Journal of Pharmaceutical and Health Sciences 2013;2(1),27-31.

Original Article

JPHS

Evaluation of DNA damage and lipoperoxidation content in red earthworm, Lumbricus rubellus exposed to zinc oxide nanoparticles

Mehran Arabi[1*], Narges Najafi[1] , Homeyra Jafarzadeh Samani[1]

[1] *Dept. of Biology, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran.*

Abstract

Manufactured nanoparticles have a wide range of application due to their advantageous properties when compared with their bulk forms. There is a growing concern regarding the safety of nanoparticles. This study aimed to evaluate the cell membrane lipoperoxidation (LPO) and DNA integrity in red earthworm Lumbricus rubellus when exposed to artificial soil systems containing different concentration of zinc oxide nanoparticles (n-ZnO).

Exposure to n-ZnO led to an increase in LPO content and DNA damage (in terms of halos) in earthworm coelomocytes. We identified a positive strong correlation ($r = +0.891$, $p<0.01$) between LPO and DNA damage/halos. In summary, we show that n-ZnO is toxic to soil organisms and may imposed a sever oxidative stress/LPO leading to DNA damages.

Keywords: Nano-ZnO, Earthworm, Coelomocyte, Oxidative stress, DNA damage

***Corresponding author**: Mehran Arabi, PhD, Division of Animal Physiology, Dept. of Biology, Shahrekord University, P.O.Box 115, Shahrekord, Iran.
Tel: +98 381 4424412 Fax: +98 381 4424419
Email address: mehranarabi@hotmail.com

Introduction

Metal oxide nanoparticles have received considerable attention regarding their extensive potential applications in medicine and other facets of human life. Because of their potential introduction into soil as well as the aquatic environment, the inclusion of a set of ecotoxicity tests in the risk characterization of nanoparticles is necessary. However, research has only recently focused on their impact on terrestrial organisms and the literature on this topic is very limited (Lapied et al., 2010; Li et al., 2011). Due to its unique optical, catalytic, semiconducting, piezoelectric, and magnetic properties, zinc oxide nanoparticles (n-ZnO) have found many technological applications (Nowack et al., 2007). Despite these advantages, limited research has been devoted to examine the toxicity and potential adverse effects of n-ZnO. Studies on related mechanisms exerted by nanoparticles in the soil ecosystem are even more limited.

Accumulation of metal compounds in individual organisms is dependent on the balance of uptake, through waterborne and dietary routes, and excretion and growth. However, high levels of total bioaccumulation do not necessarily result in toxicity, as this is dependent on that fraction of bioaccumulated metal that is in metabolically available form directly or indirectly disrupting metabolic processes (Luoma and Rainbow, 2005).

Most studies regarding ecotoxicology of nanoparticles have regarded only acute toxicity of these particles, leaving a gap in the area of genotoxicity. Hazardous compounds with potential genotoxic effects may show weak signals of toxicity in acute tests, but they are often potent to affect genetic material, leading to mutations and/or carcinogenesis. While these effects are first appear at the individual level, they may ultimately lower the fitness of an specific organism at the population level and negatively impact the health of organisms in the ecosystem in the long-term (Warheit et al., 2007).

To help narrowing the gap in ecotoxicology of nanoparticles, in this study we examine genetoxic response of red earthworm Lumbricus rubellus exposed to artificial soil contaminated with n-ZnO.

Materials and Methods

ZnO NP powder (Purity: 99.9%, nominal size: 30±5 nm) was purchased from Nanjing Emperor Nano Material Co., Nanjing, China. Earthworms Lumbricus rubellus (ATCC; 35632) were obtained from Soroosh Mohit Sabz (SMS) Co., Shahrekord, Iran. Twenty earthworms were placed in all groups including control and experimental and were fed with organic kitchen wastes in 2 L plastic boxes and watered daily. A photoperiod (of 12L:12D) was maintained and the temperature was kept at room temperature (22±1 oC). Three low concentrations of n-ZnO 40, 80 and 120 mg/kg of dry soil were applied by mixing certain amounts of nano-powder to soil.

After 96 hr, whole bodies of earthworm were homogenized in 1:10 (w/v) ice cold 100mM phosphate buffer (containing 2mM EDTA; pH 7.8), plus 0.1% Triton- X-100 in a Potter-Elvehijim homogenizer at 24,000 rpm for 25 s. The homogenate was then centrifuged at 9000 ×g at 4 C for 15 min. 100 mM PMSF was added to the resulting clear supernatant and kept at 4 C for assays.

The extent of lipoperoxidation (LPO) as an oxidative stress biomarker was measured optically. Peroxides produced in LPO process were estimated by a TBARS assay (Buege and Aust, 1978). This was performed using a malondialdehyde (MDA; a by-product of LPO process) reaction with 2-thiobarbituric acid (TBA). Supernatants were homogeneized in 150 mM NaCl. An aliquot of homogenate was added to 10% trichloroacetic acid (TCA) and 0.67% TBA to adjust to a final volume of 1.0 ml. The reaction mixture was placed in a

microcentrifuge tube and incubated for 15 min at 95 oC. After cooling, it was centrifuged at 5000 ×g for 10 min and the optical density at 532 nm was determined. LPO levels are expressed as nanomoles of MDA per milligram of protein (Buege and Aust, 1978).

DNA damage was assessed by number of halos (bright zone around coelomocyte genome) using the DNA diffusion assay for estimation of apoptosis (Apte et al., 2008). Briefly, end frosted microscopy slides were pre-coated with 90 μL of 1% normal melting. Coelomocytes from earthworm coelomic fluid were then embedded in 90 μL of 1% low melting agarose and were placed on pre-coated slide. Slide was immediately covered with cover glass and then transferred onto ice packs for 1 min. Cover glass was removed and third layer of 110 μL of 1% low melting agarose was put and was covered with cover glass, slide was then placed on ice packs for 1 min. Cover glass was removed and slide was immediately transferred to lysis solution (sodium lauryl sarcosinate 0.01%, di-sodium EDTA 1 mM, NaCl 1.25 M, Tris buffer 5 mM, pH 10), for 45 min, followed by alkaline solution (NaOH 300 mM pH>13) for 10 min. Slide was then immersed in neutralizing buffer (Tris-Cl 0.4 M, pH 7.5) for 45 min, followed by absolute ethanol for 20 min, and was visualized by staining with 75 μL (20 μg/mL). Ethidium bromide and observed under fluorescence microscope Pro-way xsz- 156, (Lawrence and Mayo) red filter. Two hundred cells per slide were counted and classified as normal (with sharp outlines and without halo) and apoptotic (with halo) depending on their DNA diffusion pattern.

Data were summarized using descriptive statistical methods. Results were analyzed using SPSS (Version 16 software). One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests used for comparing the means values between groups. Differences were considered significant at $P<0.05$.

Results

Table 1 presents the LPO/TBARS and the percentage of DNA damage after 21 days n-ZnO treatments. As seen, n-ZnO can increase the LPO as an oxidative stress biomarker in a dose-dependent manner. When the n-ZnO dose reached 120 mg/kg soil, the content of MDA were about 120.63 times higher than in the control ($P< 0.05$). (Table 1 and Figure 1).

Table 1: Alterations in lipoperoxidation (LPO) extent and DNA damage (halos) in earthworms after 21 days treatment with different concentrations of zinc oxide nanoparticles (n-ZnO).

		LPO/MDA	DNA damage/halos percentage
n-ZnO (mg/kg)	0	0.92 ± 0.12	1.8 ± 0.34
	40	1.024 ± 0.34 *	1.91 ± 0.42
	80	1.84 ± 0.32 * #	3.65 ± 1.04 * #
	120	2.03 ± 0.42 * #	4.13 ± 1.76 * #

Datas are given as mean±SD for n = 20. , Values are statistically significant at $p< 0.05$.
* compared to control group (0 mg/kg n-ZnO) , # compared to 40 mg/kg n-ZnO treated group
Unit for LPO (lipoperoxidation) is nM MDA/mg protein

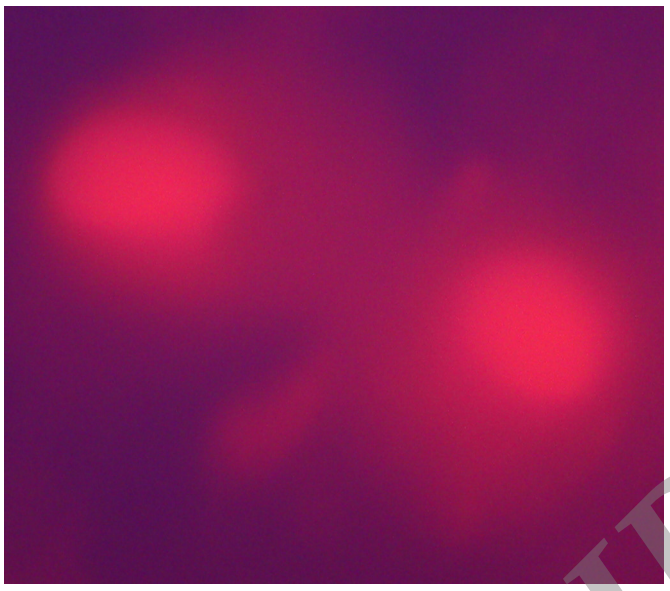

Figure 1: showing two coelomocytes with DNA damage. Halos (DNA fragments) are seen around genome of cells.

Discussion

Production, use, and disposal of nanomaterials will inevitably lead to their release into the environment (Lin and Xing, 2007). However, the potential exposure of nanoparticles cannot be comprehensively measured due to the limitations of current analytical technologies (Clark et al., 2012). Our study indicated that n-ZnO can leave serious adverse effects in red earthworms concentrations over 40 mg/kg soil.

Biomarkers are useful tools in toxicological studies, because their responses integrate spatial and temporal variations in environment, modulating the exposure of organisms to contaminants. Oxidative stress and its main consequences namely LPO are a general response to toxicity induced by many contaminants. Therefore, its assessment is included in biomonitoring programs as a nonspecific biochemical marker (Alvarez et al., 1987).

MDA is an index of LPO damages, which can cross-link cell membrane phospholipids PS and PE, PS and PS, and, PE and PE. Lack of the uniformity to these cross-links in cell membrane can generate physical forces, which may disturb the membrane lipid distributions leading to reduction in membrane integrity and fluidity (Alvarez et al., 1987).

The main mechanism of toxicity of nanoparticles is thought to be oxidative stress via reactive oxygen species (ROS)(Kohen and Nyska, 2002) resulting in degradation of lipids, carbohydrates, proteins and DNA (Kelly et al., 1998). For n-ZnO, participation of ROS in induced membrane damages has been demonstrated in the earthworm Eisenia foetida which is in agreement with our results (Hu et al., 2010). DNA diffusion test is a useful tool to be use as a biomarker of genotoxic effects on invertebrates in soil. The results of the genotoxic assay in the present study showed clearly that the earthworms were subjected to DNA damage at 80 and 120 mg/kg soil of n-ZnO. n-ZnO can induce significant damage to earthworm's DNA with doses greater than 1.0 g/kg dry soil (Hu et al., 2010).

The capacity of n-ZnO to generate ROS in vitro seems to correlate with their potential to induce cellular inflammation in vivo (Kao et

al., 2012). In addition, n-ZnO has the potential to induce genotoxicity in HEp-2 human cervix carcinoma cells increasing tyrosine phosphorylation (Osman et al., 2010).
Therefore, we concluded that n-ZnO can alter the metabolism of lipids in earthworm plasma membranes and is toxic to earthworm genomic materials.

Acknowledgements

Authors are highly grateful to Vice-president of Research, Shahrekord University for financial support of this study. We also appreciate Rasool Seidaie for his assistance in cultivation of earthworms.

References

Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa- superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 1987; 8: 338-48.

Apte AA, Manerikar RS, Ghole VS. Application of DNA Diffusion Assay in Earthworm Coelomocytes. Environ Toxicol 2008; 23 (2): 278-83.

Buege JA, Aust SD. Microssomal lipid peroxidation. Methods Enzymol 1978; 52: 302-9.

Clark K, Van Tongeren M, Christensen F.M. , et al. Limitations and information needs for engineered nanomaterial-specific exposure estimation and scenarios: recommendations for improved reporting practices. J Nanopart Res 2012; 14: 970-983.

Hu CW, Li M., Cui YB, Li DS, Chen J, Yang LY. Toxicological effects of TiO2 and ZnO nanoparticles in soil on earthworm Eisenia fetida. Soil Biol Biochem 2010; 42: 586-91.

Kao YY, Chen YC, Cheng TJ, Chiung YM, Liu PS. Zinc oxide nanoparticles interfere with zinc ion homeostasis to cause cytotoxicity. Toxicol Sci. 2012; 125(2): 462-72.

Kelly SA, Havrilla CM, Brady TC, Abramo KH, Levin ED.. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ Health Persp 1998; 106: 375-84.

Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Path 2002; 6: 620-50.

Lapied E, Moudilou E, Exbrayat JM, Oughton H, Joner EJ. Silver nanoparticle exposure causes apoptotic response in the earthworm Lumbricus terrestris (Oligochaeta). Nanomed 2010; 5: 975-84.

Li L-Z, Zhou M-D, Peijnenburg WJGM., van Gestel CAM, Jin Sh-Y, Wang Y-J, Wang P. Toxicity of zinc oxide nanoparticles in the earthworm, Eisenia fetida and subcellular fractionation of Zn. Environ Int 2011; 37: 1098-104.

Lin DH, Xing BS. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution 2007; 150: 243-50.

Luoma SN, Rainbow PS. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ Sci Technol 2005; 37: 1921-931.

Nowack B, Bucheli TD. Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 2007; 150 :5-22.

Osman IF, Baumgartner A, Cemeli E, Fletcher JN, Anderson D. Genotoxicity and cytotoxicity of zinc oxide and titanium dioxide in HEp-2 cells. Nanomed (Lond) 2010; 5(8): 1193-203.

Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicol Lett 2007; 171: 99-110.