Global Veterinaria 19 (5): 633-638, 2017
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2017.633.638

Effects of Silver Nanoparticles on *Tilapia zilli* and *Tilapia niloticus* Brain Histology and Brain Neurotransmitters

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Abstract: The use of Silver nanoparticles (Ag-NPs) has been widespread in the past few years, with unprecedented speed in many human activities including, industrial, agricultural and medical fields. This led to the spread of these compounds and their access to aquatic organisms through different sources such as sewage. Hence the importance of this research as part of a large project aimed to studying the effect of Ag-NPs on the brain of tow tilapia fish. In this work, the induced brain neurotransmitters and histopathological alterations of chronic exposure to large concentrations of Ag-NPs in two species of tilapia, *T. zilli* and *T. nilotica* were investigated. Ag-NPs were used in tow exposure concentrations 2 mg/L and 4 mg/L. Brain neurotransmitters; Adrenalin, Dopamine and Noradrenalin were decreased in fish exposed to the concentration of 4 mg/L of Ag-NPs in both *T. nilotica* and *T. zilli*. Brain hestopathological examination revealed a sever degenerative changes in the neuropil of fish exposed to 4mg Ag-NPs /L with cerebral cortex blood vessels congestion and Loss of the Purkinje cell layer in both *T. nilotica* and *T. zilli*. It could be concluded that Ag-NPs induced a neurotoxicity in both *T. nilotica* and *T. zilli* that manifested by neurochemical and neuropathological alterations that could be undertaken as a biomarkers for detection of Ag-NPs fresh water contamination.

Key words: Nanoparticles · Neurotransmitters · Neuropathology · Tilapia

INTRODUCTION

Nanoparticles (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 [2]. Recently, neurodegenerative diseases such as Alzheimer's disease have been linked to NPs as an important risk factor [1]. 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 [3]. Brain is a possible location for the NPs by either crossing the blood brain barrier (BBB) [4] or migrating along the olfactory epithelium via the olfactory nerves [5]. The Ag-NPs enabled cell entry through cellular proliferation or endothelium which causes dysfunction in mitochondria and ROS generation, leading to the destruction of proteins and nucleic acids within the cell, ultimately inhibiting cell proliferation [6]. The important mechanism of toxicity of Ag-NPs is the interaction of both nano and ionic particles of Ag with sulfur-containing proteins because of the strong silver-sulfur affinity [7].

The brain is the most suitable body organ to study the harmful alterations of oxidative stresses and this is returned to many factors. The first one is the brain is rich in the easily oxidized unsaturated fatty acids that could be oxidized producing free radicals that produce a cascade of events causing oxidative stresses. The second factor is brain tissue consumes large quantities of oxygen for oxidative phosphorylation. The third factor is the brain tissue not has a potent antioxidant defense system [8]. The changes in genes expression levels are critical biomarkers and are closer to biological responses to stress. Thus, genetic modification and gene expression can be used as an early diagnostic method to investigate the effects of environmental stress on an organism [9]. Recent studies have shown that both particles and Ag-NP cause toxicity to zebrafish cells (Danio rerio) gills with Ag-NPs causing increased toxicity [10]. Ag-NPs have

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been shown to be highly toxic to a wide range of aquatic species, including zebrafish [11]. In this study, we investigated the alterations in brain neurotransmitters and neuropathology caused by long exposure to high concentrations of Ag-NPs in two types of tilapia. *T. nilotica* and *T. zilli*.

MATERIALS AND METHODS

Nanoparticles: Ag-NPs (catalog number 576832, Sigma Aldrich, UK) of surface area 5.0 m^2 / g, particle size <100 nm, purity 99.5% and density 10.49 g/cm, were used in this study. Multi-point Brunauere Emmette Teller (BET) technique was used for investigation of Ag-NPs surface area. The value of the measured surface area was the same of manufacturer's values (5.0 m^2/g).

Preparation of Ag Nanoparticle Suspension: Ag-NPs were prepared daily in concentrations of 2 and 4 mg/L in deionized water [10].

Fish Preparation: This experiment was carried out following the ethical approve of University of Jeddah ethical committee. 90 male *T. zillii* and 90 male *T. niloticus* were used for this project. The weight of fish was 90 ± 5 g and the length was 15 ± 3 cm for each. Forty aquaria were used for rearing of the experimental fish, 10 individuals per aquarium. The aquaria water was changed daily. Water was aerated with an Eheim Liberty 150 Bio-Espumador aeration system for keeping the dissolved oxygen at 7.0±0.5 mg L^{-1}. A fish commercial diet was used for fish feeding [12] with daily feeding amount10% of fish body weight and divided on 3 times daily. The Experiment began after 15 days of fish acclimatization and extend for 30 days.

Experimental Design: The experimental fish was divided into 6 groups; the 1st was *T. niloticus* and leaved as control; the 2nd was *T. niloticus* and exposed to 2mg of Ag-NPs/L ; the 3rd was *T. niloticus* and exposed to4 mg of Ag-NPs/L; the 4th was *T. zillii* and leaved as control; the 5th was *T. zillii* and exposed to2mg of Ag-NPs/L; the 6th was *T. zillii* and exposed to 4mg of Ag-NPs/L. The individuals of each group were anesthetized on ice and killed by a spinal cord treatment after 30 days of exposure brain samples were taken for biochemical and pathological analysis [13].

Brain Neurotransmitters Analysis: Brain tissue homogenate was prepared from each sample by homogenizing one gram of each sample in 10 ml of normal saline then centrifuged at 3000 rpm and the supernatant was used for determination of the concentration of brain Adrenalin, Noradrenalin, Dopamine using Elisa kits (Cat. No. MBS281891, MBS9324341, MBS912178) that were purchased from MyBiosource (San Diego, CA 92195-3308USA) following the construction of the commercial kits.

Pathology: The brain was quickly removed and fixed in neutral 10% formalin. The samples were routinely processed for pathological evaluation [14] and examined microscopically.

Statistical Analysis: Data were represented as means ± SE. Statistical analysis was evaluated by one-way analysis of variance (ANOVA). Once a significant F test was obtained, LSD comparisons were performed to assess the significance ($p < 0.05$) of differences among various treatment groups. Statistical Processor System Support "SPSS" for Windows software, Release 16.0 (SPSS, Chicago, IL) was used.

RESULTS

Effects of Ag-NPs on the Neurotransmitters in Fish Brain Tissues: The exposure to 2mg/L Ag-NPs did not affect on adrenaline, noradrenalin and dopamine comparing to control groups. In contrary, Ag-NPs in concentration of 4mg/L induced a sever reduction in the adrenaline, noradrenalin and dopamine levels comparing either to control or fish exposed to 2mg/L Ag-NPs. There is no difference between *T. niloticus* and *T. Zillii* in adrenaline and noradrenalin levels (Table 1).

Effects of Ag-NPs on *T. niloticus and T. Zillii* brain tissues Histopathology: Histopathological examinations of the brain tissue revealed marked degenerative changes in the groups treated with 4mg Ag-NPs /L rather than groups that were treated with 2mg Ag-NPs /L. Control groups revealed normal histological structures of the optic tectum; molecular layer, granular layer and neuropil (Fig. 1), this figure was published in our group previous paper [12]. Groups of *T. nilotica* and *T. zilli* that were treated with 2mg Ag-NPs /L showed congested cerebral blood vessels, perivascular edema with moderate vacuolation in the neuropil when compared to fish of the control group (Fig. 2,3). Tilapia fish treated with 4mg Ag-NPs /L showed degenerated neurons and pyramidal cells with the neuropil showing status spongiosus (Fig. 4) and showing demyelination od the nerve axons with

Table 1: Effect of Ag-NPs on the neurotransmitters in the brain of *T. niloticus* and *T.Zillii*

Fish	Group	Adrenaline (pg/g tissue)	Noradrenalin (pg/g tissue)	Dopamine (pg/g tissue)
T. niloticus	Control	200± 25	120± 18	162 ± 31
	2mg/L	203 ± 35	116±32	157± 17
	4mg/L	170 ±19$^{*\#}$	90± 10$^{*\#}$	127± 10$^{**\#\#}$
T. Zillii	Control	190 ± 27	115 ± 30	156 ± 37
	2mg/L	193± 37	119±26	150±11
	4mg/L	163±27$^{**\#}$	93± 17$^{*\#}$	118±36$^{**\#\#}$

*: P < 0.05, **: P < 0.01, ***: P < 0.001, compared to control group of the same fish specie; $^{\#}$: P < 0.05, $^{\#\#}$: P < 0.01, $^{\#\#\#}$: P < 0.001, comparing to 2mg/L Ag-NPs exposed group of the same fish specie

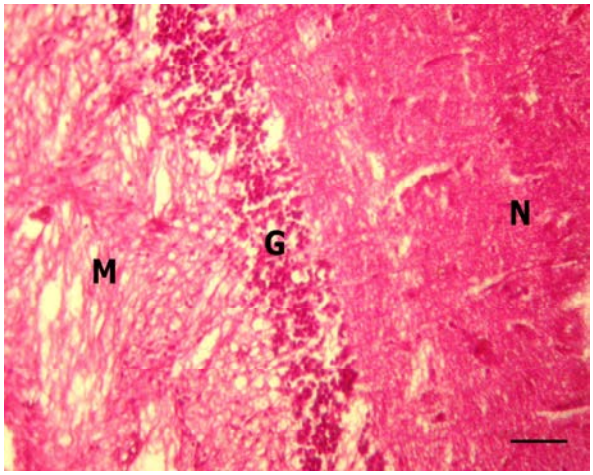

Fig. 1: Brain of control group of *T. nilotica*, showing normal histological structures of the optic tectum; molecular layer (M), granular layer (G) and neuropil (N). Stain: H&E. Bar= 50µm

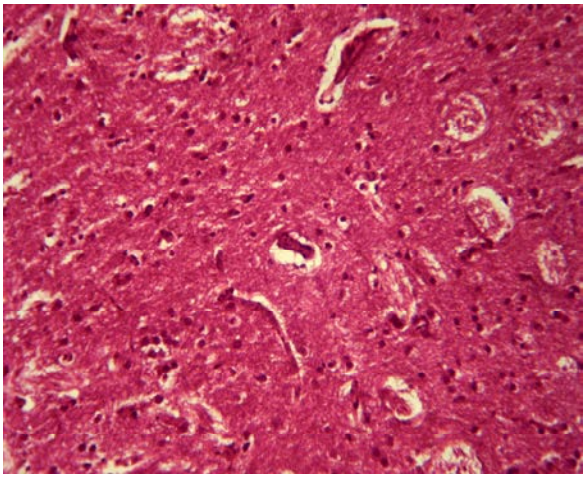

Fig. 3: Brain of Ag-NP-treated (2mg/L) *T. zillii* showing perivascular edema with moderate vacuolation in the neuropil. H&E. X400

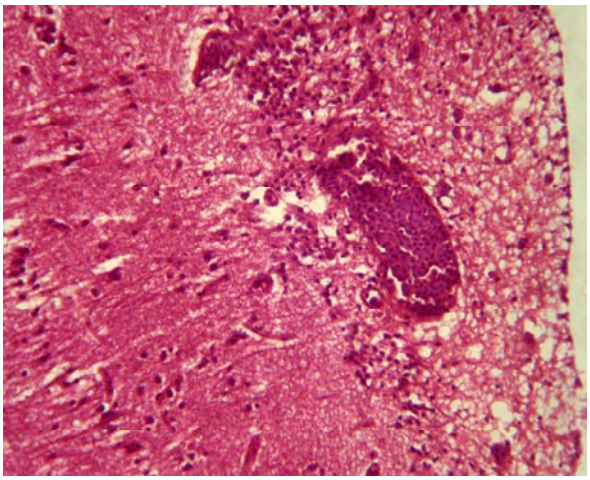

Fig. 2: Brain of Ag-NP-treated (2mg/L) *T. nilotica* showing congested cerebral blood vessels with moderate vacuolation in the neuropil. H&E. X400

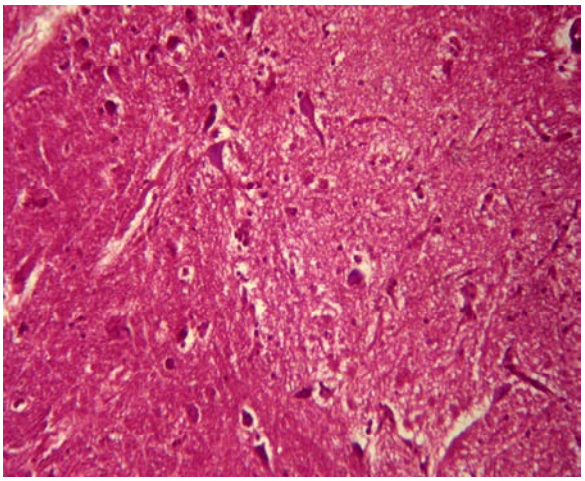

Fig. 4: Brain of Ag-NP-treated (4mg/L) *T nilotica* showing degenerated neurons and pyramidal cells with the neuropil showing status spongiosus. H&E. X400

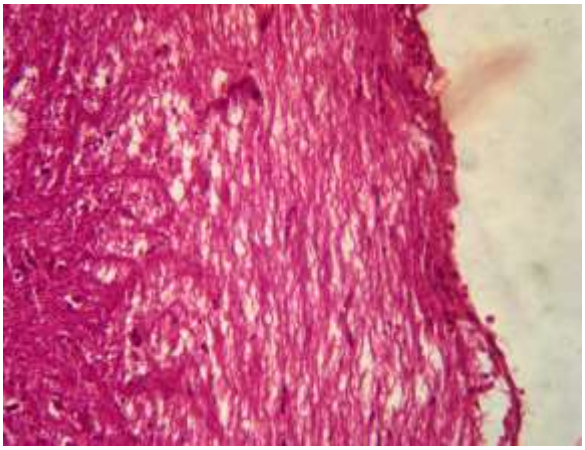

Fig. 5: Brain of Ag-NP-treated (4mg/L) *T zillii* showing demyelination od the nerve axons with congested blood vessel of the meninx primitive. H&E. X400

congested blood vessel of the meninx primitive (Fig. 5) 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 [15]. Therefore, their fate and their impact on the environment and human health need to be thoroughly investigated.

In the present study, the exposure to 2mg/L Ag-NPs didn't affect on adrenaline, noradrenalin and dopamine comparing to control groups. In contrary, Ag-NPs in concentration of 4mg/L induced a sever reduction in the neurotransmitters levels comparing either to control or fish exposed to 2mg/L Ag-NPs. Our results reported the bad effect of NPs at the highest concentrations on the levels of brain neurotransmitter, this may occurred due to the degenerative effects of NPs on the secretary cells that produce this neurotransmitter and this is obvious in the hestopathological examination. Also, we could suspect this effect may be resulted from the increased ROS that induced by NPs as proved in our previous study [10], which may be incriminated in distraction of neurotransmitter or inhabited it's production. In general this point need farther study and our explanation need farther work.

In *in-vetro* study on PC-12 cells that exposed to deferent doses of nanopartecls of manganese oxide (MnO-NPs) and Ag-NPs, dihydroxyphenylacetic acid and dopamine revealed sever depletion in a dose-dependant manner in PC-12 cells that treated with deferent doses (1-100 ug/ml) of MnO-NPs and that treated with Ag-NPs in a dose of 50 µg/mL. Meanwhile, the levels of homovanillic acid (HVA) decreased PC-12 cells that treated with 50 µg/mL either MnO-NP or Ag-NP [16]. On the same line, nanopartecls of copper (Cu-NPs) significantly reduced the levels of DA, DOPAC PC-12 cells in a dose dependant manner, this may be explained by the induction of oxidative stresses and alterations in enzymes necessary for DA pathway [17]. Treatment of adrenal medullary chromaffin cells cultures with Au-NPs and Ag-NPs in concentrations ranged from 0.01 to1 nM induced a reduction in epinephrine secretion without affecting the cells viability, that may be the ability of these particles to interfere with the second messenger pathway and vesicle secretions [18].The same results were obtained in a subsequent investigation on murine adrenal medullary chromaffin cell, that treated with Ag-NPs (1 nM) and Au-NPs (10 µg/mL). Really, these studies proved that the reduction in DA and epinephren in cells treated with NPs may be due to their interference with exocytosis [19, 20].

In this study, we noticed neuropathological changes in the fish that were treated with a 4mg Ag-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 [21-22]. Neurotoxin or progressive neurological pathologies have been linked directly to behavioral changes [12]. 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 [23]. 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 4mg Ag-NPs /L that may thus have affected the visual response and reflex of the fish. Mishra and Devi [24] 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 4mg Ag- 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 Ag-NPs in the brain tissue of various fish species [25], 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.* [26] 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 [12].

CONCLUSION

Prolonged exposure of fish to high doses of Ag-NPs could induce neurotoxicity that manifested by alterations in the concentrations of the brain neurotransmitters (adrenaline, noradrenalin and dopamine), neurodegenerative lesions in brain tissue of *T. nilotica* and *T. zilli*. The alterations could be used as an early and sensitive biomarkers for fresh water contamination with Ag-NPs.

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, acknowledge with thanks DSR technical and financial support.

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