Cerium oxide nanoparticles induced physio-biochemical, neurochemical, and morphological responses in *Cirrhinus mrigala* during short term exposure

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Abstract. Cerium oxide nanoparticle (CeO_2NPs) has wide applications in pharmaceutical, biomedical and chemical industries. Albeit of their uses, bioavailability followed by toxicity of CeO_2NPs in fresh water fishes, are yet to be understood in detail. In this evaluation, we have synthesized, characterized and assessed the biological effects (hematology, ionoregulatory, oxidative stress, histological and glutamate indices) of CeO_2NPs at different doses (2.5mg/L and 25mg/L based on $1/10^{th}$ LC_{50}) on freshwater carps *Cirrhinus mrigala*, for short term exposure of 96 h. Impact of CeO_2NPs at low concentration (2.5mg/L) confirmed a significant decrease in hematological parameters and also affecting serum Na^+, Cl^-, K^+ levels along with gill Na^+/K^+-ATPase activity. The indicated variations oxidative stress enzymes superoxide dismutase, Catalase, glutathione peroxidase with relative elevation in lipid peroxidation (LPO) (22.47 ± 0.198) compared to control groups. CeO_2NPs at high concentration (25mg/L) revealed the alterations in neurotransmitter glutamate levels compared to control groups. Rise in glucose and decrease in plasma protein levels in response to both the concentrations was noted. Microscopic observations confirmed the tissue damages and alterations in gill architecture. By integrating all results obtained by short term exposure of juvenile carps to CeO_2NPs at different doses, we reported nanoparticles have considerable deleterious effects on physiological and morphological condition of fishes.

Keywords: cerium oxide nanoparticles, *Cirrhinus mrigala,* neurotransmitter, oxidative stress

INTRODUCTION

Over the last decades, nanoparticles have become crucial scientific tool that have found application in diverse fields like nanotechnology, biotechnology, pharmacology and many others which has been owed to their potential to bridge molecular or atomic structures to vast materials (Rashmi *et al.,* 2019). This has led to unforeseen increase in engineered nanoparticles, thus raised concerns over their deleterious effects when interacted with ecosystem at large. Cerium oxide (CeO_2)/ cerium oxide nanoparticles (CeO_2NPs) are comprehensively used as additives in diesel fuel (Hendren *et al.,* 2011). However extensive use of CeO_2NPs result in no point source water pollution, leading to moderate level diverse

exposure to various flora and fauna (Cassee *et al.,* 1991).

Genotoxic effects of CeO$_2$NPs resulting in DNA damage in crustaceans at low dose of 1mg/L has been reported (Lee *at al.,* 2009). *In-vivo* findings in rat and mice have shown inhalation of CeO$_2$NPs have induced systematic toxicity in lungs and acute pulmonary toxicity (Ma *et al.,* 2012). Investigation on *Anabaena*, cyanobacteria with an EC$_{50}$ value of 6.6 mg/L exhibited cell damage and membrane disruption, rise in reactive oxygen species, lowering in chlorophyll content, and inhibition of photosynthesis (Navarro *et al.,* 2008).

The regular mode of admission of these nanoparticles include organs, olfactory, direct ingestion, entry through gills in fish and by physical defense mechanism like phagocytosis and endocytosis (Sharath *et al.,* 2020). There are documented reports suggesting that small molecules such as nanoparticles associate strongly with living system when compared to bigger molecules (Matranga and Corsi, 2012). These findings have given further reason for investigation of effect of nanoparticles. Consequently, evaluation of ecotoxicity of these CeO$_2$NPs, are crucial in their further application in nanomedicine and technology (Moore, 2006).

In this direction we adopted Indian fresh water juvenile carp, *Cirrhinus mrigala*, for the chemical – biological interactions. *C. mrigala* is widely found in Indian subcontinent and consumed extensively. *C. mrigala* is considered a sentinel fish, as it is consumed by humans widely and serving as a biomarker for the extent of xenobiotic accumulation in human beings. Studies have shown to use this fish model for similar studies (Perva *et al.,* 2020). Thus understanding the extent of biological variations caused by cerium oxide nanoparticles on *C. mrigala* will provide the knowledge of their potential appearance in food chain.

The physio biochemical studies such as hematology, ionoregulatory, oxidative stress will provide the changes in physiology happening in fish during exposure to nanoparticles, whereas neurotransmitter (glutamate) analysis will exhibit any impact on brain and gill morphological evaluation will indicate the extent of damage to gill tissue and the degree of interaction (Minghetti and Schirmer, 2016). The gill tissue is the first line of interaction to the nanoparticles, becomes an important morphological indicator of an tissue damage and entry (Shaw and Handy, 2011). Biomarkers are extensively adopted to evaluate the effect of nanoparticles on the physiology, metabolism and biochemistry of aquatic beings (Basuini *et al.,* 2016). Hematological parameters are crucial in determining the possible indication of abnormality and stress in aquatic organisms. Analysis of blood glucose levels and serum protein concentrations indicate the physiological and metabolic condition of the organism. In addition, measurement of ionic concentration like Na$^+$, Cl$^-$, K$^+$ in blood as well as gill Na$^+$/K$^+$-ATPase also acts as an important indicator of chemical induced toxicity in the organism (Saravanan *et al.,* 2011a). Stress condition leads to the generation of reactive oxygen species in fish with depletion in antioxidants. Measurement of antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase and lipid peroxidation demonstrates oxidative damage. Changes in glutamate, a neurotransmitter are investigated, as neurons are vulnerable to oxidative distortion, thus paving way for neurodegenerative problems (Li *et al.,* 2010). As higher concentration of metal oxide NPs in aquatic ecosystem will induce significant alterations in the immune system of fishes and other organisms thus disturbing the ecological balance, determining their degree of toxicity may contribute in alleviating further harm to the ecosystem. Moreover, findings of acute toxicity caused by these NPs are largely unavailable, particularly effect of CeO$_2$NPs on fresh water carps are mostly untouched.

In our present study, efforts are made to synthesize and characterize CeO$_2$NPs and identify the hematological, physio-biochemical, neurochemical and morphological responses of fresh water juvenile carp, *C. mrigala* to CeO$_2$NPs upon short term exposure of 96 hr.

MATERIALS AND METHODS

Animals
Fresh water juvenile carp, *C. mrigala* (6.8±0.8 cm of body length and 5.8±1.5 g of weight) were obtained from fish farm and were stored in

dechlorinated water for 15 days. During this phase the fishes were fed with commercial fish food. Debris and residues were cleaned once in a day. The experimental fishes were divided into three groups which were raised in 150L aquarium with continuous aeration.

Synthesis and characterization of cerium oxide nanoparticles

Cerium oxide nanoparticles (CeO$_2$NPs) were synthesized by precipitation method. Cerium nitrate hexahydrate (Ce (NO$_3$)$_3$·6H$_2$O) was dissolved in distilled water to prepare 0.1 mol/dm^3 of solution. This aqueous solution was then heated to 26°C and consequently precipitated with 0.05 mol/dm^3 of ammonium hydroxide solution. The reaction mixture was then stirred at 25°C for 22±4 hours. This reaction mixture was centrifuged at 10000 rpm, filtered and the resultant product was dried at 150°C. This sample was further calcined at 600°C for 4 hours in a muffle furnace to obtain the desired compound. The compound was further characterized by field emission scanning electron microscope (FE-SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction studies (XRD) and dynamic light scattering analysis (DLS).

20 g/l of sample solution was readied by adding CeO$_2$NPs in double distilled water and was kept for dissolving in a sonicator for 10 hours (Biotechnics). The samples were sonicated further for 1 h earlier to inoculation each day. Sedimentation of NPs was minimized by providing aeration to the sample, which held them in suspension form. However, a very minor fraction of CeO$_2$NPs were found to form aggregates.

Treatment and LC$_{50}$

The fish groups were treated to CeO$_2$NPs for short phase of 96 hr. Two varying doses of sample 2.5 mg/L and 25 mg/L based on 1/10th of LC$_{50}$ concentration (250mg/L) were added into two 120 L aquariums housing 50 fishes each, along with a control group. Food was withheld 24 hrs before sacrifice to avoid postprandial variations. Initially the mortality was recorded under dose dependent concentrations (500, 375, 250 and 125 mg/L). Since only 90% mortality was observed below 250 mgl/L, the LC$_{50}$ of SnO$_2$NPs was taken

as 250 mgl/L concentrations at the end of 96 hr observation. Further 1/10th and 1/100th value was taken for experimentation. Control and exposed fishes (2.5 mg/L and 25 mg/L) were used for biological evaluation. Nil mortality was found during the entire 96 hours of exposure to NPs. Water quality was found to be 32.4 ± 2.4 CaCo$_3$ mg/L of hardness and a pH of 7.3 ± 0.3, at a constant temperature of 22 ± 1°C with a light dark cycle of 12:12 hrs.

Sample collection and histology

The fish were euthanized with ice cold water without inducing any stress on fish. The blood samples were drawn from 50 individual fishes (2 replicates) and pool together as three replicates for further analysis. Samples for ionoregulatory studies were transferred into vial without any anticoagulant, as the presence of ions in the same might interfere with results obtained. The samples for hematological and biochemical measurement were introduced to EDTA (Ethylenediaminetetraacetic acid) vials and centrifuged at 9392 g for 20 min at 4°C. The brain samples for neurotransmitter analysis was dissected and placed in ice for analysis. Gills tissue for histopathological evaluation was immediately fixed with 10% neutral buffered formalin (NBF) overnight. Tissue sections were prepared and stained with hemotoxylin-eosin. The prepared slides were observed and photographs were recorded using Olympus CX41 microscope mounted with camera.

Hematological measurements

Red blood cells, white blood cells were counted using neubauer hemocytometer. Cyanomethemoglobin method was employed to measure the amount of hemoglobin (Hb) content in blood. Hct percentage was estimated by using the microhematocrit methodology (Nelson and Morris.1989). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined using established formulas for three replicates.

Biochemical measurements

Biochemical measurements were carried out for three replicates. Blood glucose was determined by Cooper and McDaniel 1970 method. The glucose

levels were measured as mg/dL. Plasma protein levels were determined by Lowry's method. The sample was read at 720 nm and measured in µg/ml.

Ionoregulatory studies

Plasma sodium was estimated by adding 1000 µl of precipitating reagent with 20 µl of sample. 20 µl of standard was mixed instead of sample for tube assigned as standard. The readings were taken at 550 nm and expressed as mEq/L. Similarly, potassium was estimated at 630 nm. Plasma chloride was read against blank at 505 nm and the concentration was measured in mEq/L. Gills Na^+/K^+-ATPase activity was performed and expressed as µg/h/g (Evans *et al.,* 2005). Experiments were performed for three replicates.

Oxidative stress and neurotransmitter assessment

Superoxide dismutase was determined (Das *et al.,* 2000) by blocking superoxide led nitrite formation from hydroxylamine hydrochloride and activity was measured as U/mg of protein. Catalase activity was read at 405 nm and expressed as U/mg (Goth, 1991). Glutathione peroxidase (GPx) was measured by Rotruck *et al.* (1973).The samples in triplicates were measured at 412 nm and the enzyme was reported as mU/mg of protein. Lipid peroxidation was demonstrated by using malondialdehyde (MDA) as a standard (Buege and Aust, 1978) and expressed in terms of thiobarbituric acid (TBARS). Glutamate was analyzed by development of paper chromatography (Raju *et al.,* 2004). Experiments were performed for three replicates.

Statistical analysis

All the data was statistically evaluated and put forth as Mean ± SE. In all assays, the amount of statistical significance was fixed for $P<0.05$. The significance was calculated by one way ANOVA analysis with post-hoc corrected t tests.

RESULTS AND DISCUSSION

Characterization of cerium oxide nanoparticles

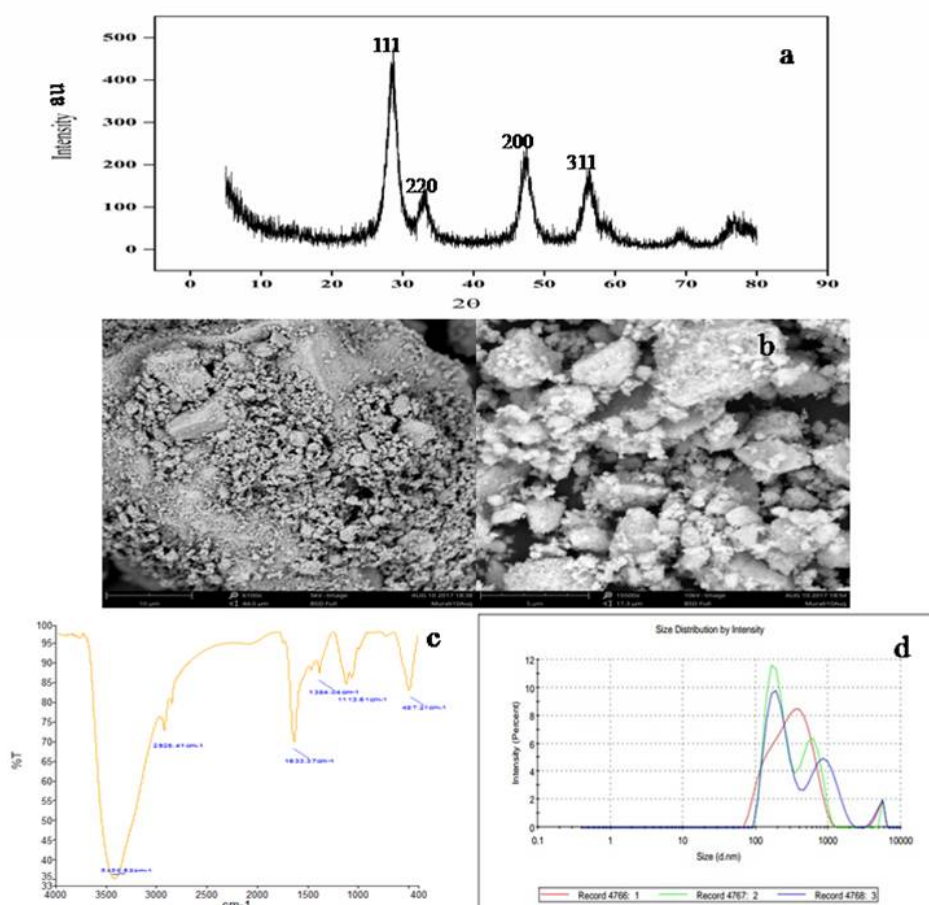The structural characterization of the synthesized cerium oxide nanoparticles was analyzed using Powder X-Ray Diffraction (PANalytical X`Pert) system. The XRD pattern of cerium oxide nanoparticles can be found in Figure 1(A). The XRD pattern established the polycrystalline property of cerium oxide nanoparticles. The XRD pattern was obtained by scanning the sample at 2θ from 10-80 degrees. Maximum intensity peaks were seen 28.65, 33.45, 48.7, 58.4, and XRD indices were 111, 220, 200, 311 crystal planes respectively. The diffraction peaks obtained in the XRD pattern reflects the pure structure of cubic cerium fluorite while comparing to the standard. The crystal planes calculated were in agreement with JCPDS No: 34-0394. The unit cell properties of the cerium oxide nanoparticles were determined using the UNITCELL software. The crystal size was found using full Width at Half Maximum (FWHM) of the respective peaks. The images of CeO₂NPs captured in Field emission scanning electron microscope (FESEM) are shown in Figure 1(B). The grains observed are similar and spherical in shape when observed from FESEM with minor aggregation and size variation from 10 and 75nm. The recorded values are similar to the investigation conducted by other research groups (Meron and Markovich, 2005). The small aggregation of spherical CeO₂NPs can be associated to consistent arrangement of the primary particles. The FT-IR spectrum of cerium oxide nanoparticles annealed at 600°C can be seen Figure 1(C). The broad spectrum band found at 3430 cm^{-1} is attributed to the OH vibrational stretching of hydroxyl groups and residual water, while the scissor bending property of the associated water molecule can be found at absorption band reading 1633 cm^{-1}. The surfactant free property of the synthesized CeO₂NPs can be confirmed by the occurrence of absorption band at 2929 cm^{-1}. The absorption band at 3430 cm^{-1} and 1633 cm^{-1} can be ascribed to the OH of the absorbed water on the surface of the sample. Further band recorded at 487 cm^{-1} is attributed to the stretching frequency of Ce-O bonds, while absorption band at 1113 cm^{-1} is corresponding to the metal oxygen (Ce-O) vibrations. The aqueous suspension of CeO₂NPs was filtered using 0.22 µm syringe associated filter and the size of the CeO₂NPs was reported by dynamic light scattering (DLS) using Malvern zetasizer nano series spectrometer. Figure 1(D) shows the size distribution for CeO₂NPs obtained

from individual run. The mean hydrodynamic size of the particles was found to be 344.2 d.nm. The hydrodynamic data obtained can be found to be independent of the primary particle size, indicating that the CeO₂NPs are agglomerated when dispersed in the double distilled water.

Effect of cerium oxide nanoparticles on hematological parameters
Table.1 demonstrates the hematological variables. The distinct and moderate variations in different blood indices were observed between control and the treated fishes. In this investigation significant ($P< 0.05$) decline of MCV, MCH, MCHC, Hct, in treated fishes were observed compared to the control fishes. Major difference in MCV and MCHC values was found in treated groups compared to the untreated fishes exposed to cerium oxide nanoparticles, while there was a definite increase in the WBC levels in control and

treated groups. Further notable changes were observed in the RBC, Hb levels. Accumulation of toxic pesticides in the body has been reported to cause deteriorating and damaging effects in the hematological and hematic systems (Seth and Saxena, 2003). The reduced MCV and RBC count reported in the present study are major indicators of anemia. The development of anemia may be ascribed to the interference of the NPs with growth and development of hematopoietic progenitor and variations in the generation of renal erythropoietin. The MCH, MCV and MCHC values indicate the damage and destruction of blood cells upon treatment with NPs. Alterations in the leucocytes indices in the stress induced animals are characterized by lymphopenia and heterophilia due to leucocytosis (Saravanan *et al.,* 2011b), whereas increased values of WBC may be because of short term spur in the specific immune responses.

Figure 1. Structural and morphological characterization of synthesized cerium oxide nanoparticles. (A) X ray diffraction pattern (B) scanning electron microscope images (C) FTIR profile (D) Dynamic light scattering analysis.

Table 1. Alterations in the haematological indices of *C mrigala* exposed to different doses (2.5 and 25 mg/L for 96 h) of cerium oxide nanoparticles with n=3.

Haematological Indices	96 h		
	Control	**2.5 mg/L**	**25 mg/L**
RBC(millions/Cumm)	0.76±0.014	0.53±0.005*	0.68±0.04*
WBC(10^3/mm^3)	17.38±0.15	34.48±1.24	35.58±0.23*
Hb(g/dL)	4.87±0.16	2.56±0.05*	4.02±0.12
Hct (%)	14.23±0.02	7.42±0.12*	10.57±0.20*
MCV(μm^3)	185.06±0.32	63.5±0.17*	34.73±0.26*
MCH(pg)	137.05±0.47	47.51±0.28*	53.66±0.34
MCHC(g/dL)	153.53±0.23	58.5±0.28*	37.99±0.26*

Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin,MCHC: Mean corpuscular heamoglobin concentration. All values are expressed as mean± SE,* *P*< 0.05 is significant.

Effect of cerium oxide nanoparticles on physio-biochemical variables

Table 2 show the effect of CeO$_2$NPs on blood glucose concentration compared to the control groups. There was a significant (*P*<0.05) increase in both the treated groups (2.5 mg/L and 25 mg/L) when compared to the control fishes. Variations in the plasma glucose levels are attributed to the stress induced hypoxia and gluconeogenesis in fishes (Lavanya *et al.,* 2011). Whereas stress induced increase in glucose concentration in animals were also associated to the enhanced production of glucose which in turn is metabolized to energy molecules (ATP) to replenish additional energy requirement, while respiratory insufficiency may also be a crucial factor leading to increased level of blood glucose. The lowering in plasma protein concentration was significantly (*P*<0.05) recorded in both the groups (2.5 mg/L and 25 mg/L) exposed to cerium oxide nanoparticles. Liver and gill tissue injury, oxidative stress, changes in hematological properties followed by protein structure conformational alterations due to NPs binding to blood and tissue proteins has been reported (Chen et al., 2011). This observation suggests the reason for decrease of protein concentration in present study. Toxicity of NPs can be the reason for lowered protein concentration as it impedes translational and transcription mechanisms.

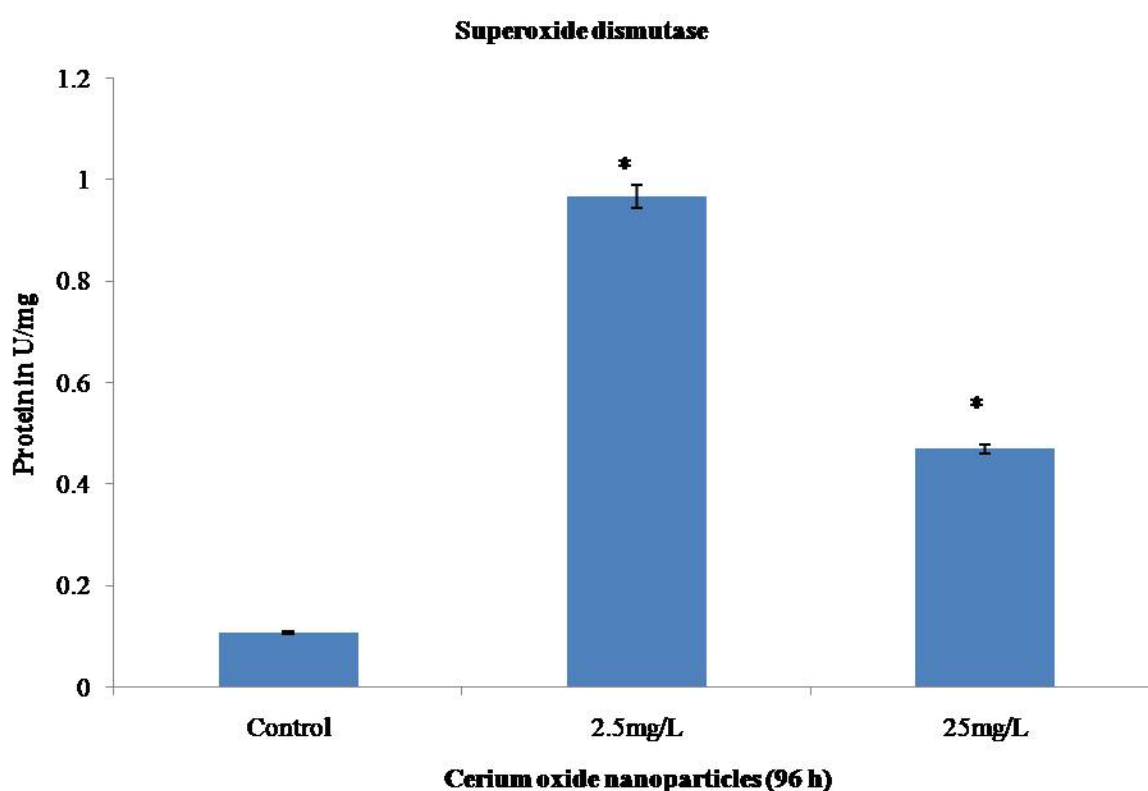***Effect of cerium oxide nanoparticles on ionoregulatory function of gills***

Table 2 shows that there was significant (*P*<0.05) decrease in gill Na$^+$/K$^+$ATPase activity in the groups exposed to both concentration (2.5 mg/L and 25 mg/L) compared to control groups. This might be attributed to the fact that toxic substances were known to inhibit the activity followed by ionic imbalances (Wood *et al.,* 1996). Toxic substances were absorbed by gill cells through proton coupled Na$^+$ channels and this reportedly blocked the Na$^+$K$^+$ATPase (Bury and Wood, 1999). The binding of toxic molecules to the amino acid containing sulfhydryl functional groups of Na$^+$/K$^+$ATPase has been demonstrated as the important factor for the inhibition of Na$^+$/K$^+$ATPase activity and was reconfirmed by downregulation of Na$^+$/K$^+$ATPase genes in the gills of zebrafish (Foyer and Noctor, 2005). Thus the present findings can be considered as the direct interaction of gills to cerium oxide nanoparticles and their uptake by Na$^+$ channels. The findings also revealed that the decreased concentration of plasma Na$^+$, K$^+$ and Cl$^-$ in the treated groups, with fishes exposed to 25 mg/L exhibiting prominent decline in ionic levels, in contrast to the control groups. This condition might be associated to the reduced intake of electrolytes or decreased efflux of the same ions from intracellular matrix to extracellular fluid. The gills of fish act as the main bridge between the surrounding aqueous environment and the blood, which is the main physiological regulator (Chandra *et al.,* 2017). This along with its other vital properties like gaseous exchange and osmoregulation makes gill tissue a suitable model to investigate the effect of toxic molecules.

Table 2. Alterations in the physio-biochemical parameters of C *mrigala* exposed to different doses (2.5 and 25 mg/L for 96 h) of cerium oxide nanoparticles with n=3.

Biochemical parameters/ Ionoregulatory indices	96 h		
	Control	2.5 mg/L	25 mg/L
Blood glucose(mg/dL)	143.66±3.4	172±4.64*	180.33±2.02*
Plasma protein(μg/mL)	14.29±0.24	2.8±0.20*	5.87±0.19*
Sodium (mEq/L)	224.83±0.44	155.33±3.52*	110.5±1.44
Potassium (mEq/L)	4.526±0.15	3.7±2.017*	2.37±0.15*
Chloride (mEq/L)	154.33±2.33	105.66±2.96	72.16±3.14*
Gills Na+K+ATPase(μg/h/g)	22.53±0.14	12.56±0.18*	8.43±0.12*

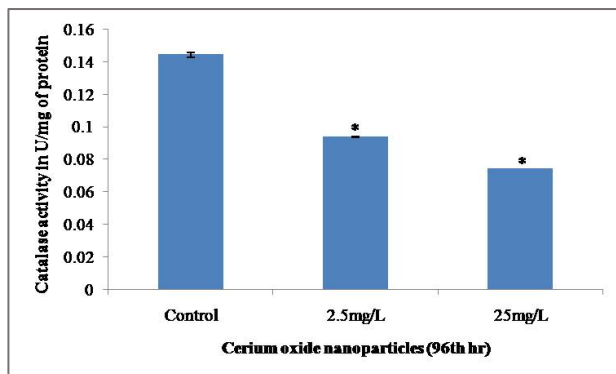All values are expressed as mean± SE, *$P< 0.05$ is significant.

Figure 2. Changes in blood SOD levels in *C mrigala* exposed to different doses of CeO$_2$NPs (2.5 mg/L and 25 mg/L, 96 h). Expressed as mean ± SE (*P<0.05- t test) with n=3.

***Effect of cerium oxide nanoparticles on blood plasma antioxidant enzyme system***

Figure 2 shows that the increase in superoxide dismutase (SOD) levels in fishes treated to both the concentrations (2.5 mg/L and 25 mg/L), this might be due to the production of SOD to compete and scavenge the excessive generation of reactive oxygen species (Puneeth and Chandra, 2020) due to cellular injury and damage from CeO$_2$NPs. Figure 3 shows the significant lowering in catalase activity in fishes treated to both the concentrations; this reveals the interference of CeO$_2$NPs to induce peroxidative damage. Catalase has been reported to translate numerous hydrogen peroxide to water and oxygen per second (El-Gendy *et al.,* 2009). Impair in catalase activity will enhance the ROS production and leading to more deterioration of tissues. Figure 4 reports the decrease in the antioxidant enzyme glutathione peroxidase (GPx) activity significantly

(*P*<0.05), when treated to both concentrations of cerium oxide nanoparticles, which are indication of augmented oxidative stress due to the interaction of NPs. GPx protects the cellular membranes by eliminating lipid peroxide and hydrogen peroxide, thus alleviating damage caused due to peroxidation. But the inhibition of GPx can be directly associated with toxicity of NPs to the fishes. Figure 5 shows that the dose dependent increase in the malondialdehyde concentration was observed. This results for increased lipid peroxidation in the treated groups, thus leading to changes in the cellular membranes. Malondialdehyde is an important biomarker of oxidative stress and damage. Increased lipid peroxidation and oxidative damage has been reported extensively in liver and other tissues in fish (Perva *et al.,* 2020).

Figure 3. Changes in plasma CAT levels in *C mrigala* exposed to different doses of CeO₂NPs (2.5 mg/L and 25 mg/L, 96 h). Expressed as mean ± SE (*P<0.05- t test) with n=3.
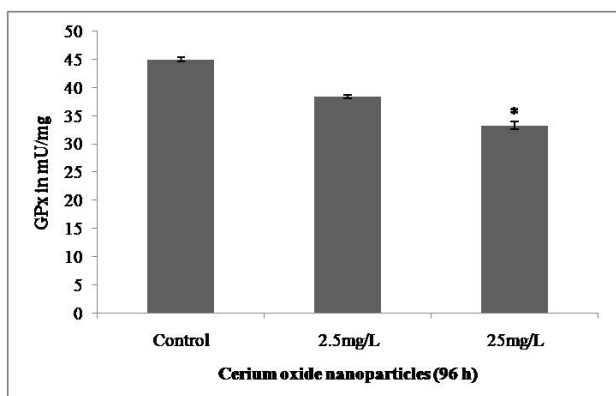
Figure 4. Alterations in GPx levels in *C mrigala* exposed to different doses of CeO₂NPs (2.5 mg/L and 25 mg/L, 96 h). Expressed as mean ± SE (*P<0.05- t test) with n=3.
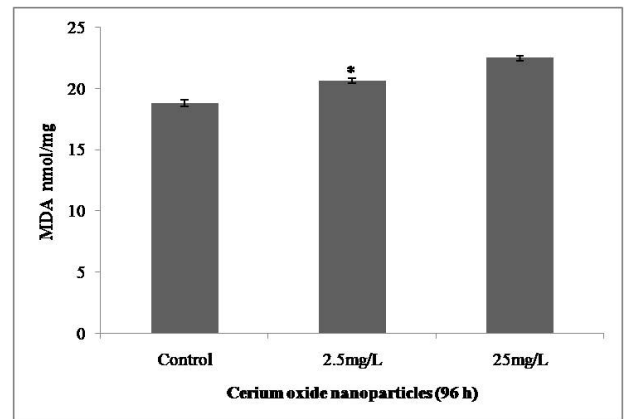
Figure 5. Changes in MDA concentration in *C mrigala,* which were exposed to different levels of CeO₂NPs (2.5 mg/L and 25 mg/L, 96 h). Expressed as mean ± SE (*P<0.05- t test) with n=3.

Effect of cerium oxide nanoparticles on glutamate levels in brain tissue
Figure 6 shows that the dose dependent variation was also found in levels of glutamate concentration, a neurotransmitter in the brain tissue. This could be associated with exo-toxicity, due to increased generation of glutamate, which might lead to depletion of nerve cells (Chandra *et al.,* 2020). The increased glutamate levels contribute to changes in brain environment which activates the glutamate receptors by permitting excess levels of calcium ions to permeate the cell (Duman *et al.,* 2019; Manev *et al.,* 1999).

Effect of cerium oxide nanoparticles by histopathological studies
Figure 7 shows gill tissue histopathological studies, which revealed several histological variations such as lifting of epithelial layer, cellular degeneration, aneurysm, hemorrhage and loss of structure. The examination exhibited cellular degeneration at both the concentrations (2.5 mg/L and 25 mg/L) whereas loss of structure, hemorrhage and absence of respiratory epithelium was observed in animals treated with higher concentration (25 mg/L). The gills are directly exposed to toxic molecules in the outer environment, which leads to pathological conditions (Mallat, 1985), thus making it a vulnerable structure. It has been reported that high concentration of ultra-small molecules is phagocytosed by the epithelial cells and this uptake makes way for the entry of such NPs resulting absorption and binding to cell walls and

cell membranes. Figure 7 (A) and 7(B) show normal structure of gill morphology in control fish, including intact primary and secondary lamellae. Figure 7(C) at lower dose 2.5 mg/L shows hemorrhage (arrow) due to gill hyperplasia which results in increased epithelial thickness and thus decline into blood stream. Aneurysm (dotted arrow) in Figure 7 (C) has been attributed to toxin induced alterations in the pillar cell structure, and thus loss of their function. Figure 7 (D), with higher concentration of 25 mg/L demonstrated, absence of respiratory epithelium and loss of structure due to interaction of NPs with gill epithelium (Figueiredo *et al.,* 2007). Figure 7 (F) shows lamellar fusion at higher concentration of 25 mg/L, owing to the thickening of gill filament epithelium from cell proliferation. The fusion of gill lamellae has been attributed to the toxin which changes the glycoprotein in the mucus covering of the cells, which further disturbs the negative charges of the epithelium and thus leads to fusion to the adjacent lamellae. Figure 7 (E) confirms lifting of lamellar epithelium (dotted arrow) indicating the defense mechanism. The separation of epithelial increases the space which withheld

diffusion of toxins from the water into the blood stream and further resulting in hemorrhage (arrow) at high doses 25 mg/L. Most of the above histological variations are result of defense mechanism against the toxic molecules thus establishing the responses of carps against cerium oxide nanoparticles.
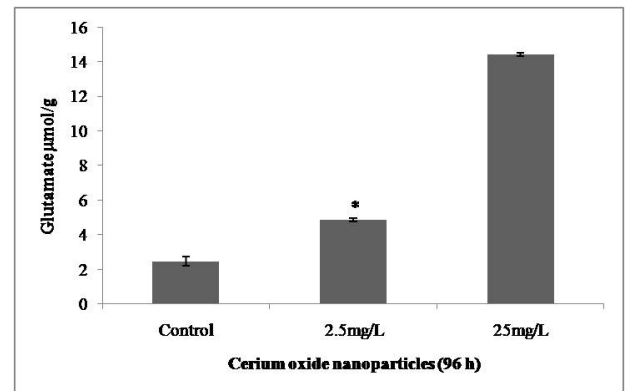
Figure 6. Variations in brain glutamate concentration in *C mrigala* exposed to different doses of CeO$_2$NPs (2.5 mg/L and 25 mg/L, 96 h). Expressed as mean ± SE (*P<0.05- t test) with n=3.
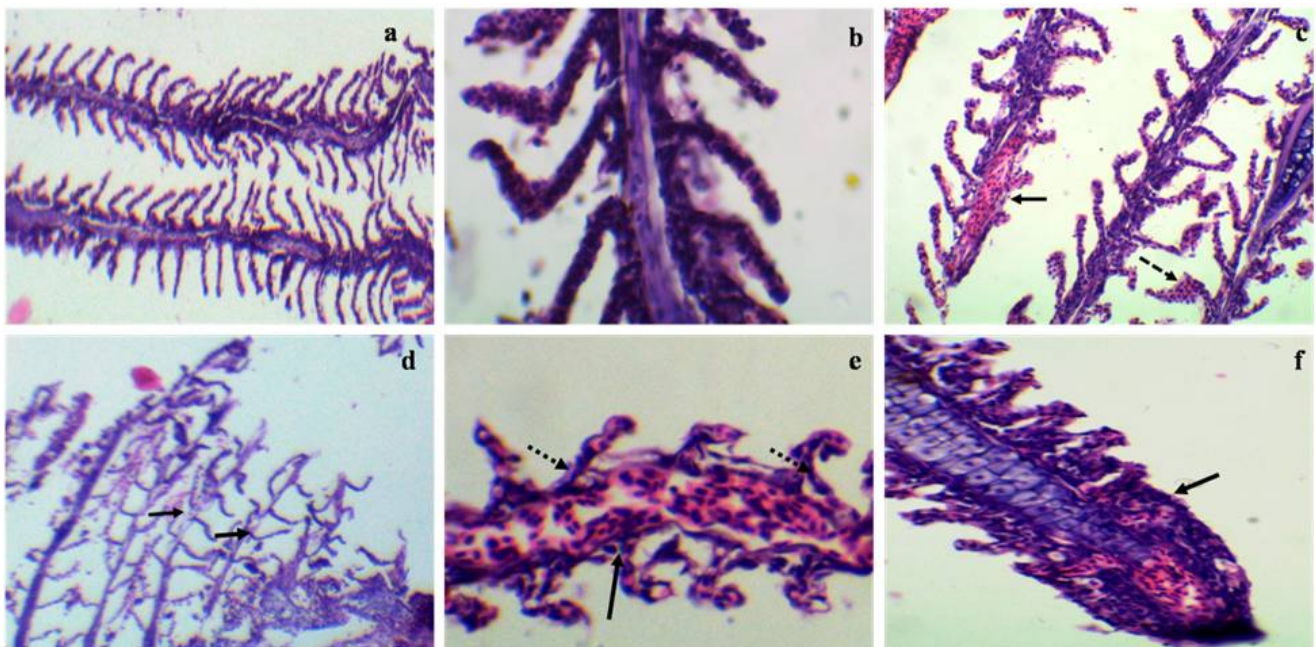
Figure 7. Histopathological analysis of *C.mrigala* Gills after 96 h. (A) 20x section from control fishes shows normal gill architecture (B) 40X section of primary and secondary lamellae from control fishes shows normal gill architecture (C) 20x section from treated fishes with 2.5 mg/L of CeO$_2$NPs shows aneursym (dotted arrow) and hemorrhage (arrow) (D) 20x section from treated fishes (25 mg/L) shows absence of respiratory epithelium and loss of structure (E) 40x section from treated fishes (25 mg/L) shows hemorrhage (arrow) and moderate lifting of epithelial and cellular degeneration (dotted arrow) (F) 40X section of treated fish (25 mg/L) show complete fusion of several lamellae.

CONCLUSION

The novelty in the work is the fact that no earlier reports have been found with regard to interaction of cerium oxide nanoparticles with fresh water fishes. The changes in haematological parameters, namely WBC and MCHC levels provide more insight into the toxic effects of CeO$_2$NPs. Biochemical markers also indicate oxidative damage through lipid peroxidation, SOD, catalase, GPx. Significant increase in the neurotransmitter, glutamate levels particularly at higher doses shows minor neurotoxicity impact of CeO$_2$NPs. Histopathological studies of fish gills, shows the direct evidence of toxicity of CeO$_2$NPs. Thus controlled discharge and neutralization CeO$_2$NPs before discharge to the environment is essential. However chronic exposure of fresh water fishes to CeO$_2$NPs have to be evaluated, and further proteomic and genomics studies might reveal effects of CeO$_2$NPs at molecular level.

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