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ORIGINAL ARTICLE

Toxicity of silver nanoparticles on the brain of *Oreochromis niloticus* and *Tilapia zillii*



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KEYWORDS

Ag-NPs;
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Abstract *Background:* Silver nanoparticles (Ag-NPs) are widely used nowadays in a variety of commercial applications including medical, health care, textiles and household supplies.

Objectives: The current study was designed to determine the median lethal dose (LC₅₀) of Ag-NPs on *Oreochromis niloticus* and *Tilapia zillii*.

Methods: Acute and sub-acute toxicity study of the Ag-NPs on brain tissues was carried out using different concentrations of the NPs at 2 mg/L and 4 mg/L. These concentrations were dispersed in deionized water with the exception of the control groups in the experiments. Biochemical and molecular analysis were conducted on tissue homogenates in order to evaluate the potential effects of NPs on the antioxidant system.

Results: The Ag-NP acute toxicity (96 h LC₅₀) values of 19.5 ± 2 and 20 ± 2.4 mg/L were reported for *O. niloticus* and *T. zillii* respectively. Fish exposed to 2 mg/L Ag-NPs did not show any significant change in the levels of reduced glutathione (GSH), total glutathione (tGSH) levels, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activity or genes expression and malondialdehyde (MDA) level. In contrary, a dose of 4 mg/L showed a significant reduction in the levels all the above-mentioned parameters except in MDA level where it was significantly induced.

Conclusion: Results indicate that exposure of *O. niloticus* and *T. zillii* to Ag-NPs (4 mg/L) has deleterious effects on brain antioxidant system, whereas a dose of 2 mg/L has no effects.

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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). Despite a wide usage of NPs in many sectors, few studies were done on their environmental impact and fate. Metallic NPs are a point of focus for many researchers because of their potential impact

on nanotechnology and society. Metallic NPs may leak into natural bodies of water in their life cycles (production, storage, transportation, consumption, disposal, or reproduction). There are not enough data about the magnitude of NPs released to the aquatic system and their influence on the living organisms. Therefore, there is an urgent requirement for more information on the ecological risks of metallic NPs. Recently; several studies have shown their interests to investigate the toxicity of NPs (Alkaladi et al., 2015). Some of these studies have proved the toxicity of NPs, such as metal oxides to bacteria, human cells, rodents and aquatics (Lin and Xing, 2007). The mechanism of NP toxicity is complicated, it may stimulate the reactive oxidative species (ROS) generation through disruption of intracellular metabolism (Warheit et al., 2006; 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). The toxicity of Ag-NPs is closely related to its transformation in biological and environmental media, including surface oxidation, release of silver ions, and interaction with biological macromolecules (McShan et al., 2014). The Ag-NPs can enter the cell through diffusion or endocytosis which causes mitochondrial dysfunction and ROS generation, this leads to protein and nucleic acid destruction inside the cell, and finally inhibition of cell proliferation (Li et al., 2013). An important toxicity mechanism of Ag-NPs is the interaction of both the ionic and nano-form of silver with sulfur containing macromolecules such as proteins due to silver's strong affinity for sulfur (Hou et al., 2013).

Brain is the most liable organ in the body for studying the adverse effects of oxidative damage and that is due to several reasons; it contains a high level of unsaturated fatty acids which are easily peroxidizable, its disproportional large consumption of oxygen per unit weight, also the brain does not possess a particular 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, genetic alteration and gene expression can be utilized as an early diagnostic method for investigating the effects of environmental stress on the organism (Dondero et al., 2006). Recent studies showed that both particulate and Ag-NPs caused toxicity to zebrafish (*Danio rerio*) gills with the Ag-NPs causing greater toxicity (Govindasamy and Abdul Rahuman, 2012). It has been demonstrated that Ag-NPs are acutely toxic to across a wide spectrum of aquatic species, including zebrafish (Griffitt et al., 2008).

No studies were performed on the toxicological effect of Ag-NPs on *Oreochromis niloticus* and *T. zillii* brain antioxidant. In the current study, we aimed to assess the changes of gene expression and activity of antioxidant enzymes in the brain tissue exposed to different levels of Ag-NPs.

2. Materials and methods

2.1. Nanoparticles

Ag-NP (cat. no 576832, Sigma Aldrich, UK) preparation was based on the manufacturer's specification for silver nanopowder of particle size < 100 nm, surface area 5.0 m²/g, density 10.49 g/cm, and purity of 99.5%. Ag-NP surface area was determined using the multi-point Brunauer Emmette Teller (BET) method. The measured surface area was 5 m²/g that no differed from the manufacturer's values.

2.2. Preparation of Ag nanoparticle suspension

The Ag nanopowder was dispersed directly in deionized water in a concentration of 2 and 4 mg/L. Ag-NPs were dispersed daily using ultrasonic vibration (40 kHz) for 30 min to prevent the NPs aggregation. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine Ag concentrations in the exposure water at zero, 12 and 24 h of exposure to verify the exposure concentrations are the same as the prepared concentrations as indicated in Table 1. The Ag-NPs' shape and size were determined using the transmission electron microscope (TEM) (JEM-1011, JEOL, Japan). Ag-NP nanoparticle was nearly spherical and fitted well with the nano-scale, and the measured particle size was close to the manufacturer's information.

2.3. Fish preparation

The Ethics Committee of King Abdulaziz 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), with daily changing of water. 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 (Afifi et al., 2016). 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 Ag-NPs dispersion of 0, 5, 10, 20, 30 and 40 mg/L were used in triplicate for the lethal toxicity study. Ten fish of each *O. niloticus* and *T. zillii* were exposed to each concentration for 24, 48 and 96 h in a 30 L aquarium with 20 L

Table 1 The actual Ag-NPs concentrations (mg/L) in the exposure water.

Concentrations (mg/L)	Time (h)				
	Zero	12	% of change	24	% of change
	M ± SD	M ± SD		M ± SD	% of change
Control	nd	nd	nd	nd	nd
2	2 ± 0.04	1.95 ± 0.01	-2.5	1.85 ± 0.05	-7.5
4	4 ± 0.06	3.84 ± 0.05	-4	3.74 ± 0.07	-6.5

nd = not detected.

of the NP treated water. The NP treated water was changed every 24 h to ensure a constant concentration of nanoparticles. The control fish was reared in distilled water free from NPs. During the experimental period, no food was supplied to fish for minimizing the absorption of NPs in food and the production of feces. The 24, 48 and 96 h sub lethal concentrations were determined using the Environmental Protection Agency (EPA) probit analysis.

2.5. Experimental design

Fish were randomly divided into six groups, 30 fish in each group (3 replicates). The first and fourth groups were left as a control for *O. niloticus* and *T. zillii* respectively; the second and third, groups were *O. niloticus* and were exposed to 2 and 4 mg Ag-NPs /L respectively. The fifth and sixth groups were *T. zillii* and were exposed to 2 and 4 mg Ag-NPs/L respectively. No food was supplied to fish during the experimental period for decreasing the absorption of Ag-NPs by food and maintaining water quality. Fish were anesthetized over ice after 15 days of the exposure. Brain was dissected, rinsed then immersed in liquid nitrogen and further kept at -80°C . The brain tissue homogenates were prepared following the method described by Puerto et al. (2009) where the supernatant was used for biochemical analysis.

2.6. Brain Ag, MDA, GSH levels and antioxidant enzymes activity assays

Brain Ag concentrations were analyzed using an inductively coupled plasma-atomic emission spectroscopy with an ULTIMA 2 apparatus (Horiba Jobin Yvon, France).

Brain tissue MDA was analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) using a TBARS assay kit (Cat. No.10009055, Cayman, USA). GSH, GST, GR, GPx, CAT and SOD activity were determined using the kits (Cat. No. NWK-GSH01, NWK-GST01, NWK-GR01, NWK-GPx01, NWK-CAT01 and NWK-SOD01) purchased from Northwest Life Science Specialties (NWLSSSTM), Vancouver, Canada.

2.7. Molecular assays and gene expressions

The expression of brain GST, GR, GPx, CAT and SOD genes was quantified using real time PCR. Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Cat. No. 74104). 0.5 μg of total RNA was used for production of cDNA by Qiagen RT-PCR Kit, (Cat. No. 205920). Five μL of cDNA, 12.5 μL of 2 \times SYBR[®] Green PCR mix with ROX from BioRad were mixed with 10 pmol/ μL of each primer for the quantified genes. The house keeping gene β -actin was used as a constitutive control for normalization. Primers were designed by using Primer3 software (<http://bioinfo.ut.ee/primer3/>) as per the published *O. niloticus* gene sequence in NCBI database. GST, Forward 5' TAATGGGA GAGGGAAGATGG3', Reverse 5' CTCTGCGATGTAATT CAGGA3'; GR, Forward 5' CATTACCGAGACGCG-GAGTT 3', Reverse 5' CAGTTGGCTCAGGATCATTGT 3'; GPx, Forward 5' CCAAGAGAACTGCAAGAACGA 3', Reverse 5' CAGGACACGTCATTCTACAC 3'; CAT, forward 5' TCCTGAATGAGGAGGAGCGA 3', Reverse 5'

ATCTTAGATGAGCGGTGATG 3'; SOD, Forward 5' GGTGCCCTGGAGCCCTA 3', Reverse 5' ATGCGA AGTCTTCCACTGTC 3' and β -actin gene Forward 5' CAAT-GAGAGGTTCCGTTGC 3', Reverse 5' AGGATTCCATAC-CAAGGAAGG 3' (XM_003445184, EU234530, EF206801, JF801726.1, JF801727.1 and EU887951). Primers were synthesized and provided by Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The conditions of PCR were initial denaturation at 95°C , 2 min, then 28 cycles of 95°C , 1 min; 60°C , 1 min; 72°C , 1 min. PCR reactions were carried out in an AbiPrism 7300 (Applied Biosystems, USA). The levels of RNA were quantified by the values of the threshold cycle (Ct). The mRNA fold changes (mRNA relative expression) were expressed relative to the corresponding mRNA mean value found in the control group and was calculated using the 2^{DDCt} method (Livak and Smittgen 2001).

2.8. Statistical analysis

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

3. Results

3.1. Lethal concentrations (LC) of Ag-NPs on fish

There was no mortality observed in the control fish. Ag-NPs exposure produced some mortality that increased with the Ag-NP concentration (Table 2). 96 h LC₅₀ was 19.5 ± 2 and 20 ± 2.4 mg/L to *O. niloticus* and *T. zillii* respectively. As in the control group, no mortality was observed in fish exposed to Ag-NPs at a concentration of 5 mg/L. 40 mg/L Ag-NP suspension caused 100% mortality with a calculated 96 h LC₉₀ of 27.3 ± 3.2 and 28.2 ± 2.9 mg/L to *O. niloticus* and *T. zillii* respectively.

3.2. The actual aqueous exposure to Ag nanoparticles

The actual Ag-NP concentrations in the experimental water column were determined at zero, 12 and 24 h of exposure. As indicated in Table 1 a non-significant time dependent loss of Ag-NPs was seen. The losses may be due to nanoparticle aggregates despite continual aeration. The experimental water was changed every 24 h to keep the nanoparticle concentration

Table 2 Lethal concentrations (mg/L) of Ag-NPs on *O. niloticus* and *T. zillii* after 24, 48 and 96 h of exposure.

Time (h)	Fish	Toxicity (mg/L)		
		LC ₁₀	LC ₅₀	LC ₉₀
24	<i>O. niloticus</i>	19.9 ± 1.5	26.5 ± 3	37.9 ± 5
	<i>T. zillii</i>	20.6 ± 2.1	26.9 ± 4.1	39 ± 7
48	<i>O. niloticus</i>	17.33 ± 1.7	24 ± 3	31.8 ± 4.9
	<i>T. zillii</i>	17.7 ± 1.4	24.6 ± 3.2	32.6 ± 3.8
96	<i>O. niloticus</i>	14.2 ± 1.1	19.5 ± 2	27.3 ± 3.2
	<i>T. zillii</i>	14.7 ± 0.9	20 ± 3.4	28.2 ± 2.9

and decrease aggregation and sedimentation of the particles to a certain extent.

3.3. Effect of Ag-NPs on Ag levels in brain tissue

Our result reported that, the level of Ag in the brain tissue of *O. niloticus* and *T. zillii* was increased. The increase was significant in fish exposed to 2 mg/L Ag-NPs ($P < 0.05$) and highly significant in fish exposed to 4 mg/L ($P < 0.001$) as compared to the control. Moreover, Ag level was highly significant increased ($P < 0.01$) in fish exposed to 4 mg/L as compared to 2 mg/L as indicated in Table 3.

3.4. Effect of Ag-NPs on MDA levels in brain tissue

Exposure of fish to 2 mg/L Ag-NPs does not effect on MDA in brain tissue of *O. niloticus* and *T. zillii* as compared to control, while, exposure of fish to 4 mg/L Ag-NPs resulted in a highly significant increase ($P < 0.001$) of MDA in brain tissue as compared to control and 2 mg/L exposed fish as shown in Table 3.

3.5. Effect of Ag-NPs on GSH and tGSH levels in brain tissue

Our result reported that, the level of GSH and tGSH in the brain tissue of *O. niloticus* and *T. zillii* was not changed in fish exposed to 2 mg/L as compared to control. In contrast, GSH and tGSH levels were highly significantly decreased ($P < 0.01$) in fish exposed to 4 mg/L as compared to control and 2 mg/L exposed fish as indicated in Table 3.

3.6. Effect of Ag-NPs on antioxidant enzymes activity in brain tissue

Our result indicated that the exposure to 4 mg/L Ag-NPs caused a significant decrease in the activity of CAT and a highly significant decrease in SOD, GST, GR and GPx activity in the brain tissues of fish as compared to control and 2 mg/L exposed fish as indicated in Table 4.

3.7. Effects of Ag-NPs on the relative gene expression of antioxidant enzymes in fish brain tissues

The relative gene expression of antioxidant enzymes in the brain tissues of *O. niloticus* and *T. zillii* is shown in Table 5. The exposure to 2 mg/L Ag-NPs did not effect on CAT, SOD, GST, GR and GPx gene expression of fish brain as

compared to control. In contrast, CAT, SOD, GST, GR and GPx gene expressions were highly significantly decreased in fish exposed to 4 mg/L as compared to control and 2 mg/L exposed fish.

4. Discussion

This study is the first trial tended to estimate the median lethal concentration (LC₅₀) of Ag-NPs on *O. niloticus* and *T. zillii*. Aqueous exposure to 5 and 10 mg/L of Ag-NP suspension did not cause any fish mortality, while, 40 mg/L resulted in 100% fish mortality. The 96 h LC₅₀ was 19.5 ± 2 and 20 ± 2.4 mg/L to *O. nilotica* and *T. zillii* respectively this indicating the toxic potential of Ag-NPs on fish. *O. nilotica* and *T. zillii* nearly have the same sensitivity to Ag-NPs. The LC₅₀ of ZnONPs on *O. niloticus* was previously determined by our study (Alkaladi et al., 2015) it was 3.1 ± 0.4 mg/L this indicating Ag-NPs less toxic on *O. niloticus* than ZnONPs. Although there are no data on LC₅₀ of Ag-NPs in *O. nilotica* and *T. zillii*, there were some reports that determined 69 h LC₅₀ of Ag-NPs in *O. mossambicus* 12.6 mg/L (Govindasamy and Abdul Rahuman, 2012), 34.6 µg/L in Japanese medaka (Chae et al., 2009), in zebra fish 84 µg/L (Bilberg et al. 2012). The 96-h LC₅₀ values for *Oryzias latipes* of 60 and 300 nm Ag-NPs suspensions were 28 and 67 µg/L, respectively (Kim et al., 2011).

In the present study, Ag particles were increased significantly in the brain tissues of Ag-NP exposed fish. The increase was significant in fish exposed to 2 mg/L and highly significant in fish exposed to 4 mg/L. The NPs were taken by gills and digestive tract absorbed to the circulation. Long circulation of nanoparticles leads to increase the chance of their passage to tissues, and hence higher cellular uptake (Li and Huang, 2008). Our results are in the same line of Lee et al. (2012) who investigated the penetration of Ag-NPs into fish brain and liver, indicating that particles entered the brain and liver of common carp exposed to 200 µg/L of Ag-NPs for 96 h. Moreover, the Ag-NPs were able to penetrate deep into the brain cells. This result indicated that the particles were carried through the body of the fish by the circulation of blood, subsequently accumulating in different important organs. Once taken up in cells, particles encounter an increasingly acidic environment as they move from early to late endosomes and finally to lysosomes, resulting in their dissolution (Nel et al., 2009). Such phenomena may have contributed to the observed increases of Ag levels in brain tissue in our study. This result goes in the same line as we find previously in rat (Afifi and Abdelazim, 2015). Several toxicity studies demonstrated the distribution of nanoparticles to the brain in some experimental

Table 3 Effect of Ag-NPs on Ag, MDA, GSH and tGSH levels in brain of *O. niloticus* and *T. zillii*.

Fish	Group	Ag (µg/g)	MDA (nmol g ⁻¹ wt.w)	GSH (µmol g ⁻¹ wt.w)	tGSH (µmol g ⁻¹ wt.w)
<i>O. niloticus</i>	Control	12 ± 1.2	1.8 ± 0.3	42.2 ± 3	67 ± 4
	2 mg/L	20.2 ± 2*	1.5 ± 0.24	40 ± 6	63 ± 4.2
	4 mg/L	33 ± 4***.##	4.3 ± 1***.###	32.1 ± 4**.#	52 ± 3**.#
<i>T. zillii</i>	Control	13.1 ± 1.4	1.6 ± 0.3	44.6 ± 4.2	69.2 ± 2.8
	2 mg/L	22.9 ± 1.6*	1.37 ± 0.16	41 ± 7.2	65.4 ± 2
	4 mg/L	32 ± 6***.##	4 ± 0.6**.#	30.5 ± 6**.#	54 ± 4**.#

wt.w: Wet weight tissue. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to control group of the same fish species; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, comparing to 2 mg/L Ag-NPs exposed group of the same fish species.

Table 4 Effect of Ag-NPs on antioxidant enzymes activity in brain of *O. niloticus* and *T. zillii*.

Fish	Group	CAT $\mu\text{M H}_2\text{O}_2$ decomposed/g tissue	SOD $\mu\text{g/mg P}$	GST nmol/mg P/min	GR U/mg P	GPx ($\mu\text{U min}^{-1} \text{mg}^{-1} \text{P}$)
<i>O. niloticus</i>	Control	38.6 \pm 3	20 \pm 3	250 \pm 19	18 \pm 3	127 \pm 15
	2 mg/L	39 \pm 5	18.1 \pm 3	230 \pm 30	16 \pm 2	1115 \pm 10
	4 mg/L	30 \pm 2*.#	8 \pm 1**.#	115 \pm 10***.###	6.1 \pm 1***.#	63 \pm 8***.###
<i>T. zillii</i>	Control	36.6 \pm 3	18.4 \pm 2.7	233 \pm 16	17.4 \pm 2.7	127.4 \pm 28
	2 mg/L	35.2 \pm 5	16.1 \pm 3	220 \pm 18	14 \pm 2	116 \pm 20
	4 mg/L	28 \pm 2*.#	8.1 \pm 2**.#	120 \pm 18***.###	8 \pm 1.9***.#	71 \pm 8***.###

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to control group of the same fish species; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, comparing to 2 mg/L Ag-NPs exposed group of the same fish species.

Table 5 Effect of Ag-NPs on mRNA expression (relative expression to β -actin) of antioxidant genes in brain of *O. niloticus* and *T. zillii*.

Fish	Group	CAT	SOD	GST	GR	GP
<i>O. niloticus</i>	Control	23.4 \pm 3	7.4 \pm 0.6	172 \pm 11	1.4 \pm 0.19	42 \pm 8
	2 mg/L	20 \pm 2.7	10 \pm 2	160 \pm 15	1.2 \pm 0.1	39 \pm 8
	4 mg/L	14 \pm 2**.#	2 \pm 0.2***.###	100 \pm 9***.###	0.8 \pm 0.1**.#	20 \pm 3***.###
<i>T. zillii</i>	Control	30 \pm 2.5	7.8 \pm 1	166 \pm 8	1.66 \pm 0.17	37.8 \pm 10
	2 mg/L	28 \pm 2.5	11 \pm 1.3	150 \pm 13	1.4 \pm 0.23	34 \pm 6
	4 mg/L	11 \pm 3**.#	2.2 \pm 1***.###	110 \pm 8***.###	0.7 \pm 0.12***.###	25 \pm 6***.###

** $P < 0.01$, *** $P < 0.001$, compared to control group of the same fish species; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, comparing to 2 mg/L Ag-NPs exposed group of the same fish species.

fish such as medaka and largemouth bass via the blood–brain barrier or olfactory neuron (Srinonate et al., 2015). Exposure to Ag-NPs significantly increased whole body silver content. It is maybe due to translocation of silver from the gills to the rest of the body or due to ingestion of particulates and gastrointestinal (GI) absorption (Griffitt et al., 2009).

MDA is an important biomarker for monitoring of lipid peroxidation and the health condition of biological membranes that rich in polyunsaturated fatty acids. In the present investigation, brain of the fish exposed to lower Ag-NP concentration didn't exhibit a significant change in MDA level, while that exposed to the highest concentration exhibited an elevated MDA levels. The increase of MDA in fish exposed to the highest Ag-NPs could be explained by the depletion of the antioxidant system that proved in this study. Also, ionic Ag that produced from Ag-NPs in brain tissue is incriminated in the distraction of the cell and mitochondrial membranes with over oxidation of the polyunsaturated fatty acids producing MDA (Ranjbar et al., 2014). There are many mechanisms that may explain Ag-NP toxicity, the important one is the induction of ROS synthesis. This mechanism was documented in mammalian cells in culture, aquatic species, and organisms (McShan et al., 2014). The content of MDA increased and the activities of antioxidant enzymes decreased in the liver of Ag-NPs-treated *O. mossambicus*. The results suggest that the balance between the oxidative and antioxidant system in the fish was broken during the exposure of Ag-NPs (Govindasamy and Abdul Rahuman 2012).

The antioxidant defense systems include a series of antioxidative enzymes and low-molecular non-enzymatic antioxidants. They protect the biological systems from

environmental stress at a molecular level (Van der Oost et al., 2003; Pandey et al., 2003). The present study, therefore, assessed the activity and gene expression of main enzymatic antioxidant as CAT, GST, GR, SOD and GPx and content of antioxidant substances as GSH and tags in response to 15-day exposure to Ag-NPs at 2 mg/L and 4 mg/L. In this study, GSH and tGSH levels varied with Ag-NP concentrations. Exposure to 2 mg/L Ag-NPs didn't produce a significant change in GSH and tGSH contents in the brain tissue, while exposure to 4 mg/L produced a significant decrease in GSH and tGSH contents. It was consistent with the generation of excessive ROS that reacted with and neutralized GSH. Although the mechanisms for AgNP toxicity remain speculative, oxidative stress may be involved (Wise et al., 2010). Our data support this view as Ag-NPs generated ROS, reduced GSH and tGSH levels. The depletion of GSH is indicative of oxidative stress since GSH acts as an electron donor to neutralize ROS (Tuncer et al., 2010). This is consistent with Hussain et al. (2005) and Piao et al. (2011) showing that in rat and human liver cells Ag-NPs exposure resulted in GSH depletion, reduced mitochondrial potential, and increased ROS levels. Alternatively, Ag-NPs were reported to interfere with the activity of GSH-synthesizing enzymes, reducing GSH levels (Piao et al., 2011). Furthermore, Ag has a strong affinity for redox-reactive and protective SH groups like those in GSH could be responsible for the observed GSH depletion (Kramer et al., 2009). It is unclear as to which mechanism is responsible for the observed depletion of GSH, perhaps all three are involved.

Our result indicated that the lower Ag-NP (2 mg/L) concentration didn't affect the antioxidant enzyme activity and gene

expression in the fish brain tissue, while they inhibited at higher Ag-NP (4 mg/L) exposure concentration. The inhibition of the all measured enzyme activity and gene expressions produced in fish exposed to higher Ag-NP concentration indicated the over accumulation of ROS, which, exceeded the scavenging ability of antioxidant defense systems and the impact of Ag-NPs on the balance of antioxidant defense system and oxidative stress in vivo under this exposure concentration. The Cytotoxic effect of Ag-NPs is closely related to cellular uptake, production of ROS, and triggering of the cellular antioxidant mechanisms, either in mammalian cell culture, aquatic species and chickens (McShan et al., 2014). Our results enforced by the results of Govindasamy and Abdul Rahuman (2012), who reported that the activity of antioxidant enzymes was reduced and the content of antioxidants was lowered in the gills and liver of *O. mossambicus* treated with Ag-NPs. The cytotoxicity of AgNPs and Ag⁺ in rainbow trout (*O. mykiss*) hepatocytes and erythrocytes was similar, such that both silver types generated ROS, decreased GSH levels, and decreased activities of GR and GST (Massarsky et al., 2014).

In the present study, SOD and CAT activity and gene expression reduced in the brain of fish exposed to the highest Ag-NPs concentrations. The SOD-CAT system provides the first defense against oxygen toxicity. SOD catalyzes the transformation of O₂ and H₂O₂, and CAT contributes to convert H₂O₂ to water and oxygen (Stanic et al., 2006). The inhibitory effect of Ag-NPs on both SOD and CAT may be explained by over production of ROS that induced by Ag-NPs. SOD activity inhibited in the liver, gills and brain of carp exposed to 100 and 200 mg/L nano-TiO₂ (Hao et al., 2009). The same result was reported by Xiong et al. (2011) who proved the inhibitory effect of ZnONPs on liver SOD in fish exposed to 5 mg/L ZnONPs. Similarly, our previous study (Alkaladi et al., 2014) reported that ZnONPs inhibited the activity and reduced the gene expression of SOD and CAT in gills and liver of *O. nilotica* exposed to 1 and 2 mg/L ZnONPs. Also, the Activity of three important antioxidant enzymes GR, GPx and GST was determined. As SOD and CAT the activity and gene expression of GST, GR and GPx reduced in Ag-NPs higher concentration. GST activity was inhibited, the depletion of GSH may explain the decreased GST activity as it conjugates GSH to harmful products of lipid peroxidation (and xenobiotic compounds) (Lushchak et al., 2001); GST activities were further reduced when GSH levels were chemically reduced in BSO-treated hepatocytes (Massarsky et al., 2014). Moreover, there was a tendency for decreased GPx activity and gene expression with increasing concentrations of Ag-NPs. As GPx eliminates hydroperoxides by reduction of GSH, the decrease in its activity may relate to the observed decreased levels of GSH (Massarsky et al., 2013). Reduction in GPx activity has been reported previously in the human liver cell line HL-7702 exposed to Ag-NPs for 24 h (Song et al., 2012). The activity of GR was also reduced, perhaps in response to the decreased GSSG levels as it recycles GSSG to replenish GSH (Massarsky et al., 2014). Ali and Ali (2015) documented that copper oxide nanoparticles (CuONP) induced the oxidative stress in the digestive gland of freshwater snail *Lymnaea luteola* L. through inhibition of GPx and GST activity and decreasing the levels of GSH. The expression of GR, GPx and GST gene was repressed in fish exposed to the highest Ag-NPs concentration. The repression effect of Ag-NPs on the antioxidant genes may be explained by the deleterious

effect of metal on DNA. The expression of antioxidant genes and the activity of enzymatic antioxidant were modulated in fish, bivalves and protozoa that were exposed to metal (Trevisan et al., 2014).

5. Conclusion

The 96 h LC₅₀ of Ag-NPs was 19.5 ± 2 and 20 ± 2.4 mg/L to *O. nilotica* and *T. zillii* respectively. Ag-NPs in exposure concentration of 4 mg/L induced a deleterious effect on brain antioxidant system of *O. nilotica* and *T. zillii*. While, Ag-NPs in exposure concentration of 2 mg/L has no effect on brain antioxidant system of *O. nilotica* and *T. zillii*.

Conflicts of interest

The authors declare no conflict of interest.

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