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GENOTOXICITY OF CERIUM OXIDE NANOPARTICLE IN ZEBRAFISH AND GREEN MUSSEL *PERNA VIRIDIS* USING ALKALINE COMET ASSAY

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Abstract

Cerium oxide nanoparticles or nanoceria has versatile application in biomedical, solar cells and gas sensors. Increasing utilization of nanoceria has raised concerns over its release to environment and potential exposure. In vitro studies have shown its genotoxic potential, but reports on aquatic life are very limited. In this study, zebrafish (Danio rerio) and green mussel (Perna viridis) was exposed to different concentration 10, 20, 50 µg/l of nanoceria for 24, 72, and 120 h and the genotoxic response was measured using comet assay. The results showed significant ($p < 0.05$) increase in tail DNA (TDNA) and olive tail moment (OTM) as measured using comet assay in exposed animals as compared to control. The highest TDNA and OTM were

measured after 120 h of exposure with 50 µg/l of nanoceria in zebrafish as well as in green mussel. The results of this study demonstrate that short-term exposure to nanoceria causes a genotoxic response in zebrafish and green mussel, hence its environmental release should be carefully monitored.

Keywords
Single Cell Gel Electrophoresis, Nanoceria, DNA Damage, Zebrafish, Green Mussel

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1. Introduction

1.1 Cerium Oxide Nanoparticles

The advancement of nanotechnology has brought hundreds and thousands of nanoparticle-based products into the market. Cerium oxide nanoparticles (CeO_2 NPs) or nanoceria is one of the most popular and widely used nanoparticles belonging to the member of lanthanide series of rare earth element cerium. Because of its novel phyico-chemical, catalytic and antioxidant nature, it is being commonly used in solar cells, petroleum refining, glass polishing agents, fuel cells, adsorbents and has numerous biomedical applications (Minarchick et al., 2013; Cassee et al., 2011). Ce atom can exist in trivalent (Ce^{3+}) as well as in tetravalent (Ce^{4+}) state, which allows it to exhibit antioxidant like activities. This redox chemistry of cerium has shown promising results in multiple sclerosis (MS) by quenching free radicals and providing neuroprotection.

In vivo and *in vitro* studies involving nanoceria has shown conflicting results. Some studies have reported nanoceria to be an antioxidant in HepG2 cells (Azari et al., 2018), male Sprague Dawley rats (Ibrahim et al., 2018), human monocytic leukemia cells (THP-1) (Patel et al., 2018), primary cultured skin fibroblasts (Pezzini et al., 2017) whereas others has shown it to induce oxidative stress in balb/c mice (Adebayo et al., 2018), brown rat (Minarchick et al., 2015), *Caenorhabditis elegans* (Zhang et al., 2011). The effects of nanoceria are influenced by factors such as size, surface charge, shape of nanoceria and physiological conditions such as pH (De Souza et al., 2018).

1.2 Biomarker Studies

Genotoxicity markers have been used as early warning signals of contaminations in the environment. Single cell gel electrophoresis or comet assay has been used extensively in the field of environmental toxicology to study the effects of nanoparticles (Mahaye et al., 20017). $nTiO_2$

alone and in combination with 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) has shown to cause genotoxic damage in mussel gill cells (Canesi et al., 2014). Studies have also been conducted in freshwater snails, *Lymnaea luteola* exposed to nTiO2, nCuO, nZnO showing concentration and time-dependent increase in DNA damage using comet assay (Ali et al. 2015, 2014, 2012). Several studies have used zebrafish to study the genotoxic effect of nanoparticles such as ZnO (Boran et al., 2016),TiO_2 (Fang et al. 2015), SiO_2 (Ramesh et al., 2013), Ag (George et al. 2012), Au (Dedeh et al., 2015). The aim of the present study was to estimate the DNA damage in an economically important edible mussel *P. viridis,* and a model organism zebrafish *D. rerio* exposed to different concentration of nanoceria.

2. Materials and Methods

2.1 Maintenance of Animals and Exposure Conditions

Green mussel *P. viridis* was collected from Galigibagh beach in South Goa, India during the low tide. Mussels were brought to the laboratory within 3 hours of collections and kept in 5 L plastic container with 3 L of seawater from the sampled site. Adult zebrafish *D. rerio* was procured from a local farm in South Goa. They were placed in a 40 L aquaria with aerated and dechlorinated water with 14h light and 10h dark. The physical parameters such as temperature and pH were maintained at 28±1ºC and 7.5±0.3 respectively. Mussels and fish were acclimatized for one week before exposing them to nanoceria. Fish were fed twice every day with commercial fish-food (tetra bits). After acclimatizing period fishes were transferred into smaller 8 L aquaria.

CeO_2 NPs (99.9% pure, <25 nm particle size (BET)) were purchased from Sigma Pvt. Ltd. It was characterize using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Nanoceria was dissolved in ultrapure water to obtain a stock suspension of 1 mg/ml. The final concentration for the treatment groups was achieved by diluting stock solution in seawater and dechlorinated water for mussels and zebrafish respectively. *D. rerio* and *P. viridis* was exposed to different concentration 10, 20, 50 µg/l of nanoceria for 24, 72, and 120 h and the genotoxic response was measured using comet assay. One third of the water volume was renewed every 24h to maintain a constant concentration of exposure concentrations. Once in two days, nine mussels were randomly collected and hemolymph and soft tissues were subjected to comet assay.

2.2 Comet Assay

Genotoxicity was assayed in the peripheral blood using comet assay following the methods of Singh et al., (1988) with slight modifications. Zebrafish were sacrificed by cutting off the tail and the peripheral blood samples were collected using a syringe previously washed with 0.1 M EDTA to prevent clotting. Around 25 µl of blood was collected from 4-5 fish and mixed with 75 µl of 0.7% low melting agarose (LMA) and poured onto a slide previously coated with 1% of normal melting agarose (NMA). DNA unwinding, electrophoresis, and staining were then performed. 200 randomly selected cells (50 cells from each of the quadruplet slides) were analyzed per sample. The percentages of DNA in the tail (TDNA) was used as the parameter to measure DNA damage by using the CASP (Konca et al., 2003). For mussels, about 1 ml of hemolymph was collected from the posterior adductor muscle of 2-5 specimens and centrifuged at 2000 rpm for 10 minutes. It was then suspended in phosphate buffer saline (1.2 M NaCl, 0.027 M KCl, 11.5 mM K_2HPO_4, 0.08M Na_2HPO_4, pH 7.3). The cell suspension was then used to make the second layer of agarose followed by the rest of the protocol mentioned above.

3. Results and Discussion

DNA damage as expressed in percentage of tail DNA (TDNA) is shown in fig. 1. DNA damage has shown concentration as well as time-dependent increase in both *P. viridis* as well as in *D. rerio*. The highest TDNA was recorded on day 5 at 50 µg/l of nanoceria. On day 5, *P. viridis* has shown 2.4 fold increase in TDNA for 50 µg/l whereas it was found to be 4.8 fold in *D. rerio*. Comparatively DNA damage in *D. rerio* was found to be more pronounced than *P. viridis*. Highest OTM in *D. rerio* (1.29±0.03) and *P. viridis* (2.41±0.20) observed at 50 µg/l on day 5 fig. 2.

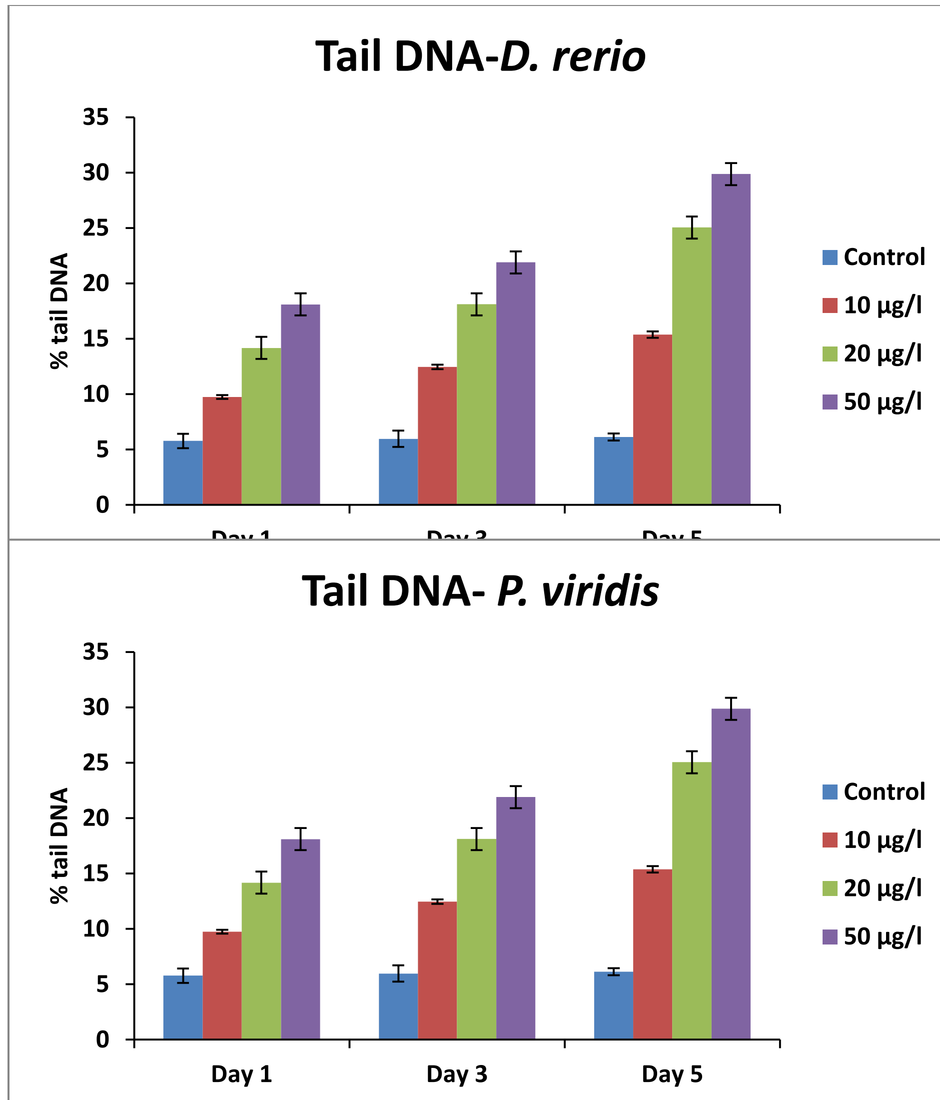

Figure 1: *Tail DNA (TDNA) in D. rerio and P. viridis exposed to difference concentrations of nanoceria*

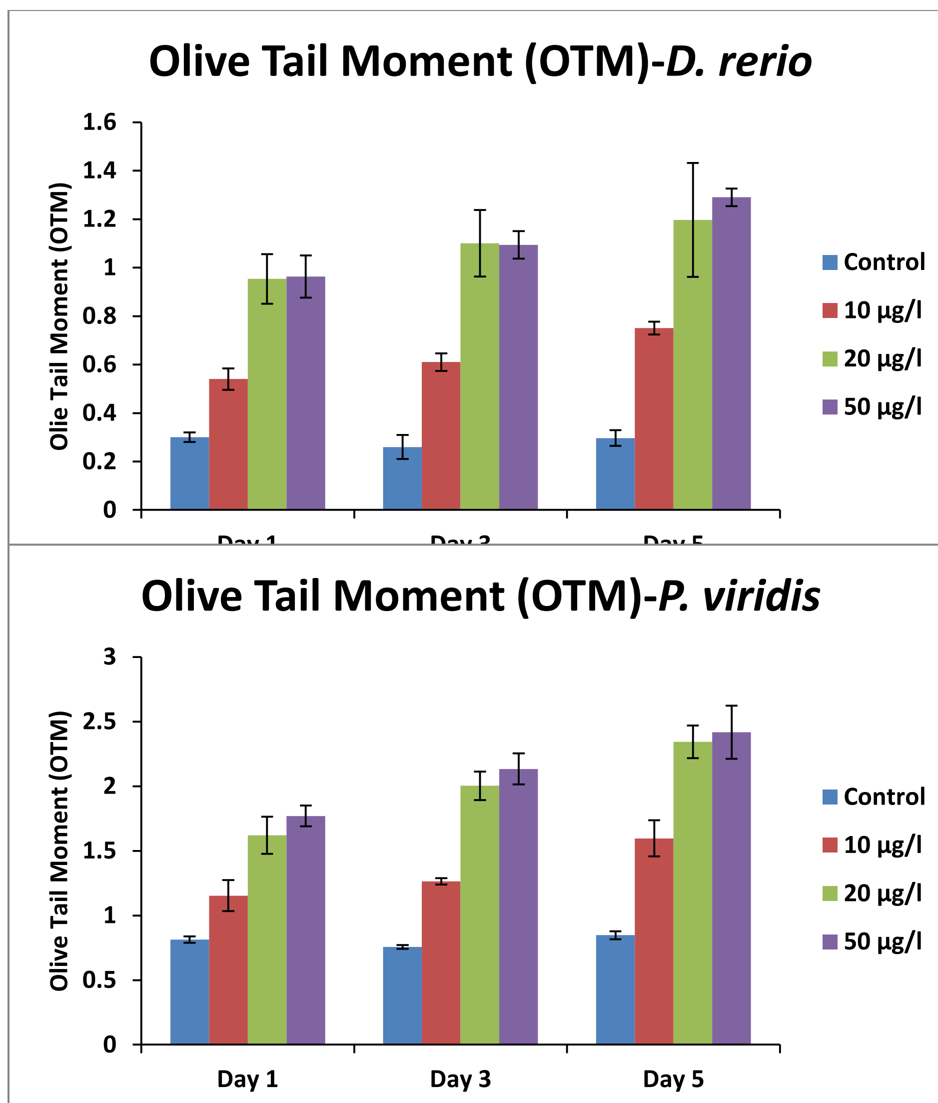

Figure 2: *Olive Tail Moment (OTM) in D. rerio and P. viridis exposed to difference concentrations of nanoceria*

Studies have reported that nanoceria causes cytotoxicity and oxidative stress in human lung cells (Park et al., 2008; Lin et al., 2006). Cerium was also reported to cause cytogenetic and development damage in Sea urchin (*Paracentrotus lividus*) embryos. Zebrafish have become a popular model to study the toxicity of nanoparticles and other environmental contaminants (Chakraborty et al., 2016). Gao et al., (2018) has studied the effet of hydroxyapatite-loaded cadmium nanoparticles (nHAP-Cd) in zebrafish and reported substantial increase in DNA

damage in exposed animal. Similar results were also reported with Titanium dioxide nanoparticles (TiO_2 NPs, Rocco et al., 2015), ZnO nanoparticles (ZnO-NPs, Du et al., 2016), iron oxide nanoparticles (IONPs, Vilacis et al., 2017). CeO_2 and its different nanocrystalline types have been tested on zebrafish (Jemec et al., 2012). The author didn't report any mortality at concentrations high as 500 mg/l, but malformation in the embryo was observed at the lowest concentration tested i.e. (100 mg/l). In another *in vivo* study, depletion of serotonin (5-HT) level was observed in the intestine of the zebrafish for exposure period longer than three days (Ozel et al., 2013). Cytotoxicity and decrease in cell viability were observed in A375 human melanoma cell line co-exposed to nanoceria and the drug doxorubicin (Sack et al., 2013).

4. Conclusion

The discharge of nanoparticles from industry and other sources to the environment need to be monitored. More biomarker studies with other nanoparticles with long term exposure are needed to have a better understanding of the mechanism of action of nanoparticles in the organisms.

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References

Adebayo, O.A., Akinloye, O., & Adaramoye, O.A., (2018). Cerium oxide nanoparticle elicits oxidative stress, endocrine imbalance and lowers sperm characteristics in testes of balb/c mice. Andrologia, 50(3). https://doi.org/10.1111/and.12920

Ali, D., Alarifi, S., Kumar, S., Ahamed, M., & Siddiqui, M.A., (2012). Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail *Lymnaea luteola* L. Aquatic toxicology, 124, 83-90. https://doi.org/10.1016/j.aquatox.2012.07.012

Ali, D., Alarifi, S., Kumar, S., Ahamed, M., & Siddiqui, M.A., (2012). Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail *Lymnaea luteola* L. Aquat Toxicol, 124-125, 83-90. https://doi.org/10.1016/j.aquatox.2012.07.012

Ali, D., Ali, H., Alarifi, S., Kumar, S., Serajuddin, M., Mashih, A.P., Ahmed, M., Khan, M., Adil, S.F., Shaik, M.R., & Ansari, A.A., (2015). Impairment of DNA in a freshwater gastropod (*Lymnea luteola* L.) after exposure to titanium dioxide nanoparticles. Arch Environ Contam Toxicol, 68(3), 543-52. https://doi.org/10.1007/s00244-015-0132-0

Azari, A., Shokrzadeh, M., Zamani, E., Amani, N., & Shaki, F., (2018). Cerium oxide nanoparticles protects against acrylamide induced toxicity in HepG2 cells through modulation of oxidative stress. Drug Chem Toxicol, 1-6. https://doi.org/10.1080/01480545.2018.1477793

Boran, H., & Ulutas, G., (2016). Genotoxic effects and gene expression changes in larval zebrafish after exposure to ZnCl2 and ZnO nanoparticles. Dis Aquat Organ, 117(3), 205-14. https://doi.org/10.3354/dao02943

Canesi, L., Canesi, L., Frenzilli, G., Balbi, T., Bernardeschi, M., Ciacci, C., Corsolini, S., Della Torre, C., Fabbri, R., Faleri, C., Focardi, S., Guidi, P., Kočan, A., Marcomini, A., Mariottini, M., Nigro, M., Pozo-Gallardo, K., Rocco, L., Scarcelli, V., Smerilli, A., & Corsi, I. (2014). Interactive effects of n-TiO2 and 2,3,7,8-TCDD on the marine bivalve *Mytilus galloprovincialis*. Aquat Toxicol, 153, 53-65. https://doi.org/10.1016/j.aquatox.2013.11.002

Cassee, F. R., van Balen, E. C., Singh, C., Green, D., Muijser, H., Weinstein, J., & Dreher, K. (2011). Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. Crit Rev Toxicol, 41(3), 213-229. https://doi.org/10.3109/10408444.2010.529105

Chakraborty, C., Sharma, A. R., Sharma, G., & Lee, S. S. (2016). Zebrafish: A complete animal model to enumerate the nanoparticle toxicity. J Nanobiotechnology, 14(1), 65. https://doi.org/10.1186/s12951-016-0217-6

Dedeh, A., Ciutat, A., Treguer-Delapierre, M., & Bourdineaud, J. P. (2015). Impact of gold nanoparticles on zebrafish exposed to a spiked sediment. Nanotoxicology, 9(1), 71-80. https://doi.org/10.3109/17435390.2014.889238

Du, J., Wang, S., You, H., Jiang, R., Zhuang, C., & Zhang, X. (2016). Developmental toxicity and DNA damage to zebrafish induced by perfluorooctane sulfonate in the presence of ZnO nanoparticles. Environ Toxicol, 31(3), 360-371. https://doi.org/10.1002/tox.22050

Fang, Q., Shi, X., Zhang, L., Wang, Q., Wang, X., Guo, Y., & Zhou, B. (2015). Effect of titanium dioxide nanoparticles on the bioavailability, metabolism, and toxicity of pentachlorophenol in zebrafish larvae. J Hazard Mater, 283, 897-904. https://doi.org/10.1016/j.jhazmat.2014.10.039

Gao, M., Yang, Y., Lv, M., Song, W., & Song, Z. (2018). Oxidative stress and DNA damage in zebrafish liver due to hydroxyapatite nanoparticles-loaded cadmium. Chemosphere, 202, 498-505. https://doi.org/10.1016/j.chemosphere.2018.03.146

George, S., Lin, S., Ji, Z., Thomas, C. R., Li, L., Mecklenburg, M., . . . Nel, A. E. (2012). Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. ACS Nano, 6(5), 3745-3759. https://doi.org/10.1021/nn204671v

Ibrahim, H. G., Attia, N., Hashem, F., & El Heneidy, M. A. R. (2018). Cerium oxide nanoparticles: In pursuit of liver protection against doxorubicin-induced injury in rats. Biomed Pharmacother, 103, 773-781. https://doi.org/10.1016/j.biopha.2018.04.075

Konca, K., Lankoff, A., Banasik, A., Lisowska, H., Kuszewski, T., Gozdz, S., Koza, Z., & Wojcik, A. (2003). A cross-platform public domain PC image-analysis program for the comet assay. Mutat Res, 534(1-2), 15-20. https://doi.org/10.1016/S1383-5718(02)00251-6

Mahaye, N., Thwala, M., Cowan, D. A., & Musee, N. (2017). Genotoxicity of metal based engineered nanoparticles in aquatic organisms: A review. Mutat Res, 773, 134-160. https://doi.org/10.1016/j.mrrev.2017.05.004

Minarchick, V. C., Stapleton, P. A., Fix, N. R., Leonard, S. S., Sabolsky, E. M., & Nurkiewicz, T. R. (2015). Intravenous and gastric cerium dioxide nanoparticle exposure disrupts microvascular smooth muscle signaling. Toxicol Sci, 144(1), 77-89. https://doi.org/10.1093/toxsci/kfu256

Ozel, R. E., Hayat, A., Wallace, K. N., & Andreescu, S. (2013). Effect of cerium oxide nanoparticles on intestinal serotonin in zebrafish. RSC Adv, 3(35), 15298-15309. https://doi.org/10.1039/c3ra41739e

Patel, P., Kansara, K., Singh, R., Shukla, R. K., Singh, S., Dhawan, A., & Kumar, A. (2018). Cellular internalization and antioxidant activity of cerium oxide nanoparticles in human

monocytic leukemia cells. Int J Nanomedicine, 13, 39-41. https://doi.org/10.2147/IJN.S124996

Pezzini, I., Marino, A., Del Turco, S., Nesti, C., Doccini, S., Cappello, V., Gemmi, M., Parlanti, P., Santorelli, F.M., Mattoli, V., Ciofani, G. (2017). Cerium oxide nanoparticles: the regenerative redox machine in bioenergetic imbalance. Nanomedicine (Lond), 12(4), 403-416. https://doi.org/10.2217/nnm-2016-0342

Ramesh, R., Kavitha, P., Kanipandian, N., Arun, S., Thirumurugan, R., & Subramanian, P. (2013). Alteration of antioxidant enzymes and impairment of DNA in the SiO2 nanoparticles exposed zebra fish (*Danio rerio*). Environ Monit Assess, 185(7), 5873-5881. https://doi.org/10.1007/s10661-012-2991-4

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res, 175(1), 184-191. https://doi.org/10.1016/0014-4827(88)90265-0

Villacis, R. A. R., Filho, J. S., Pina, B., Azevedo, R. B., Pic-Taylor, A., Mazzeu, J. F., & Grisolia, C. K. (2017). Integrated assessment of toxic effects of maghemite (gamma-Fe2O3) nanoparticles in zebrafish. Aquat Toxicol, 191, 219-225. https://doi.org/10.1016/j.aquatox.2017.08.004

Zhang, H., He, X., Zhang, Z., Zhang, P., Li, Y., Ma, Y., Kuang, Y., Zhao Y., & Chai, Z. (2011). Nano-CeO2 exhibits adverse effects at environmental relevant concentrations. Environ Sci Technol, 45(8), 3725-3730. https://doi.org/10.1021/es103309n