Journal of Animal & Plant Sciences, 32(5): 2022, Page: 1299-1305
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2022.5.0536

EVALUATION OF WATERBORNE ZINC OXIDE NANOPARTICLES INDUCED TOXICITY IN BIGHEAD CARP, *HYPOPHTHALMICHTHYS NOBILIS*

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ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) have widespread applications in various industries and cosmetics that raise concerns about their hazards to the environment. Eco-toxicological data show that nanoscale zinc oxide has harmful impacts on both aquatic species and human health. The co-precipitation method was followed to synthesize ZnO-NPs and X-ray diffraction (XRD) technique and scanning electron microscope (SEM) were used for characterization. The acute toxicity at 96h was determined by using Bighead carp as a model fish after the exposure to 11 various concentrations (2-50mg/L) of ZnO-NPs. The mean 96-h LC_{50} was measured as 22.24mg/L. Bighead carp showed different behavioral changes that lead to death during acute toxicity tests. Oxidative stress in terms of lipid peroxidation (LPO) and superoxide dismutase (SOD) was determined in fish gills and liver after chronic exposure to ZnO-NPs for 90 days and sampling was done at 15, 30, 45, 60, 75 and 90-day intervals. Significant alterations in SOD and LPO levels were determined in tissues of the liver and gills as compared to the control group. We conclude that induced toxicity mechanism of ZnO-NPs suspension in freshwater environments may be due to elevated oxidative stress.

Keywords: Bighead carp, Zinc oxide nanoparticles; Toxicity; Oxidative stress, Lipid Peroxidation

Published first online April 30, 2022. Published final October 05, 2022

INTRODUCTION

Nanomaterials are widely used in different research fields due to novel physico-chemical characteristics as compared to bulk materials (Khan *et al*., 2019). Engineered nanoparticles (NPs) can be naturally present in the ecosystem and necessarily discharged during construction, use, and disposal operations, indicating that a basic understanding of their physical state and extent of toxicity is required (Handy *et al*., 2008). For domestic applications and industries, metal oxide nanoparticles are widely used (Aitken *et al*., 2006). Zinc oxide nanoparticles (ZnO-NPs) are used in glass, ointments, cosmetics, dyes, optical filters, and cement industries (Rekha *et al*., 2010). Therefore, they enter from point and non-point sources in the aquatic environment and are taken by fish through gills, digestive tract, skin, and transported to the internal organs and tissues (Wang *et al*., 2009; Xiong *et al*., 2011) and then to humans (Yu *et al*., 2011; Handy *et al*., 2008). Due to its smaller particle size, penetration into the cell membrane becomes easier that leads to instability in cell membrane permeability and oxidative stress (OXS) (Ma *et al*., 2013). Within the cell, OXS is one of the most important mechanisms of nanomaterials toxicity that leads to the accumulation of O^{-2} (Superoxide radical) by Fenton type reactions that finally starts programmed cell death and DNA damage (Nel *et al*., 2006). Due to the key role of fish in the food web of aquatic ecosystem, they are considered as an important biomarker for assessment of entire ecosystems (Baker *et al*., 2014). Various research works have been done on ecotoxicity of nanomaterials, but only minimal information is present about bioavailability and the hazards of NPs in fish (Klaine *et al*., 2008). Carps are considered as a most common food source due to consumer preferences in Pakistan. But now a day, water pollution is becoming a major concern in the wetlands of Pakistan that adversely affects the freshwater living organisms. In intensive monoculture systems, mostly carps are cultured to reduce hunger and fulfill the global demand that enhanced variety of toxicants in aquatic systems (Gupta *et al*., 2016). Recently, research on fish has gained more attention as a model to study the toxic impacts of NPs. The present study was designed to find out acute toxicity of nanoscale zinc oxide and its effects on behavior and oxidative stress in terms of lipid peroxidation (LPO) and superoxide dismutase (SOD) in the gills and liver of Bighead carp (*Hypophthalmichthys nobilis*) after exposure to sub-lethal dose ($1/3^{rd}$ of its respective 96-h LC_{50}) for 90days.

MATERIALS AND METHODS

Preparation and characterization of ZnO-NPs: For the preparation of ZnO-NPs, sodium hydroxide and zinc sulfate were used. Distilled water was used for the

formulation of solution. For the zinc sulfate solution, sodium hydroxide was added dropwise (in a molar ratio of 1:2) under continuous stirring for 12h. The filtered precipitates were collected and washed with deionized water. Then precipitates were dried at 100°C in an oven (Shel-Lab). Fine powder was prepared using agate mortar. After that collected powder was calcined in furnace (SNOL-LHM01) for two hours at 500°C. Sample in powder form was used to calculate average crystal size by X-ray diffraction (XRD) using the Scherrer formula $d = 0.89\lambda/B \cos \theta$, where d represents the grain size; λ= wavelength of X-rays; B= full width at half maximum; θ = diffraction angle. Surface morphology of particles was checked by scanning electron microscope (SEM) (JEOL-JSM 5910).

Collection and Maintenance of Test Organisms: This experiment was done in the wet laboratory of Fisheries Research Farms of the University of Agriculture, Faisalabad, Pakistan. Freshwater fish, Bighead carp (90-day old) were placed in cement tanks after acclimation of two weeks and fed on commercial pellet fish feed (30% digestible protein and 3Kcal/g digestible energy)..

Acute toxicity: Fresh water fish, Bighead carp was selected for mean lethal toxicity (LC_{50}) and lethality tests. Different concentrations of zinc oxide nanoparticles viz. 0, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/L were tested. Ten fish were exposed to all above-described concentrations for 96-h, under constant conditions. After 24 hours, test solution was changed to fix concentrations in tanks. Sonication was done for about 60 minutes immediately prior to use to avoid particle aggregation. In the control group, no test solution was added. During the trial, the fish were not fed to reduce the absorption of the ZnO-NPs in feed and feces. During the experiment, water quality parameters like pH, temperature, total hardness, dissolved oxygen and natural 12:12 day /night photoperiod were retained (Table 1). Besides this, behavior of Bighead carp was observed in exposed and control group. Their movement, hyperactivity, equilibrium, air gulping activity and swimming pattern were checked (Almeida *et al*., 2010).

Table 1: Physico-chemical parameters used for the experiments.

Parameters	Unit	Mean	Analysis Method
Total Hardness	mg/L	150±0.38	Titration method
pH		7.5±0.04	pH meter
Temperature	°C	29±0.09	Temperature meter
Dissolved Oxygen	mg/L	6.2±0.14	Oxygen meter
Ammonia	mg/L	0.43±0.22	Titrimetric method
Electrical Conductivity	µSiemens/cm	525.32±0.10	Conductivity meter

Oxidative stress biomarkers: Freshwater fish, Bighead carp was exposed to sub-lethal dose ($1/3^{rd}$ of 96-h LC_{50}) for 90 days. After every 15 days, sampling was done and oxidative stress in terms of lipid peroxidation level (LPO) and superoxide dismutase (SOD) was assessed in the gills and liver of fish. Fish organs were homogenized separately, using chilled PBS (phosphate buffer saline) in 1/4 ratio (weight/volume) by homogenizer. After that homogenate was centrifuged at 10,000 rpm, 4°C for 15 min. For analysis of LPO and SOD, supernatant was used.

I. **Superoxide dismutase activity (SOD):** The activity of SOD was observed by its potential to suppress the process of photoreduction of Nitroblue tetrazole (NBT) at 560nm (Giannopolitis and Ries, 1977). Activity was determined in gills and liver tissues of Bighead carp.

II. **Lipid peroxidation:** The level of lipid peroxidation was checked by calculating thiobarbituric acid reactive substance (TBARS) in the gills and liver tissues (Gatta *et al*., 2000).

Water quality management: During experiments, the physical and chemical parameters viz. dissolved oxygen, pH, electrical conductivity and temperature were calculated by digital meters viz. HANNA-9146, HANNA-99301, HANNA-8424, respectively. Proper oxygen was maintained in the glass aquarium by an automatic air pump. For the maintenance of the level of pH, NaOH (to decrease level of pH) and HCL (to increase level of pH) was used. In order to maintain total hardness of test the media ethylenediaminetetraacetic acid (EDTA) and salts of magnesium sulfate ($MgSO_4$) and calcium sulfate ($CaSO_4$) were used. Sodium (Na), Magnesium (Mg), Carbon dioxide (CO2), potassium (K), calcium (Ca), and total ammonia and total hardness of test environment were recorded by following method of A.P.H.A. (2005).

Statistical analyses: To determine mean lethal concentrations of ZnO-NPs on Bighead carp, probit analysis method was done with 95% confidence interval (Hamilton *et al*., 1977) and all experiments were performed in triplicate. SPSS (Statistical Package for Social Sciences) was used to analyze statistically. Statistical differences and similarities within all variables

were determined by Analysis of Variance. To compare means, Tukey's/Student Newnan-Keul test was used.

RESULTS

Characterization of ZnO-NPs: To measure the average crystal size of samples, XRD was used. The X-ray diffraction pattern of ZnO-NPs is shown in Fig. 1. At 2θ, diffraction peaks 31.73°, 34.44°, 35.28°, 47.57°, 56.64°, 62.83°, 66.42°, 67.98° referred to as Planes of XRD as (100), (002), (101), (102), (110), (103), (112) and (201), respectively (Table 2). The average measured size was 53nm. SEM is important for sample scanning and knowledge about the shape of particles. Fig. 2 represents that zinc oxide manufactured NPs have a clear wurtzite-hexagonal crystalline arrangement.

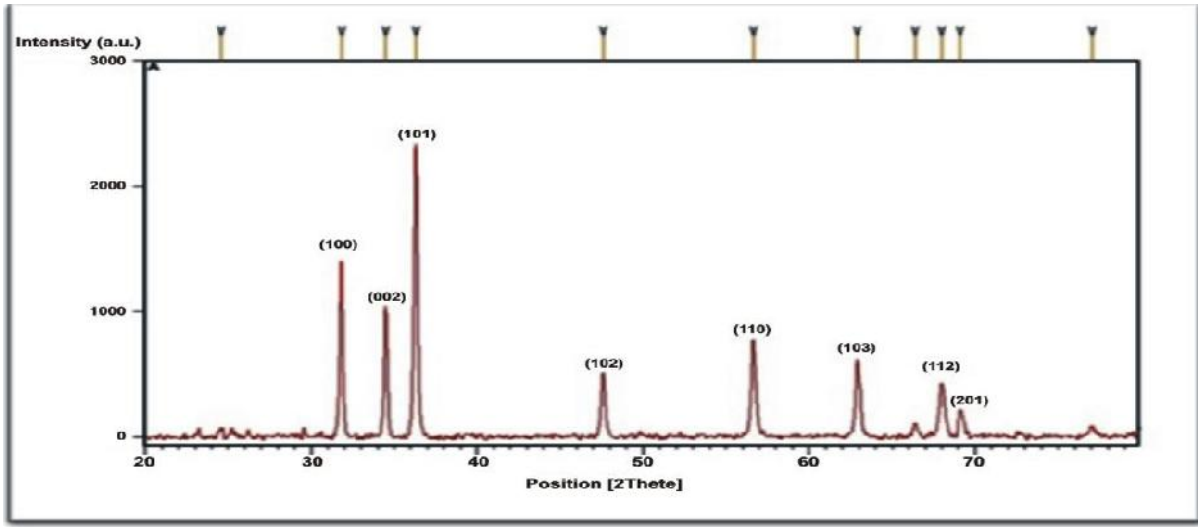

Fig. 1: X-ray diffraction (XRD) Pattern of ZnO-NPs. Peaks showed average size of the particles

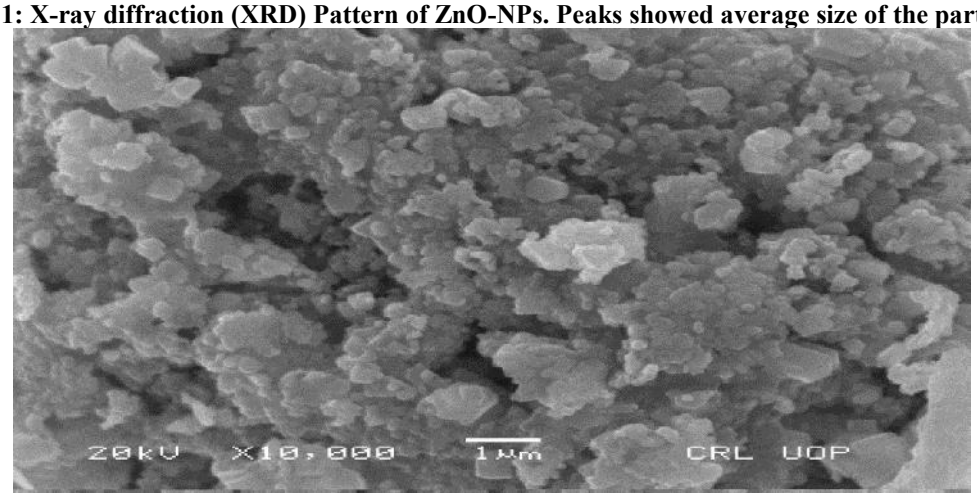

Fig. 2: Scanning electron microscopy (SEM) result of ZnO nanoparticles.

Table 2: Analysis of Peaks of X-ray diffraction Pattern of ZnO NPs.

No.	Pos. [°2Th.](deg.)	Theta (deg.)	d-spacing [Å]	FWHM [°2Th.]	Rel. Int. [%]	Hkl
1	31.7364	15.91473	2.82179	0.0974	59.45	100
2	34.2533	17.3412	2.62262	0.1248	45.0	002
3	35.2824	18.2346	2.23534	0.2166	100	101
4	48.589	23.7987	1.81945	0.2263	22.00	102
5	56.6559	28.32375	1.72794	0.0899	33.05	110
6	62.8342	31.45735	1.3767	0.1956	27.10	103
7	66.4243	33.2112	1.40286	0.2356	5.12	112
8	67.98623	33.8856	1.37954	0.1943	19.24	201

Acute toxicity of waterborne ZnO-NPs: Acute toxicity tests were conducted to calculate 96-hr LC_{50} and lethal concentration of ZnO-NPs for Bighead carp. Figure 3 shows fish mortality percentage against various concentrations.The mean 96-h LC_{50} and lethal concentration of zinc oxide nanopowder, for Bighead carp were calculated as 22.24 and 46.89 mg/L, respectively, at 95% confidence interval by using Probit analysis method (Table 3). Changes in behavior were observed after exposure to LC_{50} from 24-96 h. The behavioral changes induced in Bighead carp after ZnO-NPs exposure are present in Table 4.

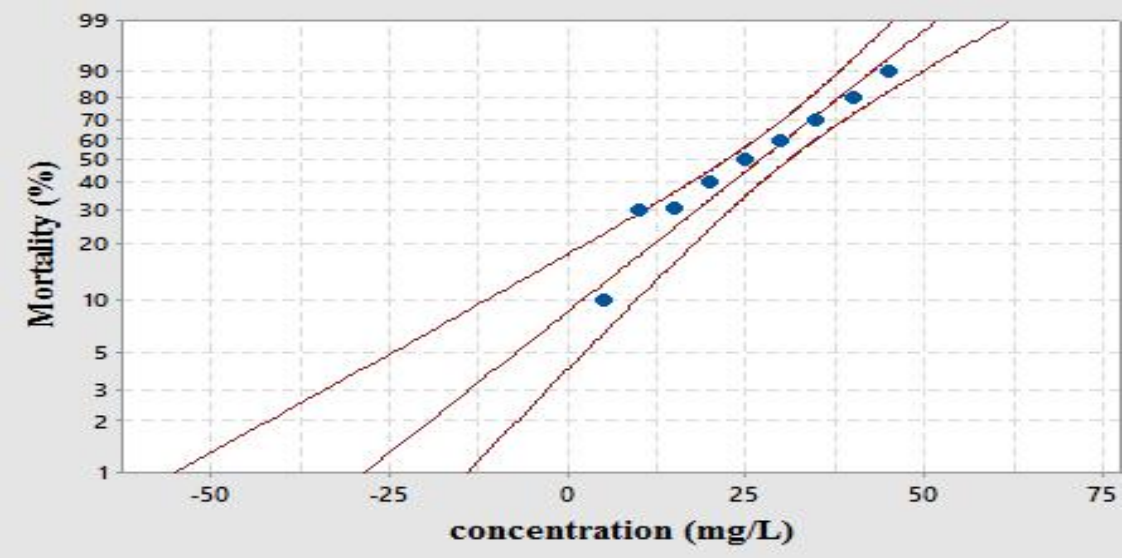

Fig.3: Probability plot for success at 95% CI

Table 3: 96h acute toxicity of ZnO-NPs (mg/L) for Bighead carp.

Fish specie	MeO-NPs	LC_{50}	95%CI (LCL-UCL)	LC_{99}	95%CI (LCL-UPL)	Pearson goodness of fit tests		
						Chi-Square	DF	P-value
Bighead carp	ZnO-NPs	22.24	17.95-26.23	46.89	40.75-57.88	2.65364	9	0.976

CI, confidence interval (mg/L); LCL, lower confidence limit (mg/L); UCL, upper confidence interval (mg/L); Lethal Conc., lethal concentrations (mg/L); DF, degree of freedom

Table 4: Behavioral response of Bighead carp exposed to LC_{50} ZnO-NPs.

Behavior	Treatment groups	
	Control	Treated
Hyper activeness	-	+
Jumping	-	+
Air gulping	-	+
Equilibrium loss	-	+
Erratic swimming	-	+
Vertical position	-	+

- Absent, + Present.

Oxidative stress Biomarkers

Superoxide dismutase (SOD) activity: Chronic exposure of Bighead carp to sublethal dose (1/3rd 96-h LC_{50}) of ZnO-NPs induced significantly variable superoxide dismutase activity in both liver and gill tissues. Liver tissues showed significant increase after 15 and 30 days followed by a sharp decrease at 45, 60, 75 and 90 days as compared to the control group in SOD activity, which increases the level of reactive oxygen species (Fig. 4A). Gills showed increased SOD activity after 15, 30, 45 and 60 days followed by significantly decreased rates at 75 and 90 days (Fig. 4B). In our present study, levels of superoxide dismutase is in the following trend: liver > gills..

Lipid peroxidation (LPO): Thiobarbituric acid reactive substances (TBARS) are synthesized as a result of lipid peroxidation, which can be easily noticed by the TBARS assay. So, this assay was used in the present research as an effective symbol to detect lipid peroxidation. Bighead carp exposed to chronic exposure to sublethal dose (1/3rd LC_{50}) of ZnO-NPs showed significant variation in TBARS level in liver and gills. Both organs showed significant increase in TBARS levels as duration increased than the control group (Fig. 5 A, B).

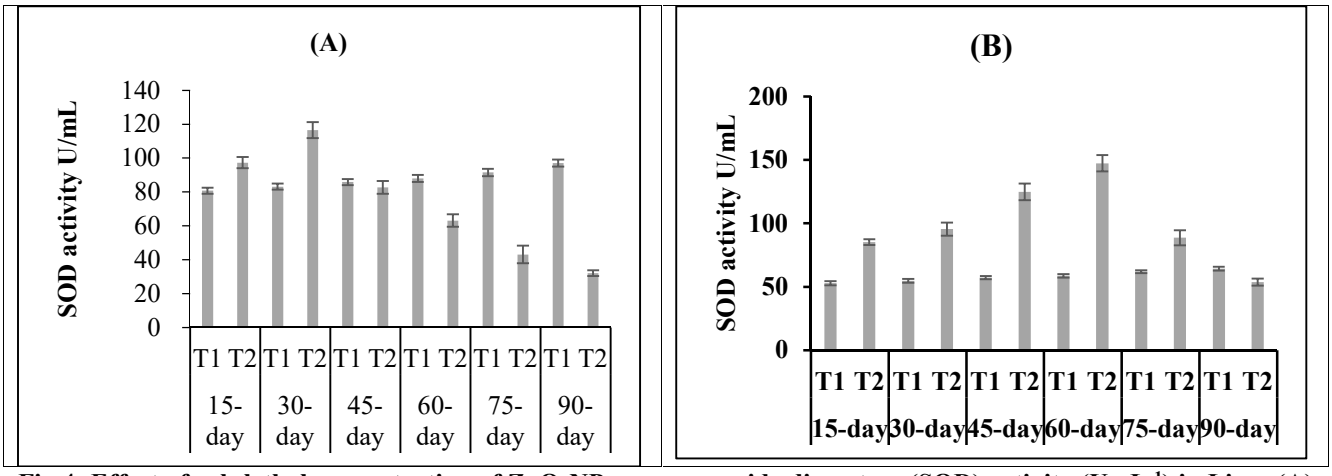

Fig.4: Effect of sub-lethal concentration of ZnO-NPs on superoxide dismutase (SOD) activity (UmL^{-1}) in Liver (A) and Gills (B) of Bighead carp.

Values represent Mean±SE (n=3). T1=control group, T2=ZnO-NPs exposed group

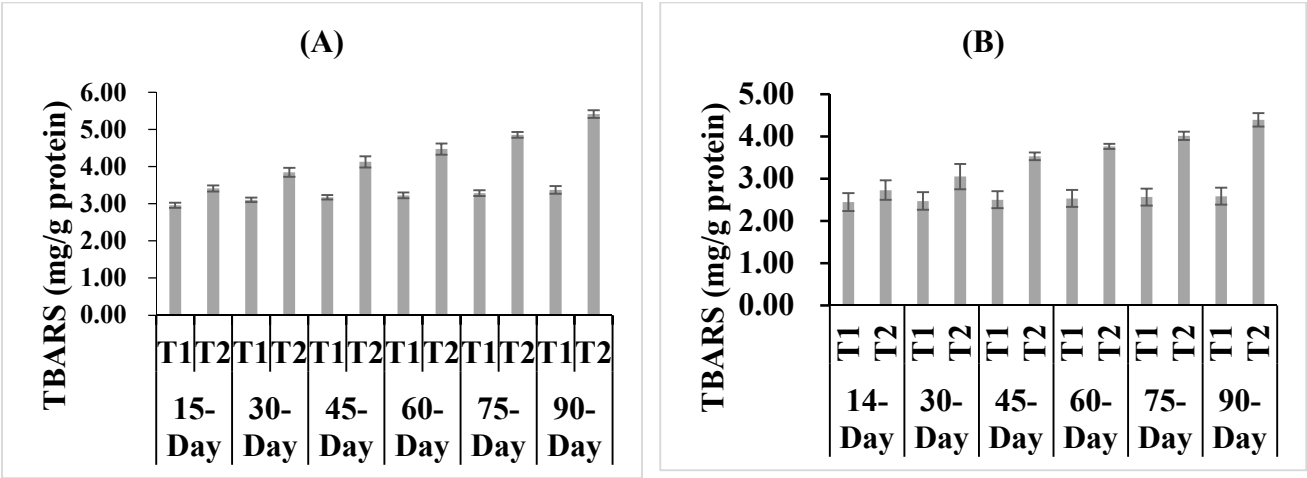

Fig. 5- Effect of ZnO-NPs on Thiobarbituric acid reactive substances (TBARS) level (mg/g protein) in Liver (A) and Gills (B) of Bighead carp.

Values represent Mean±SE (n=3). T1=control group, T2=ZnO-NPs exposed group.

DISCUSSION

Nanoparticles discharging into aquatic ecosystems may result in harmful effects on non-target animals such as fish and other aquatic organisms. The different sub-lethal concentrations of heavy metals can start accumulation of metals in target organs of fish that are settled at the last of the aquatic food chain and can disturb the food chain condition. The acute toxicity of ZnO-NPs was observed in common carp for 24 hrs and experimental fish showed the 50 % mortality rate at 30ppm concentration (Al-Taee *et al*., 2013). Subashkumar and Selvanayagam (2014) and Aziz *et al*. (2020) determined 4.897 and 31.15 mg/L LC_{50} of ZnO nanoparticle on selected carps. During present study, ZnO-NPs caused increased toxicity in Bighead carp as the particle concentrations increased. The toxicity of different types of NPs on different organisms depends upon various components viz. shape, morphology (triangular, spherical, and wire-shaped), chemical composition, size and structural properties. These mentioned factors significantly affect the physical action between target tissues and NPs (Albanese *et al*., 2012; Raza *et al*., 2016). Relatively few data are accessible in the literature that provides zinc oxide nanoparticles toxicity in fish (Kahru and Dubourguier, 2010). Toxicity is directly associated with the physico-chemical characteristics of ZnO nanoparticles. Water quality and trial species, both factors affect the acute toxicity of toxicants. Fish that are very sensitive to one metal at high or low levels may be less or even not sensitive to another metal in the environment (Hedayat and Safahieh, 2012). Thus, the outcomes of previous and our studies show that both exposure period and lethal concentrations may differ for different species. Bighead carp showed changes in

hyperactivity, jumping, increased air gulping, equilibrium loss, rapid swimming and vertical position after 24-48 h of exposure. After 48h, Bighead carp became sluggish. At the end fish become inactive with taking vertical positions for sometimes before dying. Behavioral change in this study may be due to interaction of heavy metals with neurotransmitters acetylcholine. And fast and abrupt swimming may be due to muscle spasms that might be due to respiratory dysfunction and suffocation. Oxidative stress is an accessible factor to determine toxicity, because cells react to stress by raising a number of defensive reactions that can be easily calculated as changed enzymatic or genetic expression (Kovochich *et al*., 2007). In previous studies, it was noted that SOD activity affected after the nanomaterials exposure more than the activity of other enzymes (Hao *et al*., 2009; Aziz *et al*., 2021). This is in accordance with Hao and Chen (2012) who described a time-dependent significant reduction in SOD activity after ZnO-NPs exposure in common carp. The reduction in this antioxidant enzyme is expected to be caused by inducing oxidative stress of NPs. The same observations were found in the brain tissues of *Tilapia zilli* and *O. niloticus* exposed to 2 and 4 mg/L of silver NPs (Afifi *et al*., 2016). TBARS is one of the important factors used to find out LPO levels evoked by the biological processes in the organism (Ates *et al*., 2015). In this study, the TBARS assay showed a sharp increase in liver and gills LPO levels in Bighead carp exposed to sub-lethal dose of ZnO-NP ($p < 0.05$). This is in accordance with Zhao *et al*. (2013) who reported that the lipid peroxidation level was increased significantly at different concentrations of ZnO-NPs relative to control group, Increased liver TBARS level was observed on the 7^{th} and 14^{th} days in tilapia exposed to 10 mg/L ZnO-NPs suspension ($p<0.05$) in comparison to the control group (Kaya *et al*., 2015). In the same line of our data, some authors also determined high lipid peroxidation level in fish organs (Benavides *et al*., 2016, Yuan *et al.,* 2015).

Conclusion: Bighead carp was used as a model organism to check 96-h acute toxicity of ZnO nanoparticles. Sub-lethal dose of zinc oxide nanoparticle has induced oxidative stress by causing alterations in both SOD and TBARs level due to excess of ROS (reactive oxygen species). The results develop an interest towards the security of aquatic biota and human beings.

Statement of conflict of interest: Authors have declared no conflict of interest.

Authors Contribution: SA executed the research. SA supervisor and planned the research. FL helped in compiling data and statistical analysis. HA helped in synthesis of nanoparticles.

REFERENCES

Almeida, J., C. Oliveira,C. Gravato, and L. Guilhermino (2010). Linking behavioural alterations with biomarkers responses in the European seabass *Dicentrarchus labrax* L. exposed to the organophosphate pesticide fenitrothion. Ecotoxicol. 19: 1369-1381.

Albanese, A., P.S. Tang, and W.C. Chan (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu. Rev. Biomed. Eng. 14: 1-16.

Aitken, R.J., M.Q. Chaudhry, A.B.A. Boxall, and M. Hull (2006). Manufacture and use of nanomaterials: current status in the UK and global trends. Occ. Med. 56: 300-306.

Afifi M., S. Saddick, and O.A. Zinada (2016). Toxicity of silver nanoparticles on the brain of *Oreochromis niloticus* and *Tilapia zillii*. Saudi J. Biol. Sci. 23: 754-760.

A.P.H.A. (American Public Health Association) (2005). Standard methods for the examination of water and waste water. (21st Ed). American Public Health Association, Washington, DC. pp 1220.

Al-Taee, S.K.I. and A.H.A. Al-Hamdani (2013). Pathological study of lethal concentration of N-ZnO in common carp (*Cyprinus carpio* L.). Bas. J. Vet. Res. 12: 200-207.

Ates, M., Z. Arslan, V. Demir, J. Daniels and I.O. Farah (2015). Accumulation and toxicity of CuO and ZnO nanoparticles through waterborne and dietary exposure of Goldfifish (*Carassius auratus*). Environ. Toxicol. 30: 119-128.

Aziz, S., S. Abdullah, K. Abbas and M.A. Zia (2020). Effects of engineered zinc oxide nanoparticles on freshwater fish, *Labeo rohita*: Characterization of ZnO nanoparticles, acute toxicity and oxidative stress. Pakistan Vet. J. 40: 479-483.

Aziz,S., S. Abdullah, H. Anwar, F. Latif and W. Mustfa (2021). Effect of Engineered Nickel Oxide Nanoparticles on Antioxidant Enzymes in Freshwater Fish, *Labeo rohita*. Pakistan Vet. J., 41: 424-428.

Baker, T.J., R.C.R. Tyler and T.S. Galloway (2014). Impacts of metal and metal oxide nanoparticles on marine organisms. Environ. Pollut. 186: 257-271.

Benavides, M., J. Fernandez-Lodeiro, P. Coelho, C. Lodeiro and M.S. Diniz (2016). Single and combined effects of aluminum (Al_2O_3) and zinc (ZnO) oxide nanoparticles in a freshwater fish, *Carassius auratus*. Environ. Sci. Pollut. Res. 23: 24578-24591.

Gatta, P.P., M. Pirini, S. Testi, G. Vignola and P.G. Monetti (2000). The influence of different levels

of dietary vitamin E on sea bass *Dicentrarchus labrax* flesh quality. Aquac. Nutr. 6: 47-52.

Giannopolitis, C.N. and S.K. Ries (1977). Superoxide dismutase occurrence in higher plants. Pl. Physiol. 59: 309-314.

Gupta, Y.R., D. Sellegounder, M. Kannan, S. Deepa, B. Senthilkumaran, and Y. Basavaraju (2016). Effect of copper nanoparticles exposure in the physiology of the common carp (*Cyprinus carpio*): Biochemical, histological and proteomic approaches. Fish. Aquac. 1: 15-23.

Hamilton, M.A., R.C. Russo and R.V. Thurston (1977). Trimmed Spearman-Karber method for estimating median length concentration in toxicity bioassays. Environ. Sci. Technol. 11: 714-719.

Handy, R.D., T.B. Henry, T.S. Scown, B.D. Johnston and C. R. Tyler (2008). Manufactured nanoparticles: their uptake and effects on fish- a mechanistic analysis. Ecotoxicol. 17: 396-409.

Hao, L.H., Z.Y. Wang and B.S. Xing (2009). Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in juvenile carp (*Cyprinus carpio*). J. Environ. Sci. 21: 1459-1466.

Hao, L.H. and L. Chen (2012). Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. Ecotoxicol. Environ. Saf. 80: 103-10.

Kaya, H., and M. Akbulut (2015). Effects of waterborne lead exposure in Mozambique tilapia: oxidative stress, osmoregulatory responses, and tissue accumulation. J. Aquat. Anim. Health, 27: 77-87.

Kahru, A., and H.C. Dubourguier (2010). From ecotoxicology to nanoecotoxicology. Toxicol., 269: 105-119.

Kovochich, M., T. Xia, J. Xu, J.I. Yeh and A.E. Nel (2007). Principles and procedures to assess nanomaterial toxicity. In: Wiesner, M.R., Bottero, J.Y. (Eds.), Environmental Nanotechnology: Applications and Impacts of Nanomaterials. Mc Graw Hill, New York, pp. 205-229.

Khan, I., K. Saeed and I. Khan (2019). Nanoparticles: Properties, applications and toxicities. Arab. J. Chem., 12: 908-931.

Klaine, S.J., P.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy and D.Y. Lyon (2008). Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27: 1825-1851

Ma, H., P.L. Williams and S.A. Diamond (2013). Ecotoxicity of manufactured ZnO nanoparticle - A review. Environ. Pollut. 172: 76-85.

Nel, A., T. Xia, L. Madler and N. Li. (2006). Toxic potential of materials at the nanolevel. Science, 311: 622-627.

Rekha, K., M. Nirmala, M.G. Nair, G. Manjula and A. Anukaliani (2010). Structural, optical, photocatalytic and antibacterial activity of zinc oxide and manganese doped zinc oxide nanoparticle. Physica B: Condens. Matter. 405: 3180-3185.

Raza, M.A., Z. Kanwal, A. Rauf, A.N. Sabri, S. Riaz and S. Naseem (2016). Size and shapedependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. Nanomaterials 6: 74.

Subashkumar, S. and M. Selvanayagam (2014). First report on: Acute toxicity and gill histopathology of freshwater fish *Cyprinus carpio* exposed to Zinc oxide (ZnO) nanoparticles. Int. J. Sci. Res. Pub. 4: 1-4.

Wang, H., R.L. Wick and B. Xing (2009). Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. Environ. Pollut. 157: 1171-1177.

Xiong, D.W., T. Fang, L.P. Yu, X.F. Simaand and W.T. Zhu (2011). Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebra fish: acute toxicity, oxidative stress and oxidative damage. Sci. Total Environ. 409: 1444-1452.

Yu, L.P., T. Fang, D.W. Xiong, W.T. Zhu and X.F. Sima (2011). Comparative toxicity of Nano-ZnO AND Bulk suspensions to zebra fish and the effects of sedimentation, OH. Production and particle dissolution in distilled water. J. Environ. Monit. 13:1975-1982.

Yuan, Y., S. Ge, H. Sun, X. Dong, X. Zhao, L. An, J. Zhang, J. Wang, B. Hu and G. Liang (2015). "Intracellular self-assembly and disassembly of ^{19}F nanoparticles confer respective "off" and "on" ^{19}F NMR/MRI signals for legumain activity detection in zebrafish," ACS Nano 9: 5117-5124.

Zhao, X., S. Wanga, Y. Wub, H. Youa, and L. Lv (2013). Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquat. Toxicol. 136: 49-59.