

Original Article

Effects of dietary supplementation of zinc oxide nanoparticles on some biochemical biomarkers in common carp (*Cyprinus carpio*)

Somayeh Taheri, Mahdi Banaee*, Behzad Nematdoost Haghi, Mohammad Mohiseni

Aquaculture Department, Environment and Natural Resource Faculty, Behbahan Khatam Alanbia University of Technology, Iran.

Abstract: If the dose and duration of zinc oxide nanoparticle (ZnO-NPs) supplementation optimize, low concentrations of Zn nanoparticles can replace conventional Zn sources in diets of different species of fish. Since evaluating the cytotoxicity of any nutritional supplement is one of the requirements for optimizing the dose for a specified time, we conducted this study to investigate the effects of oral administration of ZnO-NPs on oxidative stress and certain biochemical biomarkers in common carp, *Cyprinus carpio*, as an experimental model. For this purpose, ZnO-NPs were orally administered to fish for 21 days at 0 (control), 5, 10 and 15 mg kg⁻¹ feed. Administration of ZnO-NPs (15 mg kg⁻¹) significantly enhanced aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities in liver, and alanine aminotransferase (ALT), alkaline phosphatase (ALP), and LDH activities in kidney. Dietary ZnO-NPs increased glucose-6-phosphate dehydrogenase (G6PDH) activity in liver of fish. The results indicated that administration of 10 mg kg⁻¹ and 15 mg kg⁻¹ ZnO-NPs caused a significant increase in ALT and catalase (CAT) activities and malondialdehyde (MDA) levels in liver, AST and CAT activities and MDA levels in kidney. ZnO-NPs decreased the liver ALP activity. Administration of 5 mg kg⁻¹ ZnO-NPs significantly increased the cellular total antioxidant (TA) levels in various tissues. Therefore, we suggest that oral administration of 10 and 15 mg kg⁻¹ ZnO NPs caused cytotoxicity and alterations in oxidative biomarkers, but 5 mg ZnO-NPs per kg feed had no side effects on oxidative stress and biochemical biomarkers in fish.

Article history:

Received 23 August 2017

Accepted 6 October 2017

Available online 25 October 2017

Keywords:

ZnO-NPs

Oxidative stress

Common carp

Biochemical parameters

Introduction

Zinc is an essential trace element for finfish and plays a critical role in biological processes and physiological functions such as biosynthesis of hormones, enzymatic activity, and metabolism of proteins and carbohydrates (Wang and Wang, 2015). The activity of more than 300 enzymes and around 2000 transcription factors in varied species of animals is closely related to zinc (Chen et al., 2015; Wang and Wang, 2015; Swain et al., 2016). Zinc ions specifically bind to the receptors of cell membranes, carriers and channels and regulate their activity (Swain et al., 2016). This element is vital in regulating the reception of cellular signals, metabolism of secondary messengers, the activity of protein kinases

and phosphatases, as well as the binding of transcription factors to DNA (Chen et al., 2015). Therefore, zinc deficiency can lead to a lower growth rate, increased mortality, cataracts, fins and skin erosion and dwarfism (Wang and Wang, 2015). The presence of tri-calcium phosphate in fish meal, phytate or phytic acid in soybean meal and other oil seeds and grains may reduce the bioavailability minerals, such as zinc and manganese in diet of freshwater fish, including carp and rainbow trout (Hossain et al., 2003). Therefore, using zinc supplement in foodstuff may prevent zinc deficiency (Hossain et al., 2003).

Nevertheless, one of the consequences of zinc supplements in foodstuff is an increase in Zn excretion from fish body and an increase in its concentration in

*Corresponding author: Mahdi Banaee
E-mail address: mahdibanaee@yahoo.com

the environment (Swain et al., 2016). That is why researchers are looking for ways to decrease zinc content in food supplements and increase its bioavailability in diets (Swain et al., 2016). With regard to the physiological, chemical and biological properties of zinc oxide nanoparticles (ZnO NPs), their usage in food supplements could be an appropriate strategy for the aforementioned problem (Swain et al., 2016). This has recently increased interests in using Nano zinc oxide as a food supplement, a growth promoter, an antioxidant and antimicrobial compound and an immune-modulatory agent in diets of varied species of farmed animals (Swain et al., 2015).

Moreover, using metal nanoparticles as a dietary and medical supplement is considered a new approach in pharmacology (Bahrami et al., 2017). Therefore, it is essential to study the side effects of these compounds on the health of experimental laboratory models (Bahrami et al., 2017). Dietary ZnO NPs are so small that are easily absorbed by the digestive system (Swain et al., 2016) and then distributed in different tissues, especially the liver (Swain et al., 2016). This element can demonstrate its short-term effects on biochemical processes and physiological functions of cells (Muthuraman and Kim, 2015).

Regardless of nutritional and commercial aspects of aquaculture, fish can be used as a model in pharmaceutical toxicology (Chen et al., 2017). Data on the toxic effects of zinc in diets of different species of animals abound (Vandebriel and De Jong, 2012; Pandurangan and Kim, 2015a; Pandurangan and Kim, 2015b). Also, there are several studies on the toxic effects of environmental ZnO NPs on fish (Hao and Chen, 2012; Hao et al., 2013; Connolly et al., 2016; (Xiong et al., 2011; Cong et al., 2017; Fernández, García-Gómez and Babín, 2013); however, there is not much information on the toxicological effects and potential risks of ZnO NPs in high concentrations in diets of fish (Swain et al., 2016). Depending on the ZnO NPs concentration and the exposure duration, their cellular toxicity is attributed to oxidative stress, lipid peroxidation, and damage to the cell membrane and oxidative damage to DNA (Najafzadeh et al.,

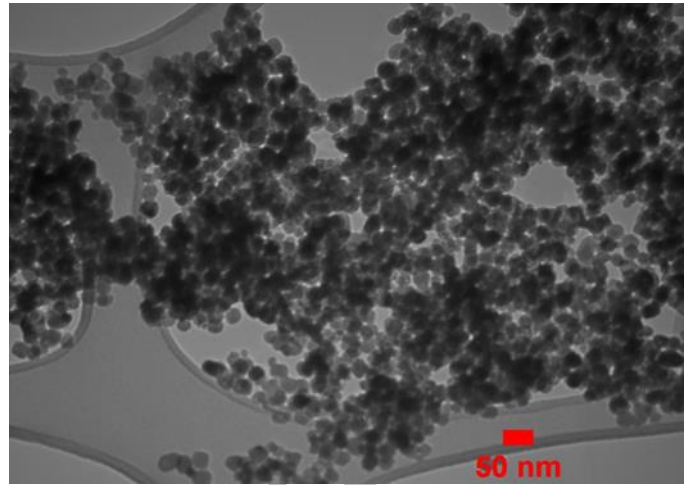


Figure 1. TEM micrographs of the Nano-ZnO powders (Adapted from Iranian Nano-materials Pioneers Company's catalog).

2013; Pandurangan and Kim, 2015a; Pandurangan and Kim, 2015b). Using ZnO nanoparticles may not sound cost-effective, but using metal nanoparticles can be used as a novel approach in treating many difficult-to-treat diseases such as cancer (Bahrami et al., 2017). Thus, the purpose of this study was to investigate consequences of using zinc oxide nanoparticles and to determine the nontoxic dose in foodstuff of common carp.

Materials and Methods

Fish: One hundred forty-four immature common carp, *Cyprinus carpio* (mean weight 20.5 ± 2.5 g) were obtained from a local fish farm (Ahvaz, Khuzestan Province, Iran) and were randomly distributed into twelve circular tanks of 80 L capacity (12 fish per each tank) at the Department of Aquaculture (Khatam Alanbia University of Technology). Prior to the experiment, fish were adapted in tap water ($24 \pm 2^\circ\text{C}$; pH, 7.4 ± 0.2 ; 50% water exchange rate/day) for two weeks. The fish were subjected to artificial light (16 L/8D). During the adaptation period, common carp were fed 2 times a day with commercial pelleted feed (3% of their body weight) according to the manufacturer's recommendations (Beyza Feed Mill, Shiraz, Iran).

Diet preparation: The formulated fish feed was enriched with nano-particles of zinc oxide (Iranian Nano-materials Pioneers Company, Iran; Table 1, Figs. 1-3). Nano-particles of zinc oxide were

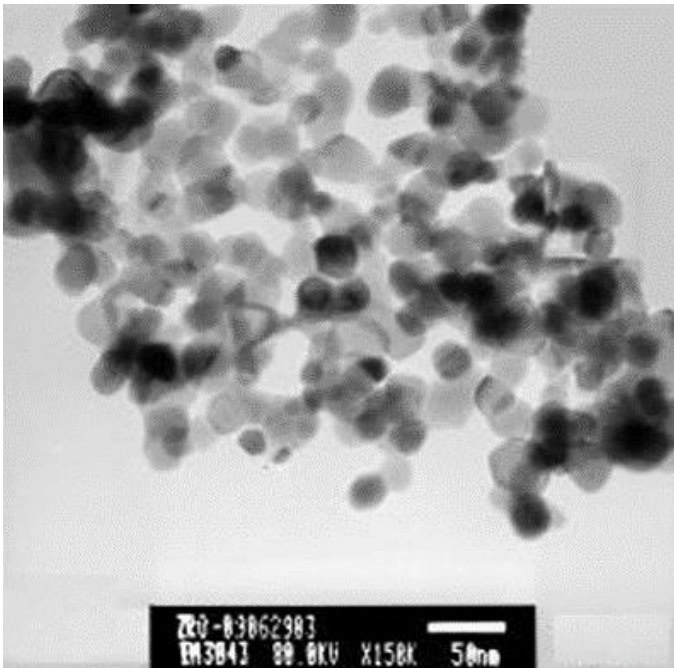


Figure 2. SEM micrographs of the Nano-ZnO powders (Adapted from Iranian Nano-materials Pioneers Company's catalog).

Table 1. Zinc Oxide Nanoparticles Physicochemical Proprieties (Adapted from Iranian Nano-materials Pioneers Company's catalog).

Zinc Oxide	ZnO
Purity	+99.9 %
Average Primary Particle Size (D50)	10-30 nm
Specific surface area (SSA)	20-60 m ² g ⁻¹
Color	White
Bulk density	5.606 g cm ⁻³

supplemented at 5, 10 and 15 mg per kg feed for a total of three treatments.

ZnO nanoparticles were prepared using distilled water and then ultrasonicated (10 min, 35 KHz, 100/400W) using an ultrasound bath (Elma, Germany) (Banaee et al., 2016). Then, solutions were added to powdered feed in order to obtain nominal concentrations of 5, 10 and 15 mg ZnO NPs per kg. Each supplemented diet was mixed in a mixer for 30 minutes and then homogenized into a paste by adding fish oil (20 mL kg⁻¹) and distilled water into the food mixer. The amount of distilled water required for pelleting (20-40% of feed weight) was then added to the mixture and further homogenized. This mixture was passed through a meat grinder, producing string shapes, which were dried in an oven at 55°C for 12 h and then broken to produce 5 mm pellets. The pellets were packed and stored at -20°C in a freezer. The

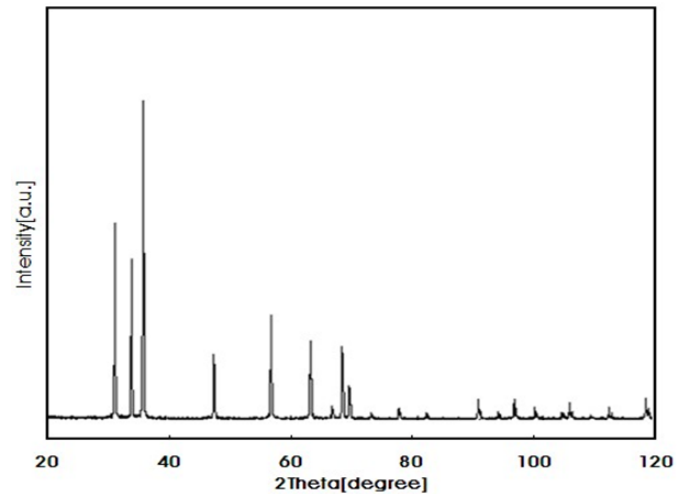


Figure 3. The X-ray powder diffraction (XRD) curves of Nano-crystalline ZnO (Adapted from Iranian Nano-materials Pioneers Company's catalog).

control diet was prepared by the same process, although no supplement was added.

Experimental design: During the experimental period, fish fed commercial pelleted feed with 0 (control), 5, 10 and 15 mg kg⁻¹ ZnO NPs supplement for 21 days. After 21 days, 12 fish per group were sampled randomly and then anesthetized with clove powder solution (200 mg L⁻¹). Fish were sacrificed by decapitation and dissected to remove the liver, and kidney. For enzymatic and biochemical analyses, tissue samples from the target organs were homogenized on ice in cold buffer 100 mM potassium phosphate (Sigma-Aldrich, Germany) pH 7.0 containing 2 mM of EDTA (Riedel-Haën, Germany). Tissue homogenates were centrifuged at 12,000x g for 15 minutes at 4°C. The supernatant was removed and frozen at -25°C for further analysis.

Biochemical Parameters Analysis: Aspartate amino-transferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were determined by the method of (Moss and Henderson, 1999). Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured by the method of Gómez-Milán and Lozano (2007). Protein levels in tissues were determined by standard procedures of (Johnson et al., 1999). For determinations test kits from Pars Azemun Co, Iran, were used. CAT activity was determined as the decrease of absorbance at 450 nm due to hydrogen peroxidase

Table 2. Effects of dietary supplementation of Zinc Oxide nanoparticles (5, 10 and 15 mg kg⁻¹ feed) on some biochemical biomarkers in liver of common carp (*Cyprinus carpio*).

Biochemical parameters	Control	5 mg ZnO-NPs per kg feed	10 mg ZnO-NPs per kg feed	15 mg ZnO-NPs per kg feed
AST (U g ⁻¹ protein)	0.51±0.07 ^a	0.53±0.21 ^a	0.58±0.11 ^a	1.27±0.33 ^b
ALT (U g ⁻¹ protein)	0.17±0.03 ^a	0.20±0.02 ^a	0.23±0.03 ^b	0.26±0.05 ^b
ALP (U g ⁻¹ protein)	1.50±0.38 ^a	0.99±0.08 ^b	0.80±0.05 ^b	0.82±0.17 ^b
LDH (U g ⁻¹ protein)	1.33±0.41 ^a	1.34±0.15 ^a	1.25±0.19 ^a	1.87±0.32 ^b
G6PDH (U g ⁻¹ protein)	8.14±1.65 ^a	18.36±2.32 ^b	16.92±1.98 ^b	16.49±2.77 ^b
CAT (kU g ⁻¹ protein)	7.31±2.17 ^a	9.10±1.36 ^a	21.38±2.91 ^b	23.15±2.79 ^b
TA (µM g ⁻¹ tissue)	10.97±2.37 ^a	26.64±5.27 ^b	11.79±3.02 ^a	10.71±0.88 ^a
MDA (µM g ⁻¹ tissue)	0.03±0.01 ^a	0.03±0.01 ^a	0.06±0.01 ^b	0.19±0.05 ^c

- Different superscripts indicate the significant difference ($P < 0.05$, Duncan's multiple comparison). Data are expressed as Means ± S.D.

- Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Lactate dehydrogenase (LDH); Glucose-6-phosphate dehydrogenase (G6PDH); Catalase (CAT); Malondialdehyde (MDA); Total antioxidant (TA).

Table 3. Effects of dietary supplementation of Zinc Oxide nanoparticles (5, 10 and 15 mg kg⁻¹ feed) on some biochemical biomarkers in kidney of common carp (*Cyprinus carpio*).

Biochemical parameters	Control	5 mg ZnO-NPs per kg feed	10 mg ZnO-NPs per kg feed	15 mg ZnO-NPs per kg feed
AST (U g ⁻¹ protein)	0.49±0.04 ^a	0.46±0.05 ^a	0.40±0.05 ^b	0.33±0.06 ^c
ALT (U g ⁻¹ protein)	0.08±0.01 ^a	0.06±0.01 ^a	0.07±0.01 ^a	0.13±0.01 ^b
ALP (U g ⁻¹ protein)	1.50±0.07 ^a	1.54±0.29 ^a	1.45±0.08 ^a	2.12±0.60 ^b
LDH (U g ⁻¹ protein)	1.55±0.26 ^a	1.65±0.14 ^a	1.75±0.20 ^a	2.02±0.41 ^b
CAT (kU g ⁻¹ protein)	2.87±0.86 ^a	3.64±0.77 ^a	4.88±1.08 ^b	5.96±0.61 ^c
TA (µM g ⁻¹ tissue)	4.09±0.98 ^a	9.18±2.83 ^c	8.06±3.75 ^{bc}	6.14±1.29 ^{ab}
MDA (µM g ⁻¹ tissue)	0.04±0.01 ^a	0.03±0.01 ^a	0.13±0.03 ^b	0.26±0.06 ^c

- Different superscripts indicate the significant difference ($P < 0.05$, Duncan's multiple comparison). Data are expressed as Means ± S.D.

- Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Lactate dehydrogenase (LDH); Catalase (CAT); Malondialdehyde (MDA); Total antioxidant (TA).

consummation as described by (Góth, 1991), although with some modifications. Total antioxidant capacity was estimated according to the ferric reducing ability of plasma (FRAP) as described by (Benzie and Strain, 1996) using TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) as a substrate. Malondialdehyde (MDA) content was assessed by modified thiobarbituric acid assay according to (Placer et al., 1996). All biochemical parameters were measured by UV/VIS spectrophotometer (model Biochrom Libra S22).

Statistical analysis of the results was carried out by one-way ANOVA; the data were checked for assumptions of normality and homogeneity (Shapiro-Wilk test) and when necessary, they were appropriately transformed. The Duncan test was used to compare pairs of means and detect significant differences ($P < 0.05$). The statistical analysis was performed at the significance level of 5%, using IBM SPSS 19. Data are presented as mean ± SD.

Results

During the experiment, mortality was not observed in the control group and fish fed ZnO NPs supplement.

Hepatic biomarkers: The activities of hepatic marker enzymes are shown in Table 2. Activities of AST and LDH were found to be significantly increased in the 15 mg kg⁻¹ ZnO NPs-administrated group ($P < 0.05$). Moreover, ALT, and CAT activities in liver tissue was enhanced by 10 mg kg⁻¹ and 15 mg kg⁻¹ ZnO NPs-supplemented diets at day 21 compared to the control group ($P < 0.05$). Administration of ZnO NPs caused a reduction in ALP activities. In this study, dietary ZnO NPS significantly increased G6PDH activity in liver of fish ($P < 0.05$). Compared with control diet, 10 and 15 mg kg⁻¹ ZnO NPs in the diet significantly enhanced malondialdehyde (MDA) levels in liver of fish ($P < 0.05$). Supplementing 5 mg kg⁻¹ ZnO NPs in the diet increased the total antioxidant levels in liver of fish (Table 2).

Kidney biomarkers: CAT activity in kidney of fish fed with 10 and 15 mg kg⁻¹ ZnO NPs-supplemented diet was significantly higher than that found in fish fed 0.0 mg kg⁻¹ ZnO NPs-supplemented diet ($P < 0.05$). AST activity was found to be elevated after the administration of 10 and 15 mg kg⁻¹ ZnO NPs. The findings demonstrated that ALT, LDH and ALP activities in kidney was enhanced in fish fed with 15 mg kg⁻¹ ZnO NPs as compared with control group ($P < 0.05$). MDA levels statistically increased in kidney of fish fed with 10 mg kg⁻¹ and 15 mg kg⁻¹ ZnO NPs compared to the control group ($P < 0.05$). The total antioxidant levels was significantly increased in kidney of fish fed with 5 mg kg⁻¹ and 10 mg kg⁻¹ ZnO NPs- supplemented diet on day 21 (Table 3).

Discussion

The required amount of zinc in diets of farmed common carp is between 15-30 mg kg⁻¹ feed which is usually added as mineral salts, including zinc oxide or zinc sulphate (Davis and Gatlin, 1996). Due to the use of oil seeds in the base diet, the bioavailability of Zn may decrease for fish (Gupta et al., 2015). Since the physiological function of zinc is affected by its way of transfer and storage in the aquaculture (Muralisankar et al., 2014), using zinc supplement in the form of nanoparticles may solve this issue. Therefore, we evaluated the influence of ZnO NPs in common carp on preventing zinc deficiency in the long term. We aimed at assessing oxidative stress biomarkers (as a general biomarker) in tissues of ZnO NPs-treated carp. Common carp were fed 5, 10, and 15 mg kg⁻¹ ZnO NPs in a 21-day experiment.

AST and ALT activities are important in cellular nitrogen metabolism, oxidation of amino acids, and liver gluconeogenesis (Murray et al., 2003). The increased activity of AST and ALT in tissues of ZnO NPs-treated fish may indicate the increased rate of proteins metabolism in cells. A similar increase of AST and ALT activities were previously reported by (Fazilati, 2013) in the serum of ZnO NPs-treated rats. In contrast, a decrease of plasma ALT and AST activities was observed in chicken broilers that were fed with ZnO NPs supplement dietary (Fathi, 2016).

ALP plays a significant role in phosphate hydrolysis and in membrane transport and it also acts as a good biomarker of stress in biological systems (Murray et al., 2003). The administration of ZnO NPs for 21 days caused a significant decrease in ALP activity in liver of fish. An increase in zinc level in liver can account for a reduced ALP activity because high levels of zinc can have deterrent effects on ALP activity (Farah et al., 2012). Increased ALP activity in kidney may be due to the effects of ZnO NPs on transphosphorylation activity as well as a metabolic dysfunction in cells.

An increase in LDH activity in liver, and kidney may be caused by metabolic stress (Muthuraman and Kim, 2015). Metabolic stress in hepatic and renal cells is dose-dependent. An increased LDH is reported in lung cells of rats and myoblast cell line of mice (Muthuraman and Kim, 2015; Kao et al., 2012). Muthuraman and Kim (2015) found that ZnO NPs increased AST, ALT, ALP and LDH activities and their mRNA expression in C2C12 cells. An increase in AST, ALT, LDH and ALP activity was observed in plasma of common carp treated with high doses of ZnO NPs (Lee et al., 2014). Zinc toxicity depends on the concentration of free ions (Kool et al., 2011). Increasing the concentration of zinc ions in the cytoplasm and influx of Zn⁺² from cytosol to mitochondria can affect permeability and stability of mitochondrial membrane, trigger caspase activation and cell apoptosis (Pandurangan and Kim, 2015b; Kao et al., 2012). Acidic lysozyme accelerates the release of Zn⁺² ions which accompanies the oxidative stress and damage to mitochondrial membrane (Fröhlich and Fröhlich, 2016). In high concentrations, ZnO NPs may disturb the homeostasis of ion in cytoplasm (Kao et al., 2012) and accordingly disturb the biochemical balance in cells.

G6PDH is the rate-limiting enzyme in the pentose phosphate pathway and a key contributor to carbohydrate and fatty acid metabolism (Murray et al., 2003). Previous studies show that alterations in G6PDH activity are critical for cells (Mehrpack et al., 2015). G6PDH plays an important role in cell growth by providing NADPH and therefore leading to

regulation of the redox activity (Stanton, 2012). Moreover, antioxidant enzyme activities are dependent on an adequate supply of NADPH. Thus, G6PDH activity should be increased to provide sufficient NADPH (Stanton, 2012). An increase in G6PDH activity following ZnO NPs administration is a cellular physiological response to cope with reactive oxygen species (ROS). Although low levels of ZnO NPs are nontoxic, increased G6PDH activity was still observed which indicates that G6PDH may act as a bio-sensor and response to very low levels of free radicals (Sauer, 1998). An increase in the activity of G6PDH can eliminate ROS by using NADPH (Sauer, 1998). However, a decrease of G6PDH activity was observed in the liver of white sucker, (*Catostomus commersonii*) exposed to zinc oxide nanoparticle (Dieni et al., 2014)

ZnO NPs have antioxidant properties (Nagajyothia et al., 2014; Nagajyothia et al., 2015). The results of this study show that administering low concentrations of ZnO NPs may enhance total antioxidant capacity of the cell by increasing the activity level of enzymatic (SOD and CAT) and non-enzymatic (protein antioxidants and glutathione) antioxidant system, reducing the ROS level and inhibiting the activity of nitric-oxide synthase and NADPH oxidase (Prasad, 2014). Furthermore, Zn inhibits the influence of lipid peroxidation products on the cellular antioxidant system (Prasad, 2014). Muthuraman et al. (2014) showed that ZnO NPs increased antioxidant enzyme activities, and their mRNA expression in the co-cultured C2C12 (mouse myoblast cell line) and 3T3-L1 cells.

Previous studies indicate that ZnO NPs may remove free radicals, increase the efficiency of the cellular antioxidant defense system, increase the enzymatic activity of antioxidant defense system and reduce malondialdehyde level (Dawei et al., 2010) and consequently protect cells against ROS and oxidative damages (Badkoobeh et al., 2013). Zn, a cofactor of superoxide dismutase (SOD), regulates the process of converting superoxide to hydrogen peroxide (Prasad, 2014). Therefore, an increase in CAT activity in hepatic and renal cells of fish which were fed 10 and

15 mg kg⁻¹ ZnO NPs could be a response to increased H₂O₂ in these cells. Saddick et al. (2015) found that *Oreochromis niloticus* and *Tilapia zillii* which were exposed to low concentrations of ZnO NPs (500 µg L⁻¹) showed an increase in CAT activity and gene expression of antioxidant enzymes in brain tissue. On the other hand, increased concentration of ZnO NPs (2000 µg L⁻¹) significantly decreased CAT activity and gene expression of antioxidant enzymes. Similarly, CAT activity decreased in liver, and intestine of zebrafish which were exposed to ZnO NPs (Xiong et al., 2011).

Zn has antioxidant properties and a key role in inhibition and removal of free radicals; therefore, it can act as an antioxidant in low concentrations (Swain et al., 2016). Nonetheless, we found that the increased amount of ZnO NPs in foodstuff increased lipid peroxidation in tissues. We suggest that an increase in MDA level in liver, and kidney could be an appropriate biomarker of lipid peroxidation in common carp after oral exposure to ZnO NPs. Our findings correspond with those of (Syama et al., 2013). They reported that ZnO NPs are not toxic at low concentration, but at higher concentrations increase ROS through increased MDA levels (Syama et al., 2013). Muthuraman et al. (2014) found that ZnO NPs increased reactive oxygen species (ROS) and lipid peroxidation (MDA) in 3T3-L1 adipocytes. An increase in MDA is reported in *C. commersonii* (Dieni et al., 2014), *C. carpio* (Hao and Chen, 2012; Hao et al., 2013), *Oncorhynchus mykiss* (Connolly et al., 2016), and zebrafish (Xiong et al., 2011) which were exposed to ZnO NPs. Cytotoxic effects of ZnO NPs, depending on their concentration and exposure duration, may be caused by ZnO NPs accumulation in the liver, the occurrence of oxidative stress, damage to DNA and an increase in lipid peroxidation (Najafzadeh et al., 2013). ZnO NPs produce free radicals, cause cellular toxicity and therefore lead to oxidative damages, inflammation and programmed cell death (Kumar et al., 2011; Umrani and Paknikar, 2014).

The results obtained from the present study showed that ZnO NPs given orally to fish could induce dose-

dependent effects on oxidative stress biomarkers and biochemical parameters. On the basis of these results, it is deduced that supplementation of 5 mg kg⁻¹ ZnO-NPs had no side effect on biochemical parameters in liver and kidney tissue of common carp. Nevertheless, to ensure the safety of using ZnO NPs as a food supplement, future studies should consider effects of these NPs in non-toxic concentrations on other physiological indicators such as growth, reproduction, the immune system.

Acknowledgments

The authors gratefully acknowledge the support offered by the Behbahan Khatam Al-anbia University of Technology. We also thank our English editor, M. Banaee for proofreading the manuscript.

References

- Badkoobeh P., Parivar K., Kalantar S.M., Hosseini S.D., Salabat A. (2013). Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats. *Iranian Journal of Reproductive Medicine*, 11(5): 355-364.
- Bahrami B., Hojjat-Farsangi M., Mohammadi H., Anvari E., Ghalamfarsa G., Yousefi M., Jadidi-Niaragh F. (2017). Nanoparticles and targeted drug delivery in cancer therapy. *Immunology Letters*, 190: 64-83.
- Banaee M., Shahafve S., Tahery S., Nemadoost Haghi B., Vaziriyani M. (2016). Sublethal toxicity of TiO₂ nanoparticles to common carp (*Cyprinus carpio*, Linnaeus, 1758) under visible light and dark conditions. *International Journal of Aquatic Biology*, 4(6): 370-377.
- Benzie I., Strain J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power. the FRAP assay", *Analytical Biochemistry*, 239(1): 70-76.
- Chen L., Groenewoud A., Tulotta C., Zoni E., Julio M.K., van der Horst G., van der Pluijm G., Snaae-Jagalska E. (2017). A zebrafish xenograft model for studying human cancer stem cells in distant metastasis and therapy response. *Methods in Cell Biology*, 138: 471-496.
- Chen L., Yang J., Zheng M., Kong X., Huang T., Cai Y.-D. (2015). The use of chemical-chemical interaction and chemical structure to identify new candidate chemicals related to lung cancer. *PLoS ONE*, 10(6), e0128696.
- Cong Y., Jin F., Wang J., Mu J. (2017). The embryotoxicity of ZnO nanoparticles to marine medaka, *Oryzias melastigma*. *Aquatic Toxicology*, 185: 11-18.
- Connolly M., Fernández M., Conde E., Torrent F., Navas J.M., Fernández-Cruz M.L. (2016). Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout. *Science of The Total Environment*, 551: 334-343.
- Davis D.A., Gatlin D.M. (1996). Dietary mineral requirements of fish and marine crustaceans. *Reviews in Fisheries Science*, 4(1): 77-99.
- Dawei A.I., Zhisheng W., Anguo Z. (2010). Protective effects of nano-ZnO on the primary culture mice intestinal epithelial cells in vitro against oxidative injury. *World Journal of Agricultural Sciences*, 6: 149-153.
- Dieni C.A., Callaghan N.I., Gormley P.T., Butler K.M.A., MacCormack T.J. (2014). Physiological hepatic response to zinc oxide nanoparticle exposure in the white sucker, *Catostomus commersonii*. *Comparative Biochemistry and Physiology, Part C*, 162: 51-61.
- Farah H.S., Al-Atoom A.A., Shehab G.M. (2012). Explanation of the decrease in alkaline phosphatase (ALP) activity in hemolysed blood samples from the clinical point of view: In vitro study. *Jordan Journal of Biological Sciences*, 5(2): 125-128.
- Fathi M. (2016). Effects of zinc oxide nanoparticles supplementation on mortality due to ascites and performance growth in broiler chickens. *IJAS*, 6(2): 389-394.
- Fazilati M. (2013). Investigation toxicity properties of zinc oxide nanoparticles on liver enzymes in male rat. *European Journal of Experimental Biology*, 3(1): 97-103.
- Fernández D., García-Gómez C., Babín M. (2013). In vitro evaluation of cellular responses induced by ZnO nanoparticles, zinc ions and bulk ZnO in fish cells. *Science of The Total Environment*, 452: 262-274.
- Fröhlich E.E., Fröhlich E. (2016). Cytotoxicity of nanoparticles contained in food on intestinal cells and the gut microbiota. *International Journal of Molecular Sciences*, 17(4): 509.
- Gómez-Milán E., Lozano M.J.S.M. (2007). Daily and annual variations of the hepatic glucose 6-phosphate dehydrogenase activity and seasonal changes in the body fats of the gilthead seabream *Sparus aurata*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 307A: 516-526.

- Góth L.A. (1991). Simple method for determination of serum catalase and revision of reference range. *Clinica Chimica Acta*, 196: 143-152.
- Gupta R.K., Gangoliya S.S., Singh N.K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, 52(2): 676-684.
- Hao L., Chen L. (2012). Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. *Ecotoxicology and Environmental Safety*, 80: 103-110.
- Hao L., Chen L., Hao J., Zhong N. (2013). Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): A comparative study with its bulk counterpart. *Ecotoxicology and Environmental Safety*, 91: 52-60.
- Hossain M.A., Matsui S., Furuichi M. (2003). Effect of zinc and manganese supplementation to tricalcium phosphate rich diet for tiger puffer (*Takifugu rubripes*). *Bangladesh Journal of Fisheries Research*, 7(2): 189-192.
- Johnson A.M., Rohlfs E.M., Silverman L.M. (1999). Proteins. In: C.A. Burtis, E.R. Ashwood (Eds.). *Tietz Textbook of Clinical Chemistry*. 3rd ed., Philadelphia: W.B. Saunders Company. 1917 p.
- Kao Y.Y., C, C.Y., Cheng, T.J., Chung, Y.M., Liu, P.S. (2012). Zinc oxide nanoparticles interfere with zinc ion homeostasis to cause cytotoxicity. *Toxicological Sciences*, 125: 462-472.
- Kool P.L., Ortiz M.D., van Gestel C.A. (2011). Chronic toxicity of ZnO nanoparticles, non-nano ZnO and ZnCl₂ to *Folsomia candida* (Collembola) in relation to bioavailability in soil. *Environmental Pollution*, 159: 2713-2719.
- Kumar A., Pandey A.K., Singh S.S., Shanker R., Dhawan A. (2011). Engineered ZnO and TiO₂ nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*. *Free Radical Biology and Medicine*, 51: 1872-1881.
- Lee J.W., Kim J.E., Shin Y.J., Ryu J.S., Eom I.C., Lee J.S., Kim Y., Kim P.J., Choi K.H., Lee B.C. (2014). Serum and ultrastructure responses of common carp (*Cyprinus carpio* L.) during long-term exposure to zinc oxide nanoparticles. *Ecotoxicology and Environmental Safety*, 104: 9-17.
- Mehrpak M., Banaee M., Nematdoost Haghi B., Noori A. (2015). Protective effects of vitamin C and chitosan against cadmium-induced oxidative stress in the liver of common carp (*Cyprinus carpio*). *Iranian Journal of Toxicology*, 9(30): 1360-1367.
- Moss D.V., Henderson A.R. (1999). *Clinical enzymology*. In: C.A. Burtis, E.R. Ashwood (Eds.). *Tietz Textbook of Clinical Chemistry*. 3rd ed., Philadelphia: W.B. Saunders Company. 1917 p.
- Muralisankar T., Bhavan P.S., Radhakrishnan S., Seenivasan C., Manickam N., Srinivasan V. (2014). 'Dietary supplementation of zinc nanoparticles and its influence on biology, physiology and immune responses of the freshwater prawn, *Macrobrachium rosenbergii*. *Biological Trace Element Research*, 160(1): 56-66.
- Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W. (2003). *Harper's Illustrated Biochemistry*, 26th Edition, McGraw-Hill, Medical Publishing Division. 818 p.
- Muthuraman P., Kim D.H. (2015). ZnO nanoparticles augment ALT, AST, ALP and LDH expressions in C2C12 cells. *Saudi Journal of Biological Sciences*, 22(6): 679-684.
- Muthuraman P., Ramkumar K., Kim D.H. (2014). Analysis of dose-dependent effect of zinc oxide nanoparticles on the oxidative stress and antioxidant enzyme activity in adipocytes. *Applied Biochemistry and Biotechnology*, 174(8): 2851-2863.
- Nagajyothia P.C., Chab S.J., Yanga I.J., Sreekanth T.V.M., Kimb K.J., Shin H.M. (2015). Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using *Polygala tenuifolia* root extract. *Journal of Photochemistry and Photobiology B: Biology*, 146: 10-17.
- Nagajyothia P.C., Sreekanth T.V.M., Tetteya C.O., Juna Y.I., Mook S.H. (2014). 'Characterization, antibacterial, antioxidant, and cytotoxic activities of ZnO nanoparticles using *Coptidis Rhizoma*. *Bioorganic and Medicinal Chemistry Letters*, 24(17): 4298-4303.
- Najafzadeh H., Ghoreishi S.M., Mohammadian B., Rahimi E., Afzalzadeh M.R., Kazemivarnamkhasti M., Ganjealidarani H. (2013). Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. *Veterinary World*, 6: 534-537.
- Pandurangan M., Kim D.H.J. (2015a). In vitro toxicity of zinc oxide nanoparticles: a review. *Nanopart Research*, 17: 158.
- Pandurangan M., Kim D.H. (2015b). ZnO nanoparticles augment ALT, AST, ALP and LDH expressions in C2C12 cells. *Saudi Journal of Biological Sciences*,

22(6): 679-684.

- Placer Z., Cushman L., Johnson B. (1996). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry*, 16(2): 359-364.
- Prasad A.S. (2014). Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. *Frontiers in Nutrition*, 1: 14.
- Sauer B.G. (1998). Effects of zinc on Glucose-6-Phosphate Dehydrogenase in buffalo rat liver cells, *Biological Sciences Department, Western Michigan University, Honors Theses*. 250 p.
- Stanton R.C. (2012). Glucose-6-Phosphate dehydrogenase, NADPH, and cell survival. *IUBMB Life*, 64(5): 362-369.
- Swain P.S., Rajendran D., Rao S.B.N., Dominic G. (2015). Preparation and effects of nano mineral particle feeding in livestock: A review. *Veterinary World*, 8(7): 888-891.
- Swain P.S., Rao S.B.N., Rajendran D., Dominic G., Selvaraju S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition*, 2(3): 134-141.
- Syama S., Reshma S.C., Sreekanth P.J., Varma H.K., Mohanan P.V. (2013). Effect of zinc oxide nanoparticles on cellular oxidative stress and antioxidant defense mechanisms in mouse liver. *Toxicological and Environmental Chemistry*, 95: 495-503.
- Umrani D.R., Paknikar K.M. (2014). Zinc oxide nanoparticles show antidiabetic activity in streptozotocin-induced Types-1 and 2 diabetic rats. *Nanomedicine*, 9: 89-104.
- Vandebriel R.J., De Jong W.H. (2012). A review of mammalian toxicity of ZnO nanoparticles. *Nanotechnology, Science and Applications*, 5: 61-71.
- Wang K., Wang W.X. (2015). Optimal dietary requirements of zinc in marine medaka *Oryzias melastigma*: Importance of daily net flux. *Aquaculture*, 448: 54-62.
- Xiong D., Fang T., Yu L., Sima X., Wentao Z. (2011). Effects of nano-scale TiO₂, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Science of the Total Environment*, 409: 1444-1452.

چکیده فارسی

تاثیر مکمل خوراکی نانوذرات اکسید روی بر برخی پارامترهای بیوشیمیایی در ماهی کپور معمولی (*Cyprinus carpio*)

سمیه طاهری، مهدی بنایی*، بهزاد نعمت دوست حقی، محمد محیسنی

گروه شیلات، دانشکده منابع طبیعی و محیط زیست، دانشگاه صنعتی خاتم الانبیا (ص) بهبهان، ایران.

چکیده:

در صورتی که دوز و مدت زمان تجویز مکمل نانو ذرات اکسید روی (ZnO-NPs) بهینه سازی شود، غلظت های پایین نانوذرات روی می تواند جایگزین منابع متداول روی در رژیم غذایی گونه های مختلف ماهیان شود. از آنجایی که ارزیابی سمیت سلولی هر گونه افزودنی خوراکی یکی از ضروریات بهینه سازی دوز مصرفی در مدت زمان مشخص است، ما این مطالعه به منظور بررسی تأثیر مصرف خوراکی ZnO-NPs بر برخی شاخص های بیوشیمیایی و استرس اکسیداتیو در ماهی کپور معمولی (*Cyprinus carpio*) به عنوان یک مدل آزمایشگاهی طراحی کرده ایم. برای نیل به این هدف، نانوذرات اکسید روی به صورت مکمل خوراکی در غلظت های صفر (کنترل)، ۵، ۱۰ و ۱۵ میلی گرم به ازای هر کیلوگرم غذا به مدت ۲۱ روز به ماهیان خورانده شد. تجویز ZnO-NPs (۱۵ میلی گرم بر کیلوگرم) به طور معنی داری فعالیت آسپارات آمینوترانسفراز (AST) و لاکتات دهیدروژناز (LDH) را در کبد و آلانین آمینوترانسفراز (ALT)، آلکالین فسفاتاز (ALP) و LDH را در کلیه افزایش داد. تجویز خوراکی ZnO NPs فعالیت گلوکز ۶-فسفات دهیدروژناز (G6PDH) را در کبد ماهیان افزایش داد. نتایج نشان می دهد که تجویز ۱۰ و ۱۵ میلی گرم ZnO-NPs منجر به افزایش معنی دار فعالیت ALT و کاتالاز (CAT) و همچنین افزایش سطح مالون دی آلدئید (MDA) در کبد و نیز افزایش فعالیت AST و CAT و سطح MDA در کلیه می شود. ZnO NPs سبب کاهش فعالیت ALP در کبد شد. تجویز ۵ میلی گرم ZnO-NPs سبب افزایش معنی دار سطح آنتی اکسیدان تام (TA) در بافت های مختلف گردید. بنابراین می توان چنین عنوان کرد که تجویز خوراکی ۱۰ و ۱۵ میلی گرم ZnO-NPs منجر به بروز سمیت سلولی و تغییر در شاخص های اکسیداتیو شد، در حالی که مصرف ۵ میلی گرم بر کیلوگرم ZnO-NPs هیچ گونه عوارض جانبی بر شاخص های بیوشیمیایی و استرس اکسیداتیو در ماهیان نداشت.

کلمات کلیدی: نانو اکسید روی، استرس اکسیداتیو، ماهی کپور، پارامترهای بیوشیمیایی.