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Evaluation of oxidative stress biomarkers and acetylcholinesterase activity in Gammarus pseudosyriacus exposed to nanosilver

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Abstract

The extensive use of nanoparticles in a variety of applications has raised great concerns regarding their biological effects and environmental fate. Silver nanoparticle, often referred to as nanosilver (n-Ag), may cause health problems because of its wide and ever growing use in many applications. n-Ag is used in treatments of wounds, disinfection of water and/or air and coatings textiles. n-Ag is shows toxic effects only when oxidized to silver ions (Ag+). There is a limited knowledge concerning environmental and health consequences of exposure to n-Ag. In the current study, we aimed to evaluate the potential ecotoxicity of n-Ag for freshwater crustacean model organism, Gammarus pseudosyriacus.

Three low concentration solutions of n-Ag (2.5, 5 and 7.5 ppm) were used over a period of 96 hr. The concentrations of n-Ag used in this study are not environmentally relevant but provide a model system to examine its ecotoxicity.

It was revealed that n-Ag imposes a severe lipid peroxidation (LPO/MDA) in Gammarus whole body extract. In addition, the activity of some antioxidant enzymes changed. Catalase (CAT) activity was decreased and glutathione peroxidase (GPx) activity significantly increased. Acetylcholinesterase (AChE) activity was also markedly decreased. An abnormal vigorous movement was observed among animals.

Briefly, n-Ag was found highly toxic to aquatic organisms and has the potential to exert a strong oxidative stress, leading to altered physiological state.

Keywords: Nanosilver, Gammarus, Oxidative stress, AChE, Lipid peroxidation

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Introduction

Nanoscience is broadly applied in various technical disciplines, including biotechnology, biomedicine, molecular medicine, pharmacology, ecotoxicology, and electronics. Agriculture, veterinary science. The food industry also benefit from nanotechnology research. With increasing use of manufactured nanoparticles, the potential for exposure of human to these substances is also increasing. Many of manufactured nanomaterials are composed of metal and metal oxides. There are reports on presence of metal-containing nanoparticles in workplaces (Peters et al., 2009), and in surface waters (Baun et al., 2009). Silver nanoparticle often referred to as nanosilver (n-Ag), may cause health problem because of its wide applications (Wijnhoven et al., 209). n-Ag is used in treatments of wounds, disinfection of water and/or air and coatings textiles (Chen et al., 2008). There is a limited knowledge concerning environmental and health consequences of exposure to n-Ag. n-Ag has been identified to be one of the most toxic nanomaterials. The cytotoxicity of n-Ag is associated with generation of reactive oxygen species (Hussain et al., 2005), and several studies suggest that n-Ag is toxic only when oxidized to silver ions (Ag+) (Benn and Westerhoff, 2008; Zhao and Wang, 2011). Despite the positive aspects of n-Ag use in industries, the toxicology and health hazards of n-Ag is not well understood (Liu et al., 2009).

Aquatic species are exposed to chemical contamination by an increasing variety of anthropogenic activities such as nanoparticle production that can induce several toxicity mechanisms, with the potential of deleterious effects. Aquatic nontarget organisms are exposed to different amounts of nanoparticles. These organisms are typically exposed to sequential pulses with different concentrations of nanoparticles. Total accumulation of metal compounds in individual organisms is dependent on the balance of uptake, through waterborne and dietary routes, excretion and growth.

There is a limited knowledge about adverse impact of nanoparticles on freshwater organisms (Luoma and Rainbow, 2005).

The genus Gammarus (Crustacea, Amphipoda) is a model organism for investigating ecotoxicity of nanoparticles. As a shredder, Gammarus plays a key role in leaf litter breakdown processes and consequently in nutrient cycling (McNeil et al., 1997). Frequent selection of Gammarus as model species is also related to its high sensitivity to various chemicals, its definite sexual dimorphism and the ease of its use in the laboratory conditions (Alonso and De Lange, 2010).

This study aimed to investigate the n-Ag impacts on a number of physiological aspects of Gammarus pseudosyriacus, including oxidative stress biomarkers and acetylcholinesterase. Although the concentrations of n-Ag used in this study are not environmentally relevant, they offer a model to examine toxicity of this substance on aquatic organisms.

Materials and Methods

Nanosilver solution was purchased from Nano-shop Co., Tehran, Iran. The particle size and purity were 40 nm and 98%, respectively, according to company brochure. Gammarus pseudosyriacus were collected using a net (by kick sampling). The mature size organisms were separated by sieving in spring 2012. Immediately after sampling, organisms were stored in plastic bottles containing ambient fresh water, and quickly transferred to the laboratory. The gammarids used during laboratory assays were collected from the Shalamzar spring (32o 02′ N, 50o 49′ E) located in Chahar-Mahal and Bakhtiari province, Iran. Amphipods were quickly brought to the laboratory in plastic vessels and then under constant aeration. A photoperiod (12L:12D)

was maintained and the temperature was kept at 12±1◦C. Organisms were fed ad libitum with alder leaves (Alnusglutinosa) in 30L glass tanks filled with water from sampling site. Freeze-dried powder of compost earthworms Eisenia foetida was given once as a dietary supplement during experiment.

Gammarids were kept in water tanks containing three low concentrations 2.5, 5 and 7.5 ppm of n-Ag, respectively. After a period of 96 hr, pools of whole animals were homogenized in 1:10 (w/v) icecold100mM 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 for15 min. One hundred mM solution of PMSF was added to the resulting clear supernatant and kept at 4◦C for assays. The supernatant was centrifuged again to obtain cytosolic fraction at 100,000 ×g at 4 C for 90 min in CAT and GPx assays (Correia et al., 2003).The special activities of oxidative stress biomarkers namely, lipid peroxidation (LPO/MDA), catalase (CAT, EC 1.11.1.6) and glutathione peroxidase (GPx, EC 1.11.1.9) were measured using spectrophotometry.
Peroxides produced in LPO process were estimated by a TBARS assay (Buege and Aust, 1978). This was performed using a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which was optically measured. Supernatants were homogeneized in150 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 for10 min and the optical density at 532 nm was determined. MDA/TBARS level is expressed as nanomoles of MDA per milligram of protein per min.
A spectrophotometric method (Aebi, 1983) was applied, which includes measuring the absorbance at 240 nm in a time interval of 1 min 30 s when the sample is added to hydrogen peroxide. The reaction can be followed by a decrease in absorbance as the peroxide is turned into oxygen and water. To perform the reaction, quartz cuvettes with a path length of 10 mm were used, adding 80 μL of each sample to hydrogen peroxide 0.072% (v/v) in 50 mM (pH 7) potassium phosphate buffer with 1 mM EDTA. The absorbance was read every 15 s. Catalase activity was calculated using an absorption coefficient for H2O2 of 0.04 mmol−1 cm−1.

The activity of GPx (Flohe and Gunzler, 1984) was measured in 50 mM phosphate buffer (pH 7.0), 2 mM GSH, 0.5 mM sodium azide, 2 U GRD, 0.12 mM NADPH, 0.2 mM H2O2, 100 μl supernatant and water to obtain a final reaction volume of 1 ml. The activity of GPx was monitored by following the decrease in NADPH concentration, which is consumed during generation of GSH from GSSG (extinction coeff. 6.2 mM-1 cm-1). The unit for GPx was nM/mg protein.min.

AChE was also measured by a certain laboratory protocol (Ellman et al., 1961). Briefly, aliquots of supernatant were incubated at 30oC for 2 min with 0.1 M phosphate buffer, pH 7.5, 10 mM DTNB as chromogen. After two min, the reaction was initiated by the addition of acetylthiocholine (AcSCh; 0.5 mM) as substrate for the reaction mixture. The final volume was 2.0 ml. Absorbance were determined at 412 nm during two min. Enzyme activity was expressed as nanomoles of AcSCh hydrolyzed per minute.

The data was summarized using descriptive statistics. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-test. Values of $P < 0.05$ was considered as statistical significance. Statistical analysis was carried out using the SPSS (version 16.50) software.

Results

N-Ag treatments altered the activity of oxidative stress biomarkers. n-Ag increased the amount of MDA/LPO in a dose-dependent manner. In higher dose 7 ppm n-Ag treatment, it was 39.82% of that in controls (Table 1). GPx activity was also augmented in all concentrations of n-Ag particularly in higher dose in comparison to control ones. Table 1 shows that CAT activity was decreased in all n-Ag concentrations. Meanwhile, AChE activity was decreased markedly in the presence of n-Ag resulting in abnormal vigorous movements among animals (Table 1).

Table 1: Changes in oxidative stress biomarkers and AChE activities after 96 hrs following treatment with different concentrations of nanosilver (n-Ag).

		AChE	Oxidative stress biomarkers		
			LPO	CAT	GPx
Negative control		9.23± 3.05	11.05± 2.01	15.45± 3.92	2.14± 0.77
Nanosilver (ppm)	2.5	7.05± 1.05	13.25± 3.10	12.22± 2.72	3.74± 0. 84
	5	6.32± 0.89*	14.55± 4.14 *	11.75± 3. 02*	4.67± 1.02*
	7.5	6.08± 1.18*	15.45± 3.09 * #	11.02± 4.22 * #	5.04± 1.51* #

Datasaregivenasmean±SDforn= 15. , Values are statistically significant at $P < 0.05$.
*compared to negative control group. , #compared to 2.5 ppm n-Ag treated group.

Unit for lipid peroxidation (LPO) is nM MDA/mg protein.min
Unit for catalase (CAT) is μM/mg protein.min
Unit for glutathione peroxidase (GPx) is nM/mg protein.min
Unit for acetylcholinesterase(AChE) is nM/min

Discussion

Aquatic life is currently being exposed to chemical contamination by an increasing variety of anthropogenic. Many studies have been done on the effects of environmental pollutants on various aquatic organisms, and on biological assessment of water quality using model organisms. Among freshwater species, crustacean amphipods Gammarus spp. are suitable organisms for ecotoxicological assessment of environmental pollutants (Prato E, Biandolino,2005).

n-Ag-based materials have a wide range of applications including spectrally selective coating for solar energy absorption, catalysis in chemical reactions, surface-enhanced Raman scattering for imaging, and antimicrobial sterilization. Because of their effective antimicrobial properties and low toxicity in mammalian cells, n-Ag have become one of the most commonly used nanomaterials in development of new products. These nanoparticles have the potential to enter the wage

pipes and the wastewater treatment plants. At present, there is a limited knowledge about the adverse effects of n-Ag in the environment (Choi et al., 2008). Some studies report that different silver textiles release variant forms of silver during washing (Lorenz et al., 2012). Biomarkers are useful tools for toxicity assessment because their responses integrate spatial and temporal variations in environments, which modulate the exposure of organisms to contaminants. Oxidative stress is a general response to toxicity induced by contaminants. Exposure to many chemical substances result in induction or inhibition of oxidative defense system. In field studies, oxidative destruction and activation of antioxidant enzymes are usually observed (Halliwel and Gutteridge 1984; Alvarez et al., 1987).

In this work, we showed that the levels of oxidative stress biomarkers were altered with n-Ag treatments. The end point of LPO process is the thiobarbituric acid-reacted MDA as an index of LPO damages, which can cross-link between cell membrane phospholipids PS and PE, PS and PS, and, PE and PE. Lack of the uniformity to these cross-links in the membrane will led to a physical force, which may disturb the membrane lipid distributions (Alvarez et al., 1987). The reduction of membrane integrity and fluidity is a consequence of enhancement of LPO process. On the other hand, with commencing LPO cascade, a significant quantities of lipid hydroperoxides (lipid-O2H) will be released, which can be explained by the activity of membranous enzyme phospholipase A2 by cleaving oxidized PUFAs from the 2-position of phospholipid glycerol backbone. Therefore they can then be metabolized by GPx to the corresponding alcohols (Halliwel and Gutteridge, 1984). Our results indicated that high LPO levels could increase the level of GPx to encounter the LPO by-products due to generation of free radicals. On the other hand, CAT activity was decreased due to changes in the structure of CAT following n-Ag generated free radicals.

A large amount and great diversity of nanoparticles have been introduced into aquatic ecosystems. During recent decades, use of biomarkers in aquatic invertebrates as indicators of contamination has been extensively developed. Animal behavior is increasingly considered as an indicator of sub-lethal exposure to toxic contaminants. Locomotor activity is one of the most vulnerable variables (Xuereb et al., 2009).

In the present work, exposure to n-Ag contamination led to a marked increase in animal movement and a vigorous and abnormal locomotion. Measurement of AChE inhibition is widely used to assess the impact of different contaminants on the aquatic invertebrates. AChE is responsible for the hydrolytic degradation of acetylcholine, which is the primary neurotransmitter in the sensory and neuromuscular systems in most animal species. This enzyme plays a key role in regulation of cholinergic nervous transmission. AChE inhibition leads to overstimulation of the central and peripheral nervous system, resulting in deleterious effects for the organism, and ultimately death (Xuereb et al., 2009). Therefore, altered locomotion behavior in Gammarus spp. may be expected as a result of the n-Ag use.

In summary, our findings revealed that application of n-Ag in different forms in human life may induce a severe oxidative stress in aquatic organisms.

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