Original Article
Middle East Journal of Cancer; July 2023; 14(3): 370-377

Evaluating Oxidative Stress Condition in Human Bladder Cancer 5637 Cell Line upon Exposure to Silver Nanoparticles

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Please cite this article as: Daei S, Ziamajidi N, Abbasalipourkabir R, Aminzadeh Z, Vahabirad M. Evaluating oxidative stress condition in human bladder cancer 5637 cell line upon exposure to silver nanoparticles. Middle East J Cancer. 2023; 14(3):370-7. doi: 10.30476/mejc.2023.95249.1759.

Abstract

Background: Bladder cancer (BC) is known as the most frequent neoplasm of the urinary system, whose prevalence has significantly increased over the past three decades. Successful treatment of BC is a highly challenging task. In this regard, several studies have demonstrated that increased level of oxidative stress may cause cancer cells death. Furthermore, silver nanoparticles (AgNPs) are recognized as one of the most widely used nanomaterials in cancer treatment. Herein, we evaluated the AgNPs-induced oxidative stress in BC 5637 cell line.

Method: In the current experimental study, using colorimetric reactions, we assessed the levels of oxidative stress parameters, including malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC), as well as the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) as antioxidant enzymes. Moreover, we performed the statistical analysis via One-way ANOVA and post-hoc Tukey tests to draw comparisons between the groups.

Results: The results indicated an increased amount of TOS, MDA, and oxidative stress index. Nonetheless, there was a remarkable reduction in SOD, GPx, and CAT activities and TAC level in the AgNPs-exposed cells compared to the control untreated ones ($P < 0.05$).

Conclusion: All in all, AgNPs have the potential to induce oxidative stress in 5637 cells. We thus concluded that AgNPs can be chosen as an antitumor agent for future investigations to treat BC.

Keywords: Metal nanoparticles, Silver, Oxidative stress, Urinary bladder, Cell line

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Introduction

Cancer is a complex and detrimental disease in which the normal cells of the body are transformed into malignant ones, leading to an uncontrolled growth

Received: April 16, 2022; Accepted: March 11, 2023

of cells and subsequently creating a mass of abnormal tissue called tumor forms.[1]

Bladder cancer (BC) is the most frequent neoplasm of the urinary system, known as a heterogeneous disease according to its histology and clinical signs. As widely reported, about 70% of the patients suffering from BC have non-muscle invasive cancer (NMIBC) at the time of diagnosis, while the remaining 30% usually have the muscle invasive (MIBC) one with a high risk of metastasis and progression, even after surgery and systemic treatment.[2] The incidence of BC is higher than that of other urological malignancies. Moreover, the prevalence of this cancer has significantly increased over the past three decades. Due to the ever-increasing prevalence of BC in developing countries, it seems that this cancer will further burden global health in the near future.[3, 4]

Despite recent recommendations and therapies concerning the treatment of this cancer, the rate of tumor recurrence is high even after surgery. As such, chemotherapy is also used to reduce it. Therefore, there is a huge demand for new options to improve cancer treatment as well as to reduce its recurrence. Recently, a promising nanoparticle-based therapy has been proposed for more effective and less toxic treatments.[5, 6]

Over the past decade, nanotechnology has been developed rapidly, accompanied by different and new applications. Due to the unique physical and chemical properties of nanoparticles, which may differ from their bulk counterparts, new perspectives on commercial and scientific applications have been provided by them. In the medical field, nanotechnology has shown promising advances in cancer diagnosis and treatment strategies, including tumor eradication and specific targeting.[7, 8]

Lots of the advantages provided by metal nanoparticles around technology and medical applications are increasingly becoming apparent.[9] That mentioned, metal nanoparticles have special properties, such as optical, electronic, thermal, and catalytic properties due to the particle size, surface to volume ratio, and space limitation.[10]

Among metal nanoparticles, silver nanoparticles (AgNPs) are recognized as one of the most widely-used nanomaterials.[8] A previous study showed that the cytotoxic mechanism of silver nanoparticles involves an elevated production level of reactive oxygen species (ROS) and malondialdehyde (MDA), as well as depletion of ATP, glutathione (GSH), and superoxide dismutase (SOD) which in turn causes cell death.[11]

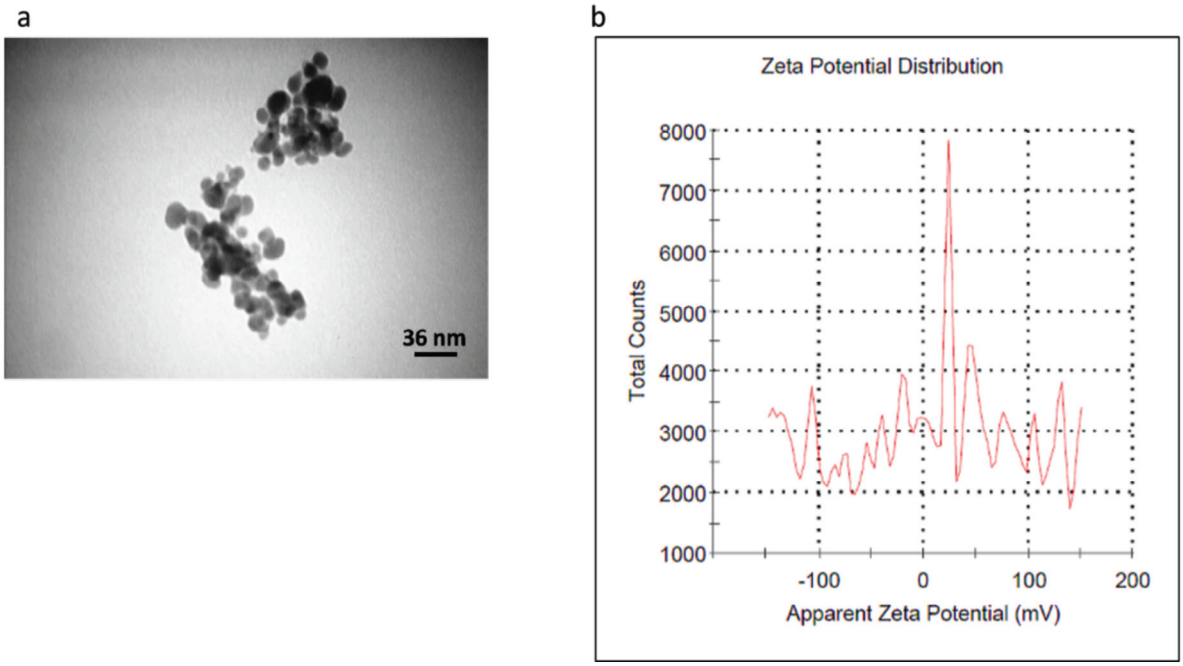

Figure 1. This figure depicts a) transmission electron microscopy (TEM) and b) zeta potential distribution of silver nanoparticles (AgNPs) from Iranian Nanomaterials Pioneers Company, NANOSANY.

Recently, the use of AgNPs as a promising anticancer agent has been verified. Furthermore, various efforts have been made to apply AgNPs to cancer treatments with positive results; for example, the induction of oxidative stress by AgNPs has been reported as one of the possible mechanisms contributing to AgNPs-induced toxicity, the mechanistic pathways are not yet exactly clear though.[12] In addition, many investigations have reported that cellular alterations stemming from oxidative stress are induced by the production of reactive oxygen intermediates (ROI) in tumor cells, which effectively enhance the cytotoxic effects of anticancer drugs.

The oxidative stress might occur in case of imbalance between ROI generation and intracellular antioxidants, such as SOD, catalase (CAT), and glutathione peroxidase (GPx).[13]

To the best of our knowledge, the effects of AgNPs have not yet been evaluated on oxidative stress condition in BC 5637 cells. In the present study, oxidative stress parameters were investigated in 5637 cells following exposure to AgNPs.

Materials and Methods

This experimental study was approved by the ethics committee of Hamadan University of Medical Sciences (ethics code: IR.UMSHA.REC.1399.203) (No. 9903271752).

Chemicals

We bought the silver nanoparticle (AgNP) (30-50 nm and coated with Poly Vinyl Pyrrolidone), with spherical shape and suspended in deionized water, from a commercial company (Nanosany Co.) (Mashhad City, Iran).

Figures 1a and 1b illustrate transmission electron microscopy (TEM) and zeta potential distribution of AgNPs.

Colorimetric assay kits, including total antioxidant capacity (TAC), total oxidant species (TOS), MDA, GPx, SOD, and CAT, as well as protease inhibitor cocktail, were purchased from Kiazist Company (Kiazist, life sciences, Iran).

Cell culture and treatment

We purchased homo sapiens 5637 cell line of BC (derived from a grade II bladder transitional cell carcinoma) from Pasteur Institute (Tehran City, Iran). The cells were grown in RPMI-1640 (KRPM500) media, to which we added 10% fetal bovine serum (FBS) (KFBS100) and 1% penicillin streptomycin (BI-1203). Afterwards, we kept them in an incubator for maintaining the cells at 37 ° C and 5% CO_2. The cells were also sub-cultured with 0.25% trypsin-EDTA treatment (KRT100) for 2 or 3 days. The desired concentrations of AgNPs were made in a serum-free culture medium.

Preparation of cell lysate

We prepared cell lysates to assess the levels of TAC, TOS, MDA, GPx, CAT, and SOD. In brief, after 24 h of incubation with the indicated concentrations (30, 50, 60 µg/ml) of AgNPs, the cells were washed twice with cold PBS and then harvested using trypsin-EDTA treatment. Subsequently, we sedimented the cells through centrifugation at 1500 rpm. Moreover, the cell pellets were dissolved in PBS or the manufacturer's buffer, including protease inhibitor cocktail. Afterwards, we lysed the cells either by incubating them at 4 ° C or freeze-thaw in triplicate. After centrifuging at 15000 rpm for 15 minutes, the resulting supernatants were collected and kept at -80°C until use. Of note, butylated hydroxytoluene (BHT) agents were also transferred into the lysis buffer for measuring MDA content.

The total protein content of lysates was quantified via Bradford method,[14] in order to normalize the oxidative stress parameters.

It should be mentioned that different concentrations of AgNPs were adopted from MTT assay according to the results of our previous study.[15]

Oxidative stress-related biomarkers assay

We determined the amount of TAC, TOS, and MDA using colorimetric reactions according to the manufacturer's protocol.

In order to give more details, CUPRAC assay, based on the reduction of cupric (Cu+2) to cuprous (Cu+1), was employed for measuring TAC index. Additionally, for determining TOS level, we assessed the ability of the samples to convert

ferrous to ferric.

MDA, a strong marker of lipid peroxidation, was assessed based on a method in which a complex is formed between MDA and thiobarbituric acid (TBA), which produces color and the absorbance is recorded at a wavelength of 532 nm.

Oxidative stress index (OSI) was calculated based on the ratio of TOS to TAC (OSI (Arbitrary/ scale) = TOS/TAC).

Antioxidant enzymes activity measurement

We determined SOD activity based on a reaction where a blue colorant, named resazurin, was changed to pink-colored resorufin by reacting with anion superoxide. Finally, the activity was expressed as inhibition rate (%).

Furthermore, a coupling reaction along with glutathione reductase and coenzyme NADPH were used for measuring GPx activity.

We measured CAT activity with the aid of its

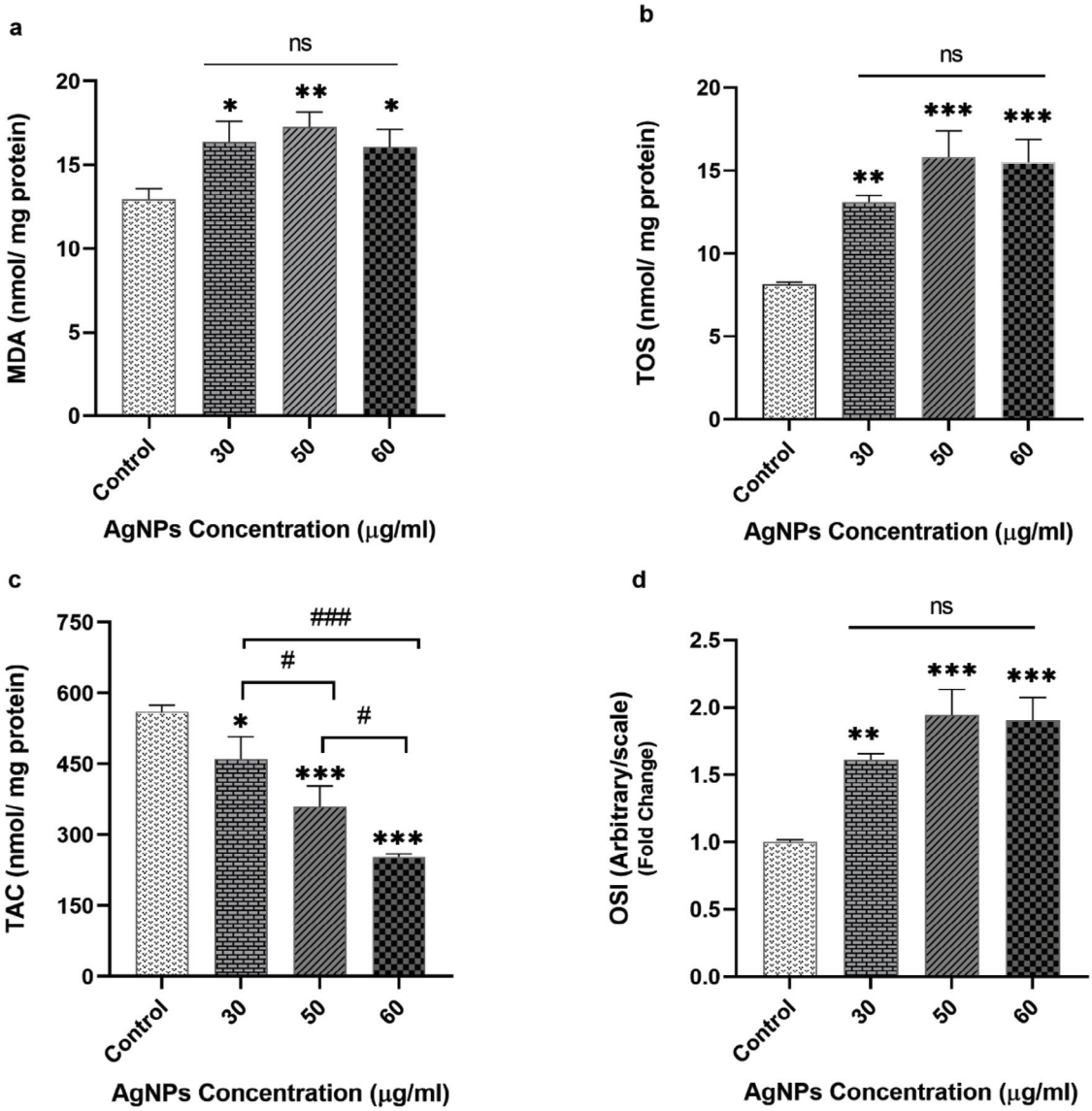

Figure 2. This figure illustrates the effects of silver nanoparticles (AgNPs) on oxidative stress parameters in 5637 cells: a) MDA; b) TOS; c) TAC; d) OSI. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared with the control, whereas #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ indicate significant differences between the groups. The results are reported as mean ± SD.

ns: Non-significant differences between the groups; MDA: Malondialdehyde; TOS: Total oxidant status; TAC: Total antioxidant capacity; OSI: oxidative stress index; SD: Standard deviation

peroxidase activity in the presence of methanol. In this method, the activity is stopped by its inhibitor and the created formaldehyde reacts with Purpald, leading to the formation of purple color. We also recorded the absorbances at a wavelength of 340 and 546 nm to measure GPx and CAT activities, respectively.

Statistical analysis

The present work utilized GraphPad Prism 9 software (San Diego, CA, USA) for statistical analyses. In addition, one-way ANOVA and post-hoc Tukey test were used for drawing comparisons between the groups; the results were expressed as mean ± standard deviation (SD). All *P*-values of below 0.05 were considered statistically significant. It is important to note that we performed all the experiments in triplicate.

Results

Oxidative stress induced by AgNPs in 5637 cells

Figure 2 represents the effects of AgNPs on modulation of MDA, TOS, TAC, and OSI levels.

MDA content markedly increased in the cells exposed to AgNPs with respect to the control ($P < 0.05$). The most remarkable increase was observed in 50 µg/mL-treated cells compared with the other concentrations. There were no significant differences between the groups concerning the level of MDA ($P > 0.05$) (Figure 2a).

TOS level significantly increased in AgNPs-treated cells compared with the control cells ($P < 0.05$); however, there were no remarkable differences between the groups ($P > 0.05$) (Figure 2b).

Treatment with different concentrations of AgNPs could significantly diminish the TAC level in a dose-dependent manner ($P < 0.05$); for example, the TAC level of 60 µg/ml-treated cells decreased to 252.7 ± 4.05 nmol/mg protein compared with the control (559.9 ± 8.46 nmol/mg protein) ($P < 0.001$). Moreover, there were significant differences in terms of TAC index between different experimental groups ($P < 0.05$) (Figure 2c).

OSI value showed a substantial increase in AgNPs-treated cells with respect to the control ($P < 0.01$); however, there were no meaningful differences between the groups ($P > 0.05$) (Figure 2d).

Effects of AgNPs on antioxidant enzymes activity

Exposure to AgNPs could reduce the activity of antioxidant enzymes in BC 5637 cells. In addition, AgNPs caused a significant reduction in CAT, SOD, and GPx activity compared with the control ($P < 0.05$) (Figure 3).

SOD activity was significantly reduced in AgNPs-treated cells compared with the control ($P< 0.01$) (Figure 3a). GPx activity also remarkably decreased in 60 µg/ml-treated cells in comparison with the control ($P < 0.05$); meanwhile, the observed reduction in GPx was not statistically significant in 30 and 50 µg/ml-treated cells ($P > 0.05$) (Figure 3b). Similar to SOD and GPx activity, that of CAT significantly decreased in the cells exposed to AgNPs in comparison with the control ($P < 0.001$). The reduction in CAT level in 60 µg/mL-treated cells was meaningfully more than that in the ones treated with 30 µg/ml ($P < 0.05$) (Figure 3c).

Discussion

The results of the present investigation revealed that AgNPs can induce oxidative stress in 5637 cells. In this regard, AgNPs decreased the activity of antioxidant enzymes (SOD, GPx, and CAT), while increasing MDA and TOS levels. Similarly, Yang et al.,[18] and also Xu et al.,[19] reported that AgNPs have the ability to reduce the antioxidant enzymes activity, such as CAT, SOD, and GPx in TPC1 as well as HeLa cells, respectively. Their results also showed that AgNPs increased lipid peroxidation, which is fully consistent with our findings. Furthermore, Erdogan et al. reported that AgNPs can significantly increase oxidative stress via reducing SOD, CAT, and GPx activity in breast cancer cells (MCF-7).[20] Surprisingly, AgNPs were considered to be antioxidants or prooxidants in some studies;[19, 21] for instance, AgNPs increased MDA amount and decreased the activity of SOD in HeLa, and SiHa human cervical cancer cells, suggesting the induction of oxidative stress.[19] Nevertheless, in contrast to our findings, it was observed that the SOD activity remarkably increased in HL-60 (human leukemia cell line) cells treated with AgNPs.[22] Moreover,

it was found that GPx activity significantly increased in HepG2, HCT, and MCF-7 cancer cells in the presence of AgNPs.[23] According to the mentioned reports, it seems as if different cell types may use enzymatic or non-enzymatic responses against an increased level of oxidative stress.[22] Hence, the reduced or elevated activity of antioxidant enzymes following exposure to AgNPs in the present report and some others,[19, 22] may be attributed to the studied cell type. Of note, the physicochemical and structural properties of nanoparticles may also affect their biological activity and toxicity. Therefore, different nanoparticles made from different compounds and chemicals might employ different pathways and mechanisms for their final biological responses. As such, scientists cannot determine the exact mechanism of toxicity only by studying a single type of nanoparticle.[24] Regarding our results and some previous investigations,[19, 22, 23] it is hypothesized that differences in the used AgNPs may alter the final outcomes.

In fact, biological systems use antioxidants SOD, GPx, and CAT against radical-mediated toxicity. Thus, antioxidant enzymes play a substantial and indispensable role in the antioxidant defense capacity.[25] Generally, inhibition of the antioxidant activity in cancer cells can reduce their ability to balance oxidative conditions and may lead to cell death. In this regard, it is revealed that AgNPs trigger apoptosis by enhancing oxidative stress in different cancer cell lines.[23] Furthermore, the apoptotic and necrotic effects of these NPs are demonstrated in the cancer cells, where oxidative damage is regarded as a possible mechanism for induction of apoptosis.[19, 26]

Actually, regulation of redox situation in cancer cells are somewhat different from that in normal cells. While the increased level of ROS in cancer cells allows them to rapidly grow and metastasize, an excessive increase in ROS production might cause elevated oxidative stress and even cell death. Additionally, cancer cells are more sensitive to a high level of ROS compared with normal ones. Accordingly, novel anticancer agents can be introduced based on these differences. Owing to the remarkable increase in the antioxidant capacity of cancer cells and the fact that ROS generation is not enough only to remove these cells, there is a substantial demand for drugs with the potential to weaken the antioxidant defense responses.[27, 28]

According to the aforementioned arguments, AgNPs have the potential to be employed as an anticancer agent. Our findings suggested that these nanoparticles reduce antioxidant status and increase TOS and MDA levels. All in all, the

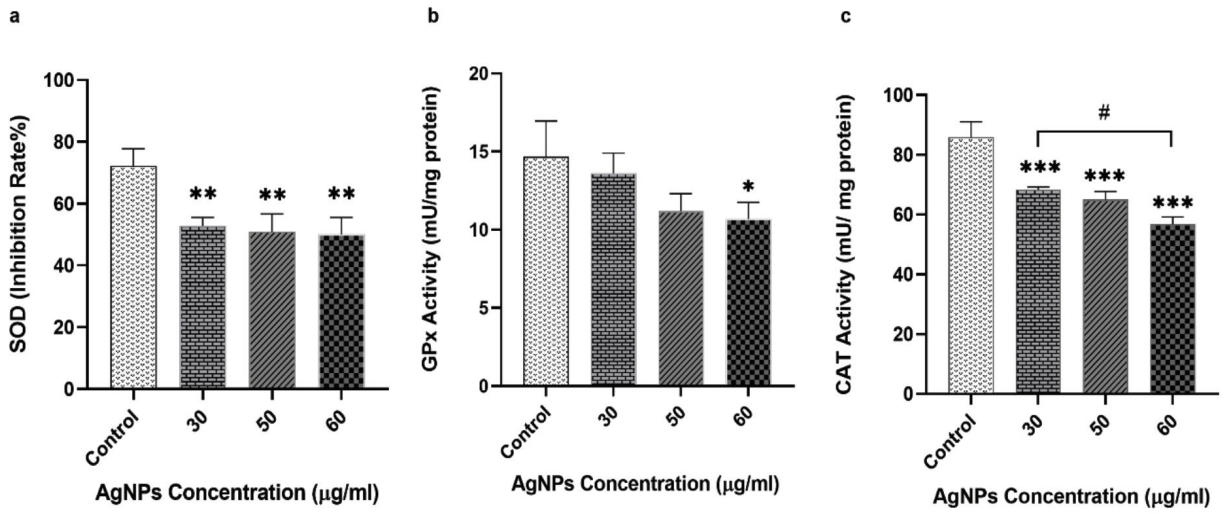

Figure 3. This figure shows the effects of silver nanoparticles (AgNPs) on antioxidant enzymes activity in 5637 cells: a) SOD; b) GPx; c) CAT. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared with the control, while #$P < 0.05$ indicates significant differences between the groups. The results are reported as mean ± SD.

AgNPs-mediated cytotoxicity,[15] may be related to the increased level of oxidative stress.

Although our study clearly revealed the pro-oxidant effects of silver nanoparticles, further experiments, such as PCR array, are needed to prove this issue. It is also necessary to investigate the antioxidant effects of N-acetyl-cysteine against AgNPs.

Conclusion

This study demonstrated that AgNPs lead to oxidative stress through an increase in MDA and TOS levels along with a significant reduction in antioxidant enzymes activity and TAC level. Hence, it seems as if the elevated oxidative stress is related to the cytotoxicity of AgNPs in 5637 cells. Meanwhile, further research is still required to evaluate oxidative stress-mediated apoptosis related to the AgNPs toxicity in BC 5637 cell line.

Conflict of Interest

None declared.

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