. D. and Henry, T. B. 2013. Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout *(Oncorhynchus mykiss). Aquat. Toxicol.,* **126**: 104-115.
3) Bancroft, J. D. and Gamble, M. 2008. *Theory and Practice of Histological Techniques*. 5th ed., Churchill Livingstone. New York, London, Philadelphia
4) Deng, X., Luan, Q., Chen, W., Wang, Y., Wu, M., Zhang, H. and Jiao, Z. 2009. Nanosized zinc oxide particles induce neural stem cell apoptosis. *Nanotechnology,* **20**: 115101.
5) Groman, D. B. 1982. *Histology of the striped bass*. American Fisheries Society. Monograph, n3. Bethesda, Maryland: American Fisheries Society.
6) Feng, X., Chen, A., Zhang, Y., Wang, J., Shao, L. and Wei, L. 2015. Central nervous system toxicity of metallic nanoparticles. *Int. J. Nanomedicine,* **10**: 4321–4340.
7) Hanley, C., Layne, J., Punnoose, A., Reddy, K. M., Coombs, I., Coombs, A. and Wingett, D. 2008. Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. *Nanotechnology,* **19**: 295103
8) Hao, L., Chen, L., Hao, J. and Zhong, N. 2013. Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp *(Cyprinus carpio)*: A comparative study with its bulk counterparts. *Ecotoxicol. Environ. Safety.,* **91**: 52-60.
9) Hussain, S. M., Javorina, A. K., Schrand, A. M., Duhart, H. M., Ali, S. F. and Schlager, J. J. 2006. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. *Toxicol. Sci.,* **92**: 456-463.
10) Kermer, P., Liman, J., Weishaupt, J. H. and Bahr, M. 2004. Neuronal apoptosis in neurodegenerative diseases: from basic research to clinical application. *Neurodegener Dis.,* **1**: 9-19.
11) Koziara, J. M., Lockman, P. R., Allen, D. D. and Mumper, R. J. 2006. The blood–brain barrier and brain drug delivery. *J. Nanosci. Nanotechnol.,* **6**: 2712-2735.
12) Lin, D. and Xing, B. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environ. Poll.,* **150**: 243-250.
13) Mishra, A. and Devi, Y. 2014. Histopathological alterations in the brain (optic tectum) of the fresh water teleost Channa punctatus in response to acute and subchronic exposure to the pesticide Chlorpyrifos. *Acta Histochem.,* **116**: 176-181.
14) Moore, M. N. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?. *Environ. Int.,* **32**: 967-976.
15) Nel, A., Xia, T., Madler, L. and Li, N. 2006. Toxic potential of materials at the nanolevel. *Science,* **311**: 622-627
16) Reddy, K. M., Feris, K., Bell, J., Wingett, D. G., Hanley, C. and Punnoose, A. 2007. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Appl.*

Phys. Lett., **90**: 213902.

17) Saddick, S. Afifi, M. and Abu Zinada, O. A. 2015. Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of Oreochromis niloticus and Tilapia zillii. *Saudi J. Biol. Sci.,* . http://dx.doi.org/10.1016/j.sjbs.2015.10.021

18) Sharma, H. S. and Sharma, A. 2007. Nanoparticles aggravate heat stress induced cognitive deficits, blood-brain barrier disruption, edema formation and brain pathology. *Prog. Brain Res.,* **162**: 245-273.

19) Shi, H., Magaye, R., Castranova, V. and Zhao, J. 2013. Titanium dioxide nanoparticles: a review of current toxicological data. *Part Fibre Toxicol.,* **10**: 15.

20) Valdiglesias, V., Costa, C., Kiliç, G., Costa, S., Pásaro, E., Laffon, B. and Teixeira, J. P. 2013. Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles. *Environ. Int.,* **55**: 92-100.

21) Win-Shwe, T. T., Yamamoto, S., Ahmed, S., Kakeyama, M., Kobayashi, T. and Fujimaki, H. 2006. Brain cytokine and chemokine mRNA expression in mice induced by intranasal instillation with ultrafine carbon black. *Toxicol. Lett.,* **163**: 153-160.

22) Zhang, Q. L., Li, M. Q., Ji, J. W., Gao, F. P., Bai, R., Chen, C. Y., Wang, Z. W., Zhang, C. and Niu, Q. 2011. In vivo toxicity of nano-alumina on mice neurobehavioral profiles and the potential *mechanisms. Int. J. Immunopathol. Pharmacol.,* **24**: 23S-29S