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Testing Toxicity and Antidote Effect of Selenium Nanoparticles with *Paramecium caudatum*

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Abstract

A simple method for assessment of the toxicity and antidote effect of selenium nanoparticles with *Paramecium caudatum* is presented. Light microscopy in combination with computerized video tracking is employed for the determination of the survival time of *P. caudatum*. Up to 800 mg/L, selenium nanoparticles are not acutely toxic. With respect to a potential antidote effect, the lethality of silver nanoparticles, silver nitrate, sodium hydrogen selenite, and sodium selenite to *P. caudatum* was decreased and survival time was extended upon pre-treatment with selenium nanoparticles. Taken together, these findings suggest that administration of selenium nanoparticles attenuates exposure to toxicants. Selenium nanoparticles could be a good functional additive for food management in animals.

Keywords

Selenium Nanoparticles, Toxicity, Antidote Effect, *Paramecium caudatum*

1. Introduction

Protozoan cells are often used as bioindicators of chemical pollution and their toxicity in an aqueous environment [1]. *Paramecium caudatum* is one of the widely used ciliate models as bioindicators for scientific research. Komala reported the acute toxicity test of *P. caudatum* is highly sensitive to investigate the direct toxicity of chemical compounds [2]. Basically, *Paramecium* simplifies the study of physiological processes and effects of water pollutants such as mineral oil, pesticides, metals, and others by monitoring locomotor behavior, morphol-

ogy and mortality [3] [4] [5] [6] [7]. A sensitive measure of stress by water contamination [8] is locomotion, depending on the movement of the cilia mostly controlled by the action potential of the cell membrane [9] [10]. In this study, the antidote effect of selenium nanoparticles on *P. caudatum* is evaluated in a simple fashion.

Generally, selenium is a functional microelement that is involved in many physiological functions, and it has several therapeutic effects by participating in the basic composition of some enzymes and amino acids. Earlier in our experiments, we investigated how probiotic yogurt bacteria transform the inorganic selenium compounds into organic compounds. It has been found that certain bacteria have been defending against the toxicity of selenite ion; elemental selenium was produced within the cell and stored as small, nano-sized spheres (SeNPs). During the fermentation, the transparent nutrient solution becomes red in the selenium nanoparticles produced by the proliferating bacteria. The nanoparticles formed in the bacterium can be recovered and used after purification of the cell wall after purification. The spheres produced measured 100 to 500 nanometers depending on the bacterial species. With animal studies with sheep, chicken and fish, and human studies, it was proved that this selenium form has a significantly better antioxidant effect than other selenium compounds; it cannot be overdosed and is the least toxic form of selenium [11] [12] [13]. Selenium has long been known for its ability to reduce the harmful effects of metals [14] [15]. For example, Hao *et al.* reported that selenium supplement prevented abnormal changes in the levels of reduced glutathione, mitochondrial membrane potential, and Ca^{2+}-ATPase activity of chromium (VI)-induced damage in chicken brain [16]. Also, selenium is able to alleviate the symptoms of mercury toxicity in cell culture [17], fish [18], and adult mice [19]. Selenium supplementation reduces hepatic oxidative stress induced by silver nanoparticles (AgNP) in rats [20]. Besides, selenium nanoparticles protect against As(III)-induced cell death and DNA damage by reducing the production of As(III)-induced reactive oxygen species [21]. Recently, there has been an increasing ecological and global public health concern associated with environmental contamination by some metals. Therefore, the studies of antidote substances are increasing in scientific research. Based on the information mentioned above, the authors focused on a simple and rapid method to determine the antidote effect of biosynthesized selenium nanoparticles.

2. Materials and Methods

Selenium nanoparticles were produced according to Eszenyi *et al.* and Prokisch and Zommara [22] [23]. In the preparation, 20 mL of 10,000 mg/L sodium selenite stock solution was added into 980 mL MRS broth, then 10 mL of *L. acidophilus* culture was added, and the mixture was incubated at 37˚C for 24 - 36 h. The culture was centrifuged at 6000 rpm for 15 min, and the pellets were washed with water. Finally, cells were lysed by hydrochloric acid 37% (m/m) for 5 days

at room temperature. The mixture was centrifuged at 6000 rpm for 15 min, washed with water and filtered by vacuum filtration. After purification, the suspension of selenium nanoparticles contained 800 mg/kg selenium in the form of 250 nm size red elemental selenium (Figure 1).

Stock cultures of *P. caudatum* were cultured in tap water supplemented with yogurt powder (1 g/L). Yogurt was made by starter culture (Lyofast Y 250) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* were obtained from SACCO Srl (Italy). Fresh cultures were initiated by seeding 100 ml of freshwater with 1 mL of a stationary phase paramecium culture. The cultures were maintained at room temperature for 15 - 30 days.

2.1. Determination of Toxic Level of Selenium Nanoparticles and Toxicants

A solution of silver nanoparticles with a concentration of 20 mg/L and particle size of 10 - 20 nm was purchased from Dr. Juice (Miskolc, Hungary). Silver nitrate ($AgNO_3$) was obtained from Reanal Laborvegyszer. (Budapest, Hungary). Sodium hydrogen selenite ($NaHSeO_3$) was obtained from VWR International (Lutterworth, Leics, UK) and sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$) were obtained from Scharlau Chemie (Barcelona, Spain).

Stock solutions of 20 mg/L of silver nanoparticles and silver nitrate, 80.0 mg/L of sodium hydrogen selenite, and sodium selenate decahydrate were prepared and then diluted in serial dilutions (until 10^{-10}). 20 µL fresh culture of *P. caudatum* was exposed with 20 µL solutions on the glass slide in control groups. LC95 values of every toxicant were determined. Surviving time and the effect of concentration are applied to calculate the LC50 concentration values. The NOEL (No Observed Effect Level) concentration was defined as the concentration when the surviving is longer than 20 min.

2.2. Determination of Antidote Effects of Selenium Nanoparticles

500 µL fresh culture of *P. caudatum* was treated with 500 µL selenium nanoparticles (800 mg/L) at room temperature for 2 h in experimental groups. At the end of the treatment, 20 µL of the treated culture was placed on the middle of

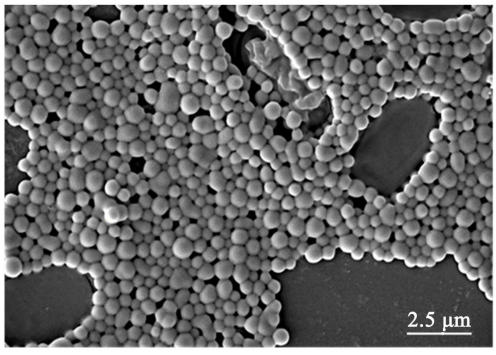

Figure 1. The scanning electron microscopic (SEM) image of applied selenium nanoparticles.

the glass slide, and while observing under a microscope 20 μL toxicants were added. Then, the survival time, locomotor behavior, and morphological changes of *P. caudatum* are continuously observed under the microscope for 20 min. After more than 20 min, the solution on the glass slide became dry out and the cells were started to die. The location of selenium nanoparticles inside the cell is described by light microscopy attached to a CCD camera, and scanning electron microscope (Hitachi S-4300).

3. Results and Discussion

3.1. The Toxic Level of Selenium Nanoparticles and Toxicants

The concentration of selenium nanoparticles higher than 800 mg/L was shown to exert no effects on locomotion and morphology of *P. caudatum*. Within 5 min of treatment, selenium nanoparticles started to be accumulated inside the cells. Figure 2 shows the black spots are inside *P. caudatum* which are red with a proper illumination (white light from the side). Also, scanning electron microscopic image with X-ray mapping shows the location of selenium nanoparticles in the cell (Figure 3). These results indicated no morphological changes in *P. caudatum*.

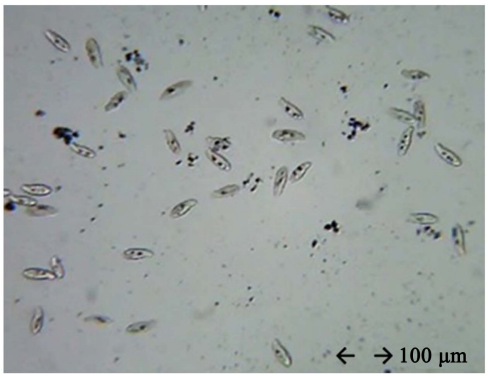

Figure 2. Light microscopic image of *P. caudatum* treated with selenium nanoparticles (800 mg/L).

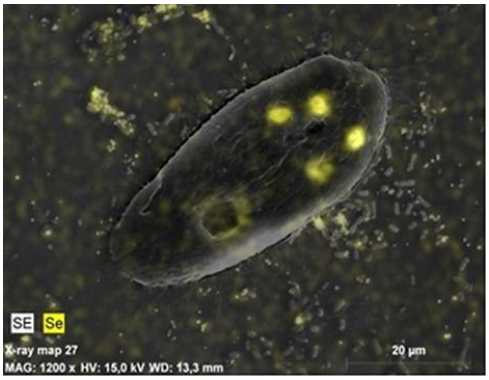

Figure 3. Scanning electron microscopic (SEM) image of *P. caudatum* treated with selenium nanoparticles. The X-ray fluorescent mapping shows the location of selenium in the pictures. The selenium spheres and *Lactobacillus* cells are visible as well.

For toxicants, results showed that ≥1.25 mg/L of silver nanoparticles, ≥2.5 mg/L of silver nitrate, ≥10.0 mg/L of sodium hydrogen selenite, and ≥20.0 mg/L of sodium selenate have a lethal effect on *P. caudatum* in the control groups as presented in **Figure 4**. The survival time vs. concentrations has a special shape, which is similar to the pH titration curve. Its inflection point gives the LC50 value. All *P. caudatum* died within 18 min due to cell lysis. In the present experiments, we observed that these toxicants affected the test organism, *P. caudatum* in a concentration-dependent manner.

Silver especially AgNPs are known for their antibacterial effect and are used in various nanomedicine and biomedical applications. However, there is increasing concern related to the biological impact of the use of AgNPs on a large scale, and the possible toxicity to the environment and health [20]. Several works show that silver nanoparticles exert an inhibitory effect in all biological systems such as the virus [24], bacteria [25], protozoa [26], algae [27], and fungi [28] regardless of their structural or physiological characteristics, at the cellular level. For instance, 30 - 50 μg/L of silver nanoparticles have a rapid toxic effect on *Paramecium* within 15 min [29]. In other words, AgNPs have also the capacity to kill cells with different levels of complexity, both prokaryotic and eukaryotic. Most of the reports show an effective concentration near 0.1 mg/L of silver [27] [28]. It is classified that sodium selenite is highly toxic, and sodium selenate is moderately toxic to fingerlings of tilapia [30]. Generally, selenium bioavailability depends on their

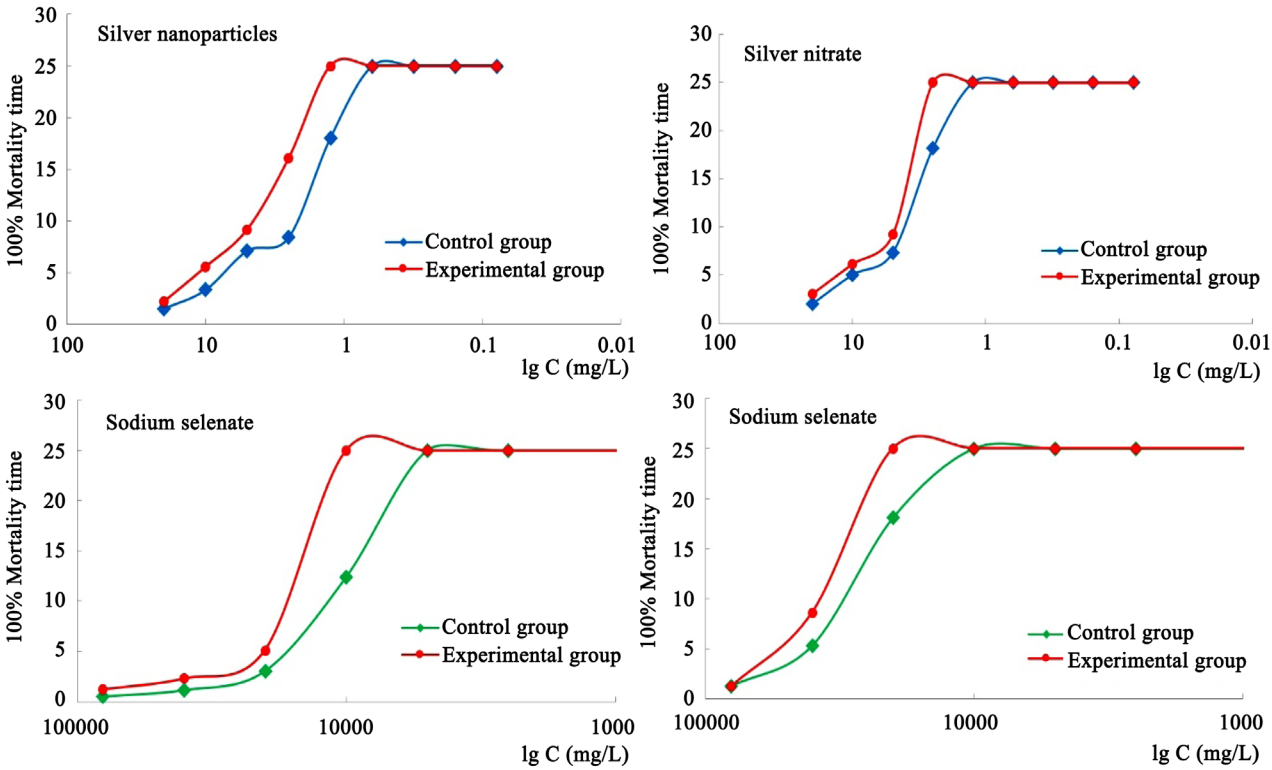

Figure 4. The survival time of *P. caudatum* and the concentration of toxicants on a logarithmic scale. Experimental groups were pre-treated with selenium nanoparticles, and control groups were not treated.

size, chemical type, and dose in the source. The elemental selenium nanoparticles have very good bioavailability as indicated by enhancement of serum antioxidant status and selenium concentration in blood and tissues [31], and much lower toxicity as indicated by median lethal dose, acute liver injury, survival rate, and short-term toxicity [32] compared to other chemical types.

3.2. The Antidote Effects of Selenium Nanoparticles

The lethal concentration of toxicants was significantly decreased by 2 times in the experimental groups which were pre-treated with selenium nanoparticles (800 mg/L). Namely, the lethal concentrations were found as ≥2.5 mg/L, ≥5.0 mg/L, ≥20.0 mg/L, and ≥40.0 mg/L for silver nanoparticles, silver nitrate, sodium hydrogen selenite, and sodium selenate, respectively (**Figure 4**). There were no changes in locomotion and morphology of *P. caudatum* in the experimental groups under the exposure of previously determined lethal concentrations of the toxicants. Particularly, *P. caudatum* did not exhibit any alteration in their shape, blebbing, lysis, and swimming. And their survival time was extended by the administration of selenium nanoparticles (**Figure 4**). The changes in the experimental group are compared with the control groups in **Figure 4**. These results are theoretically consistent with the results of other research methods. For instance, bulk selenium (selenite) treatment in a mouse model was more toxic than selenium nanoparticles in terms of deleterious effects on mice growth, liver functions, and hepatic lipid peroxidation [33], and the selenium nanoparticles dramatically reduce the death incurred by acute toxicity associated with bulk selenium up to 4 times in a rodent model [34]. Also, selenium nanoparticles protect against As(III)-induced cell death and DNA damage [21], and supplementation of selenium with lead reduced the lead level in serum [19]. Selenium antagonizes the toxicity of arsenic and cadmium mainly through sequestration of these elements into biologically inert complexes and/or through the action of selenium-dependent antioxidant enzymes [35]. Besides, Ansar *et al*., reported selenium supplementation reduces the AgNP-induced hepatotoxicity in rats [20]. Basically, the toxicity of AgNPs is explained due to the release of Ag ions in the system or extensive systemic distribution of Ag in tissues causing oxidative stress, protein or DNA damage, and apoptotic cell death [36] [37] [38]. Selenium restores the activity of important antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase [39]. Therefore, the antidote effect of selenium nanoparticles can be explained by the restoration of target selenoprotein activity and restoring the intracellular redox environment. Basically, impairment of the thioredoxin and glutaredoxin systems allows for proliferation of cytosol and mitochondrial reactive oxygen and nitrogen species which lead to mitochondrial injury/loss, lipid peroxidation, calcium dyshomeostasis, impairment of protein repair, and apoptosis [40]. For instance, mercury binds to the selenocysteine binding site of thioredoxin reductase 1 in the cytosol and thioredoxin reductase 2 in the mitochondria dramatically inhibiting their function [41]. Basically, metals have a lower affinity for thiol groups and a higher affinity for sele-

nium containing groups. In this case, selenium supplementation, with limitations, may have a beneficial role in restoring adequate selenium status and mitigating the toxicity of mercury [42].

Different changes in the locomotion and morphology of *P. caudatum* were observed for the exposure of the silver and bulk selenium during the experiment. *P. caudatum* affected by silver mainly showed alteration of their shape by developing irregular blebbing of the cell membrane before cell lysis. There were occurred single or multiple blebs in the cell membrane. The process of blebbing is a common phenomenon during apoptosis. At the same time, fragmentation and disintegration of macronucleus were also observed with increased time of exposure. These morphological changes in the experimental group lasted longer than the control group. Similar kind of changes on the cell membrane of *P. caudatum* was observed with xenobiotics, monocroptophos (>90 mg/L), and fenthion in *P. caudatum*. The complete cell lysis occurred within 17 min on average ranging from 5 to 20 min [6] [43] [44].

The effect of bulk selenium mainly showed the locomotion changes. Particularly, due to the high dose, the swimming of *P. caudatum* stopped completely, but his ciliates kept moving until cell lysis. As the concentrations decreased, sequential changes such as the swimming speed and changes (circular movement), immobile, and cell lysis were observed. In other words, *P. caudatum* died after they became immobile. Gradual decrease in the swimming speed, with the increased time of exposure, is due to the effect of the toxicants on the cellular metabolism and morphological changes. Similar changes like reduction in the swimming speeds, altered morphology, and generation times were observed when *P. caudatum* was exposed to a higher concentration (>350 mg/L) of acephate [7] [44].

4. Conclusion

Through our study, we found *P. caudatum* is a suitable and practical microorganism for testing toxicological research. The locomotion, morphology, and death of *P. caudatum* are easily detectable under a microscope. We proved that biosynthesized selenium nanoparticles are antidotes against silver and bulk selenium induced toxicity, the *P. caudatum* can tolerate 2 times higher concentrations of tested toxicants if they were pre-treated with selenium nanoparticles. The developed method is simple, rapid, and economical. This method will be suitable to test the acute toxicity of toxic metals, metalloid compounds, mycotoxins, and insecticides also.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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