Iranian Journal of Pharmaceutical Sciences
2020: 16 (2): 57-68
www.ijps.ir

Original Article

Selective Toxicity of Ag/Tio$_2$ Nanoparticles of Waste Water of Industrial Factories on Muscle Mitochondria Isolated from *Solendactylus* Scallop

Farahnaz Tanbakosazan[a], Pirouz Derakhshi[a], Parviz Abroomand Azar[b], Khadijeh Ashtari[c,d,e], Parvaneh Naserzadeh[f], Enayatollah Seydi[*g,h], Jalal Pourahmad[f*]

[a]Department of Chemistry, Faculty of Science, Islamic Azad University, Science and Research Branch, Tehran, Iran, [b]Chemical Faculty, Islamic Azad University, Tehran North Branch, Tehran, Iran, [c]Radiation Biology Research Center, Iran University of Medical Sciences, Tehran, Iran, [d]Department of Medical Nanotechnology, Faculty of advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran, [e]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran, [f]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [g] Department of Occupational Health and Safety Engineering, School of Health, Alborz University of Medical Sciences, Karaj, Iran, [h]Research Center for Health, Safety and Environment, Alborz University of Medical Sciences, Karaj, Iran.

Abstract

Industrial wastewater is of global concern due to its severe effects on the environment. Compared with municipal wastewater, industrial wastewater generally contains the high concentration of toxic or no biodegradable pollutants. In recently year, scientific showed that scallop could filtration wastewater. Therefore, it was decided to determine the mechanistic toxicity of wastewater contained NPs (Ag and TiO_2) towards isolated mitochondria via reliable methods. Isolated muscle scallop mitochondria were obtained by differential ultracentrifugation on before and after exposure to wastewater. Our results showed that two NPs (Ag and TiO_2) induced mitochondrial dysfunction via an increase in mitochondrial reactive oxygen species (ROS) generation, lipid peroxidation (LPO) and mitochondrial membrane potential (MMP) collapse. Finally, Ag-NPs and TiO_2-NPs have reduced the level of glutathione (GSH) and also induced apoptosis. Our results suggest that wastewater contained NPs -induced toxicity is the result of a disruptive effect on the mitochondrial respiratory chain, increasing the chance of cell death signaling.

Keywords: Stress Oxidative; Isolation Mitochondria; Scallop; Wastewater; ROS; Ag/ TiO_2-NPs**.**

1. Introduction

The volume of industrial wastewaters is increasing due to economic growth. Furthermore, Industrial wastewaters are a complex, and are considered as one of the major health concerns and threats [1-3]. It has

Corresponding Authors: Jalal Pourahmad, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Tel: +98 (21) 2255-8786
E-Mail: j.pourahmadjaktaji@utoronto.ca
Cite this article as: Tanbakosazan F, Derakhshi P, Abroomand Azar P, Ashtari K, Naserzadeh P, Seydi E, Pourahmad J, Selective Toxicity of Ag/Tio2 Nanoparticles of Waste Water of Industrial Factories on Muscle Mitochondria Isolated from Solendactylus Scallop, 2020, 16 (2): 57-68.

been shown that industrial wastewater has higher levels of toxic contaminants compared to municipal waste [4]. Fats and heavy metals and also oil are among the most important industrial wastewater [4, 5]. Many techniques have been proposed for treatment of industrial wastewater. One of the most important techniques is the use of nanoparticles (NPs). In recent years, NPs have attracted much attention in various fields. One of these applications is the treatment of wastewater, which has attracted many researchers. TiO_2 and Ag are NPs that are used in sewage treatment [6-8]. The advantage of using NPs in wastewater treatment is reactivity and very high surface contact level, and also better removal ability [9]. Despite the use of nanoparticles in sewage treatment, comprehensive toxicological studies are not available in relation to their toxicity.

Scallop is a group of marine animals found in the sea and in the oceans around the world. Studies have shown that scallop is used for treatment of wastewater [10-12]. It is reported that the scallop have been used to remove phosphate. Also, they are considered as low-cost alternative adsorbent for dyes, this indicates the importance of using scallop[11]. Therefore, more research is needed on the snails. In addition, the effects of NPs on the snails have not been studied. One of the most important mechanisms by which NPs cause damage to living organisms is through the generation of free radicals (such as reactive oxygen species; ROS) [13, 14]. By using *in vivo* and *in vitro* studies, the toxicity of nanoparticles can be thoroughly investigated [15]. It has been shown that marine organisms are susceptible and vulnerable to excessive ROS generation and oxidative stress caused by environmental pollutants [16]. It is reported that Complexes I and III in the mitochondrial respiratory chain (MRC) are considered as main generating sources of ROS [17].

The mechanism of the effect of industrial wastewater on aquatic organisms such as scallop has not been well studied. Accordingly, we determine the accumulation of TiO_2-NPs and Ag-NPs on scallop tissue(i), the toxicity effect of TiO_2-NPs and Ag-NPs on scallop mitochondria (ii); and evaluate the effect of TiO_2-NPs and Ag-NPs on oxidative stress (through ROS, LPO, GSH levels assay) and mitochondrial damage (MMP collapse assay) (iii); and finally evaluate apoptosis signaling (IV).

2. Materials and Methods

2.1. Chemicals

All of the materials were purchased from Sigma-Aldrich Co. (Taufkirchen, Germany). And Chemicals were of the highest commercial grade available.

2.2. Animals

We collected samples of Solendactylus [S. dactylus] on Bandar Abbas in the Persian Gulf (n=10). The samples were kept in seawater of the sampling station for 24h finally transfers in laboratory. (Classification/Names: Bivalvia | Veneroida | Solenidae; Environment: Benthic. Tropical Distribution**:** Western Indian Ocean: Kuwait, Persian Gulf).

2.3. Simulation of Water Industrial Factories Contains Ag/Tio_2

In the first step, we collect wastewater, and determined concentration of TiO_2 and Ag NPs. Trace metals in the water samples were determined using ICP-MS. The water sample was introduced, after filtration and acidification, into ICP-MS instrument by conventional pneumatic nebulization, using a peristaltic pump with a solution uptake rate of about 1 ml/min. The nebulizer gas flow, sample uptake rate, detector voltages and lens voltage were optimized for a sensitivity of about 50,000 counts/s for 1 ng/ml solution of Indium. In the second step, we collected S. dactylus with seawater samples from the sea-surface at <1m depth. In the third step, we give information of TiO_2 and Ag concentration of industry waste water thus simulated new system for TiO_2 and Ag that same natural industry waste water. We designed the tank dimensions, 1× 0.5×0.5; we enter 1 m^3 volume of water with same natural industry concentration (the water contained just TiO_2 and Ag concentration).

2.4. Analysis of Ag/Tio_2 Content

Muscle of scallop were thawed and about (spiked values 0.07g respectively) of each tissue were weighed, digested and analyzed for Ag/TiO_2 content using A 10 mg L^{-1} (Agilent Technologies, Courtaboeuf, France) elemental analysis. For that, tissue of interest was digested in ultrapure nitric acid overnight. Then 0-3 ml of H_2O_2 (H_2O_2, 30% (v/v) was added to the solutions and heated at about 25 ºC in a high-pressure reaction container in an oven chamber until the samples were completely digested. Finally, the remaining solutions were diluted to with 3 mL of nitric acid (HNO3, 67% (v/v). Weir 2012 was used to analyze the Ag/TiO2 concentration in the samples. The detection limit of Ag/TiO2 was 0.1 µg/ml. Data are expressed as nano grams per gram fresh tissue.

2.5. Preparation of Mitochondria

About 3g of muscle tissue were finely chopped in four to five times the volume of homogenization buffer. The minced tissues were homogenized with a glass homogenizer with a Teflon pestle and then centrifuged at 3000×g for 20 min at 4 ºC to remove nuclei, unbroken cells, and other non-subcellular debris. The supernatants were centrifuged at 15,000×*g* for 45 min. The dark packed lower layer (mitochondrial fraction) was re-suspended in the media culture and re-centrifuged twice at 15,000×*g* for 45 min. Mitochondrial sediments were suspended in media culture at 4 ºC before the assay. Aliquots of the suspension were used to determine parameters of oxidative stress.

2.6. Protein Concentration

Mitochondrial protein concentration was determined by the Coomassie blue protein-binding method using bovine serum albumin (BSA) as the standard [18].

2.7. Estimation of Complex II Activity

The activity of mitochondrial complex II (succinate dehydrogenase) was assayed through the measurement of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction and absorbance at 570 nm was measured with an ELISA reader (Tecan, Rainbow Thermo, Austria [19].

2.8. Quantification of Mitochondrial ROS Level

Isolated mitochondria were incubated in media culture of DMEM. a sample was taken and 2, 7-dichlorofluorescin diacetate (DCFH-DA) was added (final concentration, 10 mM) to the mitochondria and was then incubated for 15 min. toxic materials-induced ROS generation in isolated mitochondria was determined through the fluorescence and light scattering were analyzed for at least 10000 counts per sample in the flow cytometry using a BD Biosciences FACS Calibure TM flow cytometer. Samples were gated on the forward/side scatter to exclude cell debris and clumps. A flow cytometer with the Flowing software-2-5-1, equipped with a 488 nm argon ion laser, was used and fluorescence signals were obtained using a 530 nm band pass filter (FL-1 channel) [20].

2.9. Mitochondrial Membrane Potential (MMP) Assay

The mitochondrial uptake of the cationic fluorescent dye, rhodamine 123, was used for determination of mitochondrial membrane potential (MMP). The mitochondrial suspensions (500 mg protein/mL) were incubated and then 10 mM of rhodamine 123 was added to the mitochondrial solution in the media culture. Fluorescence and light scattering were analyzed for at least 10000 counts per sample in the flow cytometry using a BD Biosciences FACS Calibure TM flow cytometer. Samples were gated on the forward/side scatter to exclude cell debris and clumps. A flow cytometer with the Flowing software-2-5-1, equipped with a 488 nm argon ion laser, was used and fluorescence signals were obtained using a 530 nm band pass filter (FL-1 channel) [20].

2.10. Measurement of GSH Content

The mitochondrial fractions (500 mg protein/ml) were incubated for 1 hr at 30 ºC and then 0.1 mL mitochondrial fractions was added into 0.1 mol/l phosphate buffer and 0.04% DTNB in a total volume of 3 mL (pH ≈ 7.4). The developed yellow color was read at 412 nm using a spectrophotometer (UV-1601 PC, Shimadzu, Japan). Reduced glutathione (GSH) content was expressed as mg/mg protein [21].

2.11. Determination of Lipid Peroxidation

The content of the lipid peroxidation marker (malondialdehyde, MDA) was determined using the method of Zhang *et al*.

The amount of MDA formed in each of the samples was assessed by measuring the absorbance of the supernatant at 532 nm with an ELIZA reader (Tecan, Rainbow Thermo, Austria). Tetra methoxy propane (TEP) was used as standard and MDA content was expressed as nmol/mg protein [22].

2.12. Determination of Apoptosis and Necrosis

The percentage of apoptosis versus necrosis from tissue in duration 2 h, were measured. In the early stages of apoptosis phosphatidylserine (PS) is translocated from the inner side of the plasma membrane to the outer layer. Annexin V is a calcium-dependent phospholipid binding protein with a high affinity for PS. Therefore, be used as a sensitive probe for the exposure of PS on the cell membrane and hence as a marker of apoptosis. Briefly, double-staining by Fluorescein isothiocyanate (FITC)-Annexin V/propidium iodide (PI) was performed. After washing twice times in PBS, the tissue was re-suspended in binding buffer. FITC-Annexin V was added to a final concentration of 5 µM and the cells were incubated in the dark for 10 min. The cells were then washed again in PBS, centrifuged at 300 g and re-suspended in binding buffer. Before flow cytometric analysis10 µM PI in binding buffer was added to each sample. The fluorescence signals of Annexin V and PI were measured by flow cytometry on the FL1 and FL3 channels.

2.13. Statically Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD as the post hoc test. Results were presented as mean ± S.D. of triplicate samples. The minimal level of significance chosen was $p<0.001$.

3. Results and Discussion

3.1. Results

3.1.1. Determination of Ag /TiO_2

Finally, the content of Ag /TiO_2NPs in

Table 1. ICP assay after exposure of TiO_2 and Ag NPs. Values are mean ± SD. $^{***}P < 0.001$significantly different from respective the Scallop before groups.

TiO_2 and Ag concentration in scallop muscle		
Tissue	**Concentration of TiO_2 (µg/g)**	**Concentration of Ag (µg/g)**
Scallop before	1.2±0.2	1.32±0.03
Scallop after	3.15±0.21***	3.28±0.023***

Table 2. GSH content and mitochondrial isolated mitochondria. Isolated mitochondria (0.5 mg/mL) were incubated for 1 h. Values represented as mean ± SD (n = 3). $^{*}P < 0.05$ compared with control mitochondria.

Groups	**GSH (15 min)**
Control	1564.3±77.9
TiO_2-NPs	1441±19.21*
Ag-NPs	1486±13.81*

waste water in the muscle scallop was measured 10 days pre and post-exposure to the nanoparticles are listed in Table 1. In the experimental groups, the Ag /TiO_2were mainly accumulated in muscle scallop (Table 1).

3.1.2. Succinate Dehydrogenase (SDH) Activity

The measure for mitochondrial functionality was also assessed using the MTT test after 1 h incubation of mitochondria obtained from scallop. Figure 1 illustrates a significant decrease in mitochondrial metabolic conversion of MTT to formazan following the incubation of mitochondria from scallop.

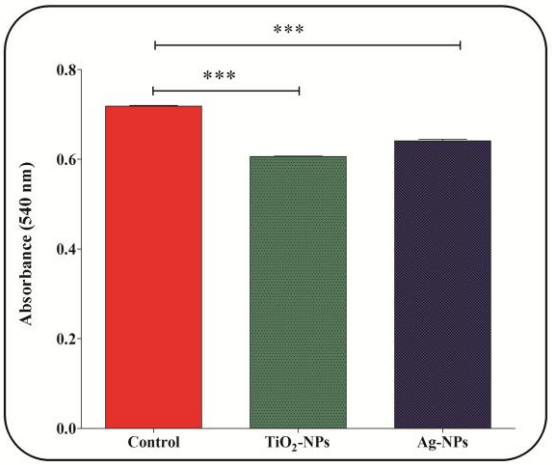


Figure 1. Succinate dehydrogenase (SDH) activity was measured using MTT dye as described in Materials and methods. Isolated mitochondria (0.5 mg/ml) were incubated for 1 h. Values represented as mean ± SD (n = 3). *** $P < 0.05$ compared with control mitochondria.

3.1.3. Mitochondrial ROS Production

As shown in figure 2, TiO_2-NPs and Ag-NPs induced significant ROS formation in scallop mitochondria. As demonstrated in figure 2, TiO_2-NPs and Ag-NPs induced increased DCF fluorescence intensity (H_2O_2 production) as the DCF peak shifted rightward on the x-axis.

3.1.4. Mitochondrial Membrane Potential (MMP)

As shown in figure 3, MMP significantly decreased in all mitochondrial test groups (exposed to TiO_2-NPs and Ag-NPs) in a concentration- and time-dependent manner.

3.1.5. Mitochondrial Glutathione (GSH) Level

As presented in table 2, GSH levels was decreased after incubation of scallop mitochondria with TiO_2-NPs and Ag-NPs compared to control.

3.1.6. Mitochondrial Lipid Peroxidation (LPO) Level

As shown in figure 4, LPO level increased due to the exposed of TiO_2-NPs and Ag-NPs treated group, (Figure 4). Furthermore, we found a notable positive correlation between ROS and LPO levels.

3.1.7. Apoptosis Assay

To determine if apoptosis is involved in the cytotoxic effects of TiO_2-NPs and Ag-NPs, apoptosis was determined by annexin V/PI staining by flowcytometry. Our results showed that TiO_2-NPs and Ag-NPs induced apoptosis in mitochondria obtained from the scallop (Figure 5).

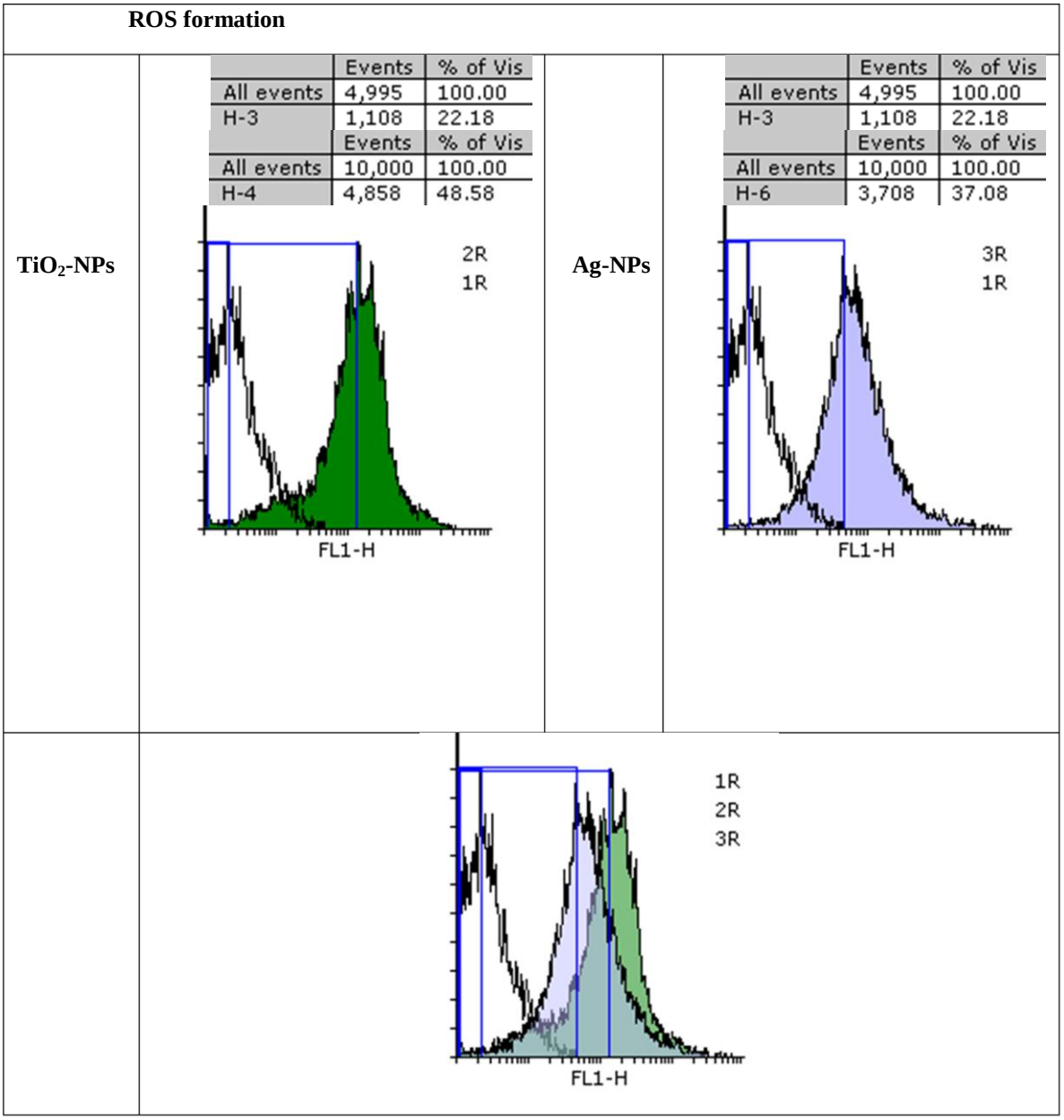


Figure 2. ROS formation in scallop mitochondria. ROS formation was evaluated after 15 min incubation. ROS formation was determined by flowcytometry using DCFH-DA as described in the "Materials and methods" section. FL1: the fluorescence intensity of DCF.

3.2. Discussion

Research has shown that the volume of sewage from the industries has been rising and is considered one of the important environmental problems. Industries wastewater contains a variety of compounds [23, 24]. Heavy metals are one of the most important compounds that have caused many concerns due to the accumulation of living organisms [25]. In recent years, the use of scallop as increased as marine species. Scallop are among the marine compounds used to treatment of wastewater [26]. Until now, the mechanism of the effect of industrial wastewater and NPs (for wastewater treatment, and remove several pollutants in

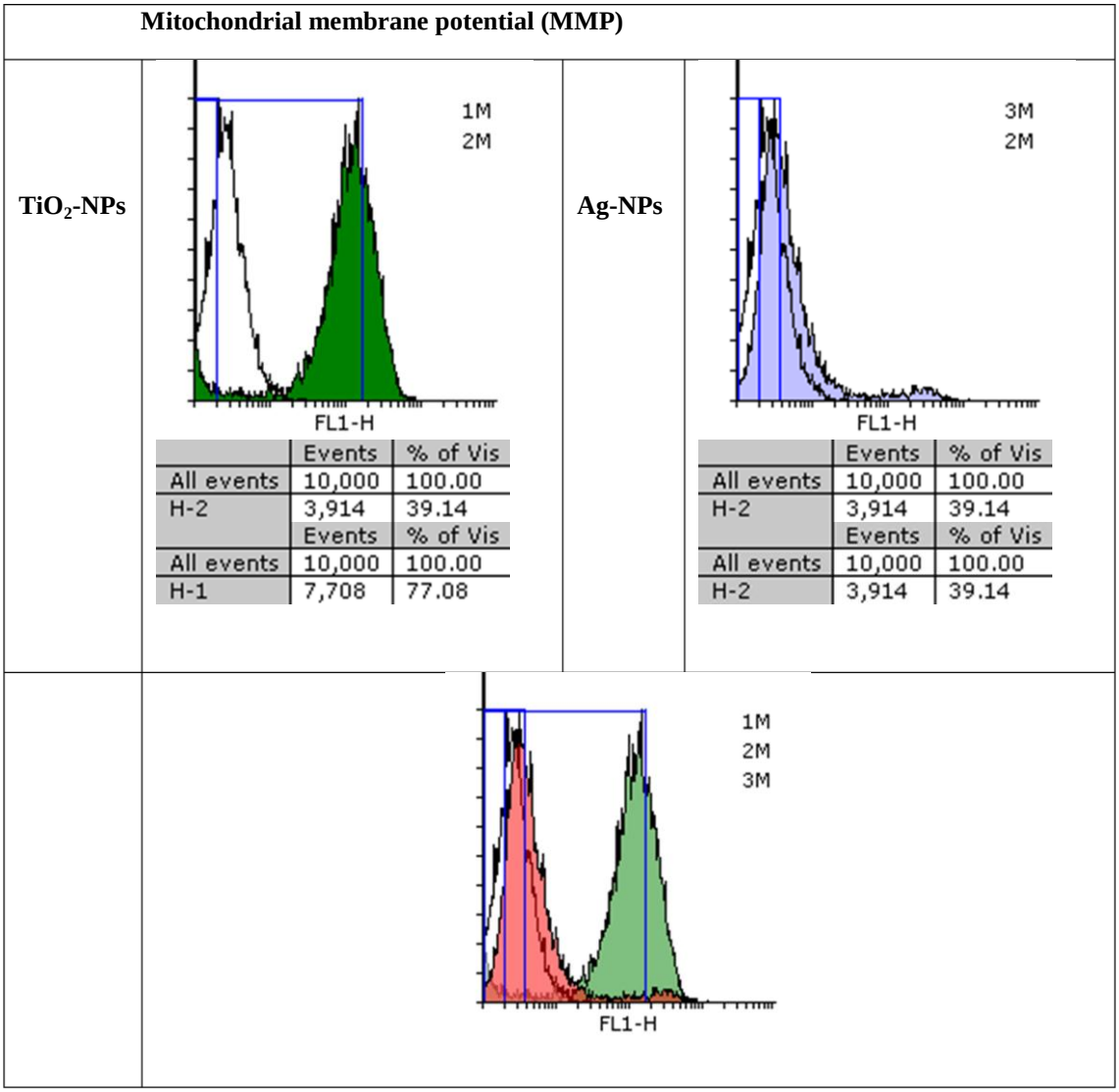


Figure 3. Mitochondrial membrane potential (MMP) collapse was measured by rhodamine 123 as described in the Materials and methods section.

water)on aquatic organisms such as scallop has not been well considered. Therefore, the aims of the present study were to: (i) determine the accumulation of TiO_2-NPs and Ag-NPs on scallop tissue; (ii) determine the toxicity effect of TiO_2-NPs and Ag-NPs on scallop mitochondria; (iii) evaluate the effect of TiO_2-NPs and Ag-NPs on oxidative stress (through ROS, LPO, GSH levels assay) and mitochondrial damage (MMP collapse assay); and (IV) evaluate apoptosis signaling.

At first, the results of ICP assay showed that TiO_2-NPs and Ag-NPs have the ability to accumulate in the muscle scallop. These results are in agreement with previous studies showing that different nanoparticles have accumulation capability in the tissues [15, 27, 28]. In the following, the results showed a significant reduction in mitochondrial complex II activity in TiO_2-NPs and Ag-NPs groups compared to control group. Previous studies have shown that NPs through oxidative stress (OS) are able to increase the generation of

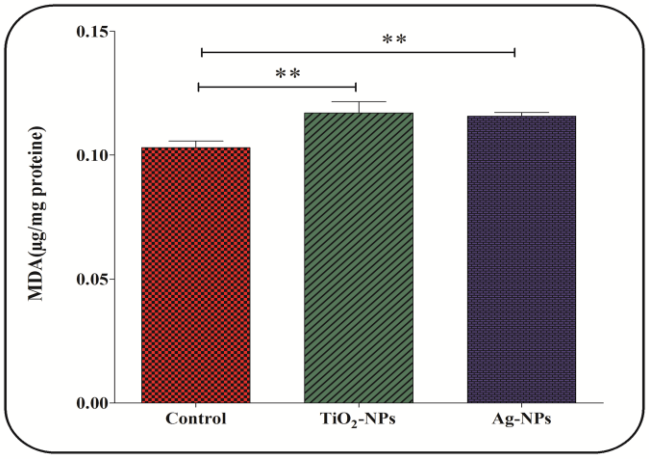
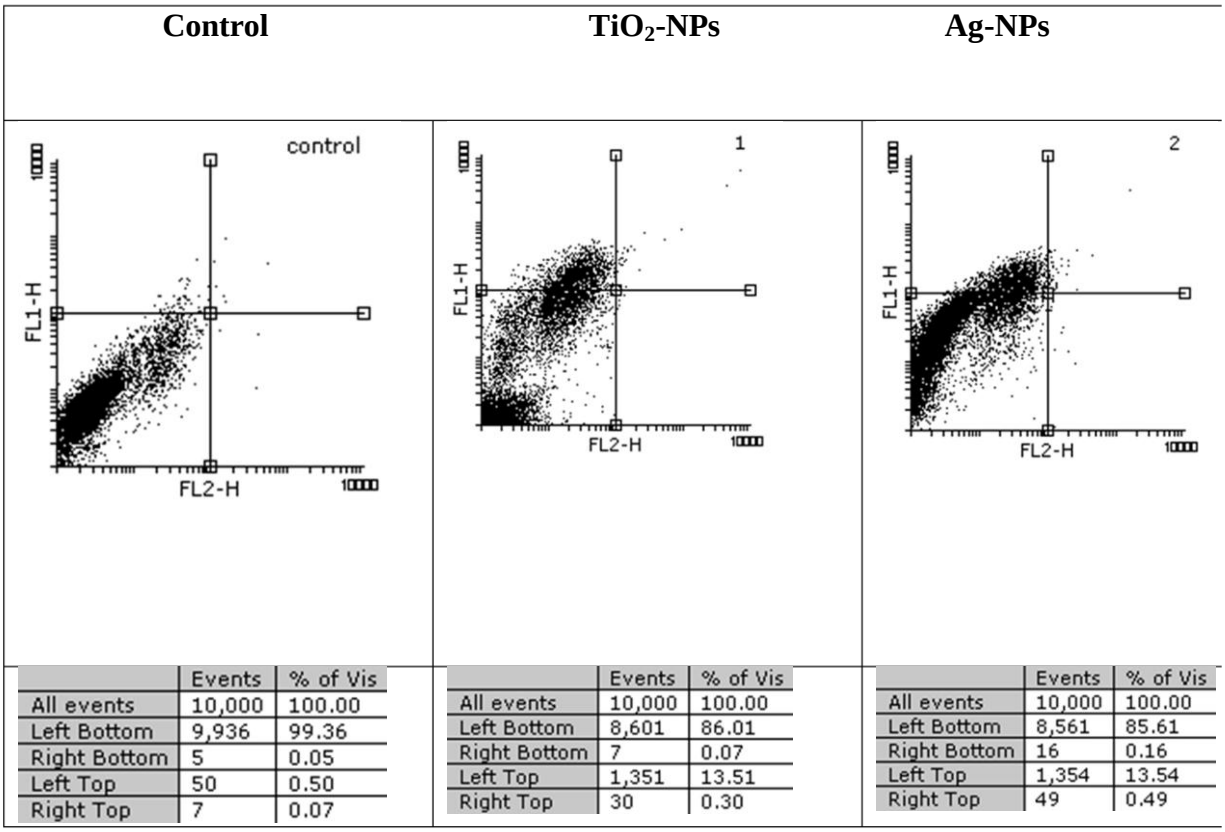


Figure 4. Lipid peroxidation (LPO) and mitochondrial isolated mitochondria. Isolated mitochondria (0.5 mg/mL) were incubated for 1 h. Values represented as mean ± SD (n = 3). $^{**}P < 0.01$ compared with control mitochondria.



	Events	% of Vis
All events	10,000	100.00
Left Bottom	9,936	99.36
Right Bottom	5	0.05
Left Top	50	0.50
Right Top	7	0.07

	Events	% of Vis
All events	10,000	100.00
Left Bottom	8,601	86.01
Right Bottom	7	0.07
Left Top	1,351	13.51
Right Top	30	0.30

	Events	% of Vis
All events	10,000	100.00
Left Bottom	8,561	85.61
Right Bottom	16	0.16
Left Top	1,354	13.54
Right Top	49	0.49

Figure 5. Apoptosis and Necrosis.

ROS. ROS play different roles at different levels [15, 29, 30]. Furthermore, metallic-NPs exhibit toxicity through ROS generation [29]. Additionally, flowcytometry assay with DCFH-DA probe shows the increase level of ROS in scallop mitochondria. ROS are generated during the production of ATP through the MRC and an imbalance between oxidant and antioxidant systems. Complexes I and III in MRC are considered main

generating sources of ROS [17]. Additionally, ROS and lipid hydroperoxides as a oxidant agents play a role in the induction of cell death via the OS [31]. Results showed a significant increase in LPO in TiO_2-NPs and Ag-NPs groups.

It has been shown that lipid peroxidation levels increase by the generation of ROS [31]. The results suggest that LPO is affected by ROS. Therefore, the results show that TiO_2-NPs and Ag-NPs with mitochondrial dysfunction, increases in ROS and LPO level can cause damage to the scallop, which plays an important role in the treatment of wastewater. Furthermore, ROS by attacking mitochondrial membrane phospholipids decline the MMP. It ultimately leads to the activation of cell death factors and cell death signaling [32, 33]. Flowcytometry assay with 123probe shows the increase collapse of MMP in scallop mitochondria. Using isolated mitochondria, it has been shown that there is a direct relationship between ROS generation and MMP level [34]. Our results are in agreement with these results. In this research, results showed that the level of GSH in the scallop mitochondria was reduced by exposure to NPs ($TiO_2$and Ag). Therefore, NPs (TiO_2 and Ag) have been able to increase the oxidative status (by increasing ROS) and also decreasing GSH. Finally, the results showed that the TiO_2-NPs and Ag-NPs induced apoptosis in the mitochondria isolated from scallop. Previous studies have shown that ROS generation and collapse in MMP activates signaling of apoptosis [35, 36]. Our results are in agreement with these results.

4. Conclusion

In conclusion, our results indicate that mitochondria are targeted in scallop muscle by TiO_2-NPs and Ag-NPs. This leads to changes in scallop mitochondria which subsequently induce apoptosis signaling.

References

[1] Cēbere B, Faltiņa E, Zelčāns N, Kalniņa D. Toxicity Tests for Ensuring Succesful Industrial Wastewater Treatment Plant Operation. *Environ. Clim. Technol*. (2009) 3: 41-47.

[2] Reemtsma T, Putschew A, Jekel M. Industrial wastewater analysis: a toxicity-directed approach. *J. Waste. Manag*. (1999) 19: 181-88.

[3] Xiao N, Qin L, Zhang X, Li Y, Zhang F. Industrial wastewater biological toxicity research status (2016).

[4] Mutamim NSA, Noor ZZ, Hassan MAA, Olsson G. Application of membrane bioreactor technology in treating high strength industrial wastewater: a performance review. *Desalination* (2012) 305: 1-11.

[5] Barakat M. New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem*. (2011) 4: 361-77.

[6] Gehrke I, Geiser A, Somborn-Schulz A. Innovations in nanotechnology for water treatment. *Nanotechnol. Sci. Appl*. (2015) 8: 1-17.

[7] Lu H, Wang J, Stoller M, Wang T, Bao Y, Hao H. An overview of nanomaterials for water and wastewater treatment. *Adv. Mater. Sci. Eng*. (2016) 2016.

[8] Samanta H, Das R, Bhattachajee C. Influence of Nanoparticles for Wastewater Treatment-A Short Review. *Austin. Chem. Eng*. (2016) 3: 1036.

[9] Yunus IS, Harwin, Kurniawan A, Adityawarman D, Indarto A. Nanotechnologies in water and air pollution treatment. *Environ. Technol. Rev*. (2012) 1: 136-48.

[10] Shiraishi T, Tamada M, Saito K, Sugo T. Recovery of cadmium from waste of scallop processing with amidoxime adsorbent synthesized by

graft-polymerization. *Radiat. Phys. Chem*. (2003) 66: 43-47.

[11] Shirzad-Siboni M, Khataee A, Joo SW. Kinetics and equilibrium studies of removal of an azo dye from aqueous solution by adsorption onto scallop. *J. Ind. Eng. Chem*. (2014) 20: 610-15.

[12] Yeom SH, Jung K-Y. Recycling wasted scallop shell as an adsorbent for the removal of phosphate. *J. Ind. Eng. Chem*. (2009) 15: 40-44.

[13] Fu PP, Xia Q, Hwang HM, Ray PC, Yu H. Mechanisms of nanotoxicity: generation of reactive oxygen species. *J. Food Drug Anal*. (2014) 22: 64-75.

[14] Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed. Res. Int*. (2013) 2013: 942916.

[15] Khosravi Y, Salimi A, Pourahmad J, Naserzadeh P, Seydi E. Inhalation exposure of nano diamond induced oxidative stress in lung, heart and brain. *Xenobiotica*. (2018) 48: 860-66.

[16] Winston GW. Oxidants and antioxidants in aquatic animals. *Comp. Biochem. Physiol. C. Pharmacol. Toxicol*. (1991) 100: 173-76.

[17] Lee S, Tak E, Lee J, Rashid MA, Murphy MP, Ha J, Kim SS. Mitochondrial H2O2 generated from electron transport chain complex I stimulates muscle differentiation. *Cell. Res*. (2011) 21: 817-34.

[18] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. (1976) 72: 248-54.

[19] Zhao Y, Ye L, Liu H, Xia Q, Zhang Y, Yang X, Wang K. Vanadium compounds induced mitochondria permeability transition pore (PTP) opening related to oxidative stress. *J. Inorg. Biochem*. (2010) 104: 371-78.

[20] Faizi M, Salimi A, Rasoulzadeh M, Naserzadeh P, Pourahmad J. Schizophrenia induces oxidative stress and cytochrome C release in isolated rat brain mitochondria: a possible pathway for induction of apoptosis and neurodegeneration. *Iran J Pharm Res*. (2014)13:93-100.

[21] Sadegh C, Schreck RP. The spectroscopic determination of aqueous sulfite using Ellman's reagent. *MURJ*. (2003) 8: 39-43.

[22] Zhang F, Xu Z, Gao J, Xu B, Deng Y. *In vitro* effect of manganese chloride exposure on energy metabolism and oxidative damage of mitochondria isolated from rat brain. *Environ. Toxicol. Pharmacol*. (2008) 26: 232-36.

[23] Bilińska L, Gmurek M, Ledakowicz S. Comparison between industrial and simulated textile wastewater treatment by AOPs – Biodegradability, toxicity and cost assessment. *Chem. Eng. J*. (2016) 306: 550-59.

[24] Ebrahiem EE, Al-Maghrabi MN, Mobarki AR. Removal of organic pollutants from industrial wastewater by applying photo-Fenton oxidation technology. *Arab. J. Chem*. (2017) 10: S1674-S79.

[25] Lu F, Astruc D. Nanomaterials for removal of toxic elements from water. *Coord. Chem. Rev*. (2018) 356: 147-64.

[26] Shirzad-Siboni M, Mohagheghian A, Vahidi-Kolur R, Pourmohseni M, Yang J-K. Preparation and characterization of Scallop shell coated with Fe3O4 nanoparticles for the removal of azo dye: Kinetic, equilibrium and thermodynamic studies. *Indian J. Chem. Technol*. (2018) 25: 40-50.

[27] Ho D, Wang CH, Chow EK. Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine. *Sci. Adv*. (2015) 1: e1500439.

[28] Karpeta-Kaczmarek J, Dziewiecka M, Augustyniak M, Rost-Roszkowska M, Pawlyta M. Oxidative stress and genotoxic effects of diamond nanoparticles. *Environ. Res*. (2016) 148: 264-72.

[29] Abdal Dayem A, Hossain MK, Lee SB, Kim K, Saha SK, Yang GM, Choi HY, Cho SG. The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles. *Int. J. Mol. Sci*. (2017) 18.

[30] Dhanwal V, Katoch A, Singh A, Chakraborty S, Faheem MM, Kaur G, Nayak D, Singh N, Goswami A, Kaur N. Self-assembled organic nanoparticles of

benzimidazole analogue exhibit enhanced uptake in 3D tumor spheroids and oxidative stress induced cytotoxicity in breast cancer. *Mater. Sci. Eng. C. Mater. Biol. Appl*. (2019) 97: 467-78.

[31] Elyoussoufi Z, Habti N, Motaouakkil S, Cadi R. Paraphenylenediamine induces apoptosis of Murine myeloma cells in a reactive oxygen species dependant mechanism. *J. Toxicol. Environ. Health. Sci*. (2012) 4: 76-84.

[32] Goyal S, Amar SK, Dubey D, Pal MK, Singh J, Verma A, Kushwaha HN, Ray RS. Involvement of cathepsin B in mitochondrial apoptosis by p-phenylenediamine under ambient UV radiation. *J. Hazard. Mater*. (2015) 300: 415-25.

[33] Zhou Y-j, Zhang S-p, Liu C-w, Cai Y-q. The protection of selenium on ROS mediated-apoptosis by mitochondria dysfunction in cadmium-induced LLC-PK1 cells. *Toxicol. In Vitro*. (2009) 23: 288-94.

[34] Pyatrikas DV, Fedoseeva IV, Varakina NN, Rusaleva TM, Stepanov AV, Fedyaeva AV, Borovskii GB, Rikhvanov EG. Relation between cell death progression, reactive oxygen species production and mitochondrial membrane potential in fermenting Saccharomyces cerevisiae cells under heat-shock conditions. *FEMS. Microbiol. Lett*. (2015) 362: fnv082.

[35] Bauer D, Werth F, Nguyen HA, Kiecker F, Eberle J. Critical role of reactive oxygen species (ROS) for synergistic enhancement of apoptosis by vemurafenib and the potassium channel inhibitor TRAM-34 in melanoma cells. *Cell. Death. Dis*. (2017) 8: e2594.

[36] Kamogashira T, Fujimoto C, Yamasoba T. Reactive oxygen species, apoptosis, and mitochondrial dysfunction in hearing loss. *Biomed. Res. Int*. (2015) 2015: 617207.