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SILVER NANOPARTICLES INHIBIT PROLIFERATION AND MIGRATION OF PROSTATE CANCER CELLS: AN EXPERIMENTAL REPORT

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Abstract – *Objective:* *Prostate carcinoma is a serious public health in men with increasing incidence and mortality rates. The present study aimed to investigate cytotoxic activities of silver nanoparticles (AgNPs) against PC-3 human prostate cancer cell line in vitro.*

Materials and Methods: *The antiproliferative effect of AgNPs on PC-3 cells was assessed using various concentrations of AgNPs (5-80 µg/mL) by MTT assay. We have also investigated the effect of AgNPs on the migration property of cancers cells using a wound-healing assay. Then, obtained results were analyzed using ANOVA and Student's* t*-test.*

Results: *The AgNPs diminished significantly the viability of PC-3 cells in a dose and time-dependent manner. According to the dose-dependent viability curve, the IC50 (50% inhibiting concentration) at 48 h was calculated to be 65.7 µg/mL. Migration assay also confirmed the antimetastatic potential of the AgNPs against PC-3 cells. After 24 h, the migration rate was 2.5 fold lower for AgNPs-treated cells compared to control cells.*

Conclusions: *Our findings support the use of AgNPs as an appropriate option for future therapeutic application in prostate cancer therapy.*

KEYWORDS: *Prostate cancer, Silver nanoparticles, Viability, Metastasis.*

INTRODUCTION

Prostate cancer with an estimated of almost 1.4 million new cases and 375,000 deaths worldwide was the second abundant malignancy and the fifth cause of cancer-related mortality among men in 2020 [1]. Based on GLOBOCAN 2020 estimates, prostate cancer counting 14.1% and 6.8% of all new cases and death caused by cancer in men, respectively[1]. It can be localized or metastasized to the bones and lymph nodes, depending on its severity[2]. Various factors such as age, genetics, ethnicity, hypertension, obesity, environmental toxins, chemical hazards, and radiation appear to be involved in the pathogenesis of prostate cancer, although the exact mechanism is still unclear[3]. Current therapeutic approaches for prostate cancer include chemotherapy, radiotherapy, surgery cryosurgery, and hormone replacement therapy[4]. Resistance to treatment remains the biggest challenge in cancer today because the effectiveness of therapy ultimately surrenders to the resistance pathways implemented by cancer cells[5]. Furthermore, these therapeutics have

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been attributed to serious side effects including toxicity on healthy cells, vomiting, nausea, and hair loss [5]. To avoid these undesirable effects, the search for new agents for cancer treatment has been the goal of numerous studie [6]. In this regard, nanotechnology can play a significant role in overcoming the limitations of conventional treatment strategies, being considered the star technology of the twenty-first century[7]. Nanoparticles, with a particle in a size range of 1–100 nm, thanks to their special physical, chemical, and biological properties, have recently received more attention. Among the commonly used nanoparticles, silver nanoparticles (AgNPs) are widely used in a variety of business areas, including medical, pharmaceutical industries, agricultural, wound dressings, food packages, and electronic[8-10]. In addition, AgNPs have numerous biomedical applications and exhibit anti-biofilm, antimicrobial, antioxidant, anti-inflammatory, anti-angiogenic, and anticancer activities (Figure 1)[11,12]. In modern anticancer therapy, AgNPs play a noteworthy role, especially in the detection and diagnosis of malignant tumors, as well as regulated and externally stimulated drug delivery systems [13]. The use of silver nanoparticles is influenced by the type of application, the properties of the particles, and the organisms. The size, morphology, and surface characteristics of AgNPs make them suitable for cancer cells internalization and affect cell physiology[14]. The great potential of AgNPs in cancer management is related to their role in triggering oxidative stress, membrane damage, mitochondrial dysfunction, DNA damage, resulting in cell death[15].

Fig. 1. Biomedical application of silver nanoparticles.

According to evidence, the cytotoxicity of AgNPs is affected by particle size, shape, concentration, and the degree of agglomeration or aggregation. Moreover, regardless of the physicochemical properties of AgNPs, their cytotoxicity depends on the organism's variety. Indeed, the toxic response is not the same in every cell line. The present study, therefore, investigated the cytotoxic effect of AgNPs on the viability and migration rate of PC-3 prostate cancer cell line *in vitro*.

MATERIALS AND METHODS

Cell Culture

Human prostate cancer cell lines PC-3 were purchased from the Pasteur Institute of Iran. The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% penicillin/streptomycin (Sigma-Aldrich; St. Louis, MO, USA) to confluence at 37°C and 5% CO_2 atmosphere.

Preparation and Characterization of AgNPs

In this assay, US silver nanoparticles (AgNPs) with 99.99% purity were used. Morphology and microstructure of AgNPs were investigated with transmission electron microscopy (TEM) (Philips CM30, Amsterdam, The Netherlands) with light background and the size of AgNPs measured using ImageJ software. The crystal structure of the nanoparticles was determined using the X-ray diffraction (XRD) technique (Siemens D500, Munich, Germany). This assumption was further validated by Fourier transform infrared spectroscopy (FTIR) device (Nexus 670; Northglenn, CO, USA).

Preparation of Different Concentrations of AgNPs Solution

An amount of 2 mg of AgNPs powder was weighed and 10 mL of complete culture medium (DMEM containing 10% FBS and 1% penicillin/streptomycin) was added to prepare a 200 µg/mL stoke solution. Then, the AgNPs mixture was sonicated for 15 minutes, filtered, and purified. The different concentrations (5, 10, 20, 50, 100 µg/mL) were prepared by diluting the base solution and stored in a refrigerator at 4°C.

Cell Viability Assay

The viability of cells was analyzed using the MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide)] (Sigma-Aldrich; St. Louis, MO, USA) assay to determine the cytotoxic effect of the AgNPs at different concentrations. PC-3 cells (5×10^3 cells/well) were seeded in a 96-well plate in 5% CO_2 at 37°C to a confluence of 85%. Then, the cells were washed with (phosphate-buffered saline, pH 7.4) PBS, treated with various concentrations of AgNPs (5-80 µg/mL), and incubated at 37°C in a humidified incubator. Following 24, 48, and 72 hours of treatment, cells were washed with PBS and then incubated with 20 µL of MTT (5 mg/mL in PBS) in a fresh medium for a further 4 h at 37°C. After that, MTT was removed, and the resulting formazan was dissolved in 100 µL of dimethyl sulfoxide (DMSO) with gentle shaking at 37°C for 15 minutes. The absorbance (OD) was read at 570 nm with 630 nm as reference wavelength (OD blank) using an enzyme-linked immunosorbent assay (ELISA) plate reader. The OD values were converted into percentages of cell viability rate based on the subsequent formula:

Cell viability rate (%) =
(OD treatment-OD blank)/
(OD control-OD blank) × 100

The results were obtained as the mean of three independent experiments. GraphPad Prism (La Jolla, CA, USA) was used for calculating a 50% reduction in cell viability (i.e., IC50 values).

Scratch Assay

Wound healing assay was used to determine cell migration of prostate cancer cells upon silver nanoparticles treatment. PC-3 cells (1×10^5 cells/well) were seeded into 24-well plates and kept under 5% CO_2 at 37°C to a confluent monolayer. Then, the scratch was made with a sterile 200-µl pipette tip and washed with PBS to remove floating cells. After that, the cells in the presence and absence of AgNPs were cultivated. The images of cells were captured at 0 hours. After 24 h of continuous culture, the medium was aspirated from the cells. Then the cells were washed with PBS, fixed using 4% formaldehyde, stained with crystal violet, and the images of migrating cells were photographed under a phase-contrast inverted microscope. The gap widths were measured using ImageJ software in each case. All experiments were repeated three times.

Statistical Analysis

All assays were performed in triplicate, and each experiment was repeated at least three times. All statistical analyses were conducted using the Student's *t*-test or one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons using the GraphPad Prism software (La Jolla, CA, USA). Data were considered significant at p-value <0.05 and the results were expressed as mean ± SD.

RESULTS

Characterization of Silver Nanoparticles

Characterization of AgNPs was carried out using TEM which indicated a spherical shape and smooth surfaces of the nanoparticles with sizes about 20 nm and tend to agglomerate (Figure 2a). The data obtained from XRD showed the peak location corresponds to the peak in the JCPDS 04-0783 reference card and some extra peaks in the graph, probably due to the presence of stabilizers added to the nanoparticles process (Figure 2b).

Cytotoxicity Effect of AgNPs in PC-3 Cells

The cell viability test is an essential method for a toxicological investigation that may offer information on cell death, survival, and metabolic activities, as well as explain the cellular response to drugs and toxic compounds [16]. The *in vitro* cytotoxic impact of the synthesized AgNPs was evaluated against prostate cancer PC-3 cells at varying concentrations using an MTT assay. Results indicated that AgNPs have potential cytotoxicity (anticancer effect) against the examined cell line. As shown in Figure 3, incubation of PC-3 cells with AgNPs significantly decreased cell viability in a dose and time-dependent manner. The IC50 values of AgNPs for PC-3 cells were calculated to be 73, 65.7, and 32 µg/mL following 24, 48, and 72 h of treatment, respectively. After being treated for 72 h, the viability decreased dramatically, and only 22% were viable at 80 µg/mL (Figure 3a), representing that the prostate cancer cells have lost their ability to proliferate at these concentrations.

The Effect of AgNPs on the Migration of PC3 Cells

As cancer cell migration plays a key role in disease progression [17], we examined the effect of AgNPs on the migration ability of PC-3 cells using an *in vitro* scratch wound-healing. The 24 h treatment

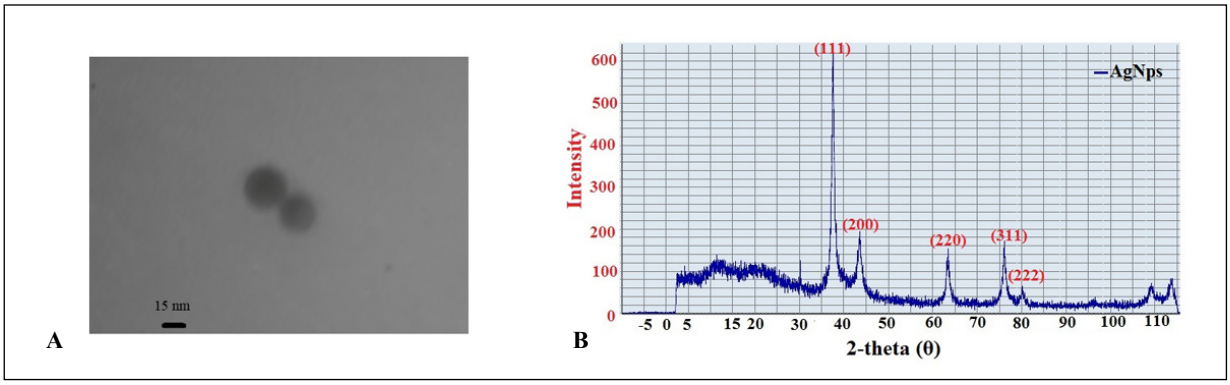

Fig. 2. Characterization of AgNPs by TEM and XRD. *A*, Smooth surfaces of the tested silver nanoparticles (TEM image). Several fields were photographed and were used to determine the diameter of nanoparticles. The average range of observed diameter was 20 nm. *B*, The X-ray powder diffraction (XRD) peaks of silver nanoparticles with the pattern peaks.

with AgNPs considerably ($p<0.001$) reduced the migration rate of PC-3 cells compared with untreated control cells, from 60% to 24% (Figure 4).

DISCUSSION

Prostate cancer is currently considered one of the most common causes of cancer morbidity and mortality in men worldwide. Due to the poor efficiency as well as side effects of traditional cancer treatments, new therapeutic approaches as alternatives or in combination with conventional therapies are demanded. Nanoparticles have recently demonstrated a novel approach to overcome cancer, including prostate cancer. Silver nanoparticles are widely used in anti-microbial studies, nanotechnology, biotechnology drug delivery, cancer treatment, and biomedical field[3,9,18]. The current researches reported the mechanisms of cytotoxicity of AgNPs, including inhibition of cell viability, inducing oxidative stress, and genes expression involvement in processes that lead to AgNPs mediated apoptosis in tumor cells[15]. The toxicity of nanoparticles is shown to be influenced by their size, surface charge, and shape[19]. Particle sizes of AgNPs in our work displayed a diameter of ≈20 nm. The particle size is critical for nanoparticle aggregation in tumor tissues through the tumor capillary's vascular gap. Hence, nanoparticles must be smaller than the vascular gap of the tumor capillary to accumulate in the tumor (up to 400 nm)[20]. According to reports, decreasing the particle size increases the surface area of the particles, which facilitates particle diffusion through cells[18,20]. In this context, Avalos *et al*[21] in examining the impact of different sizes of AgNPs (4.7 and 42 nm) interacting with HepG2 and leukemia cells, found that small AgNPs are much more cytotoxic than large AgNPs.

Our results revealed also spherical, uniform, and smooth surfaces of nanoparticles with tend to

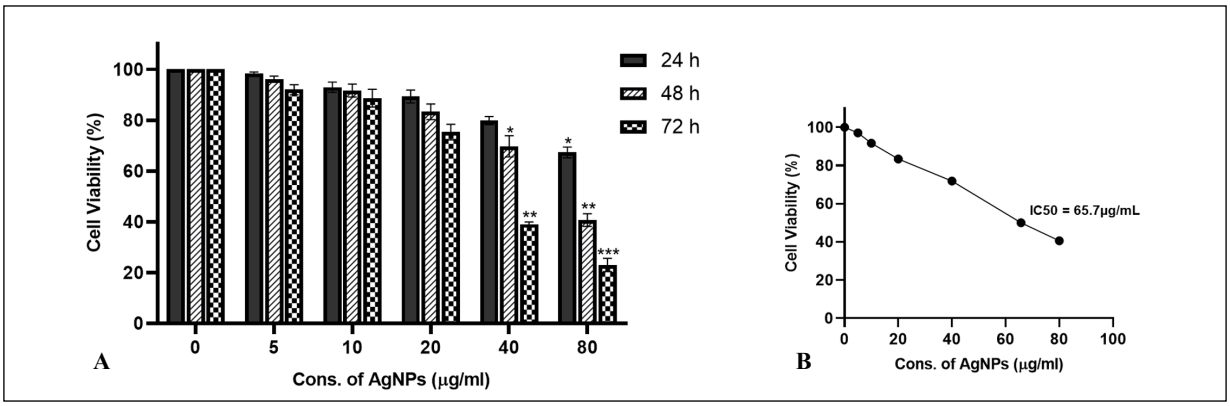

Fig. 3. The anti-proliferative effect of AgNPs induced PC3 cell line. After AgNPs treatment, the MTT data revealed a decrease in cell viability ratio. *A*, The AgNPs induction in PC3 cells significantly decreased the number of viable cells compared with the control group in a time a concentration manner. *B*, Dose-dependent viability curve at 72 h (*$p< 0.05$, **$p< 0.01$).

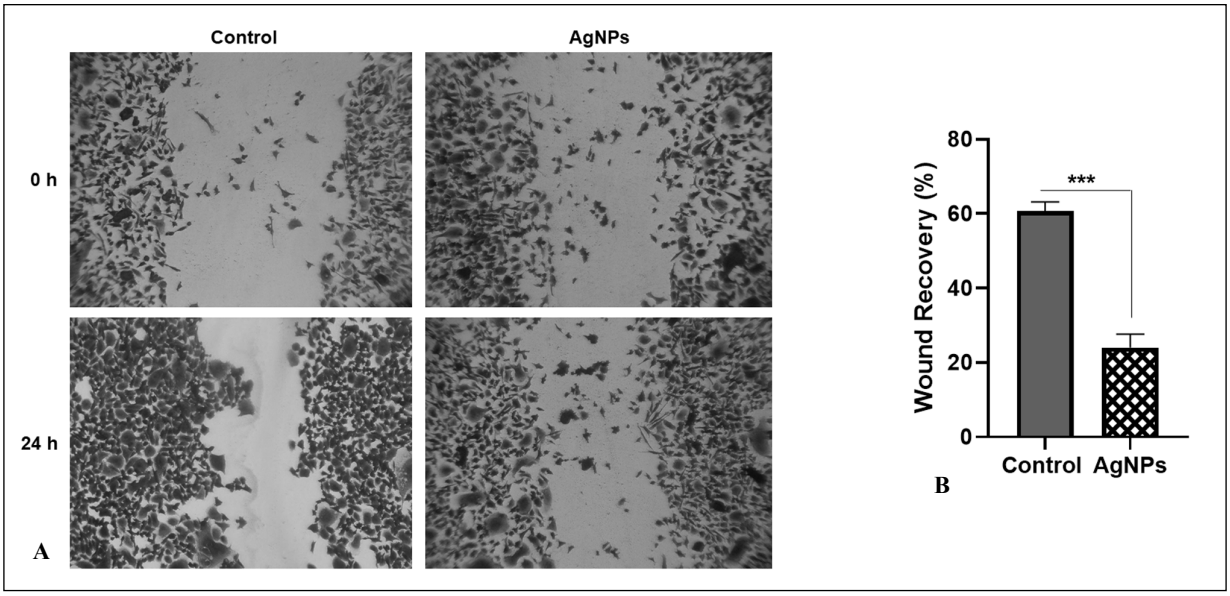

Fig. 4. Effect of AgNPs on PC3 cells migration. *A*, Cell migration measurement with scratch wound assay in AgNPs (65.7 µg/mL). *B*, Percentage of wound healing was measured and presented on a histogram using ImageJ software (***$p < 0.001$).

agglomerate, according to the TEM image. One of the most important factors in cellular internalization is the shape of nanoparticles[22]. For instance, exocytosis of nanoparticles with sharp shapes due to their entry into the endosome membrane is lower than the spherical particles[23]. Moreover, the ellipsoidal nanoparticles showed lower cell uptake compared to spherical NPs[22].

In our experiment, the half-maximal inhibitory concentration (IC50) of AgNPs was determined 65.7 µg/mL after 48 h via MTT assay. The results suggest that AgNPs were able to reduce the cell viability of PC-3 cells in a dose and time-dependent manner. Jeyaraj et al[24] demonstrated the effect of AgNPs against human breast cancer MCF-7 cells. They reported IC50 value at 20 µg/mL for AgNPs with 22 nm in size in slightly agglomerated form[24]. In another study, IC50 values of synthesized AgNPs against PC-3 were found to be 173.21 µg/mL[25]. Krishnaraj et al[26] have stated that at 100 µg/ml concentration, AgNPs had comparatively higher toxic effects (40%) than the other treated MDA-MB-231 cells. We observed the lowest viability of PC-3 cells (22%) after 72 h treatment with 80 µg/ml concentration of AgNPs.

Uncontrolled and increased cell proliferation is a major hallmark of cancer and inhibition of this process will be a great success in the development of anticancer drugs. In agreement with our results, other research groups have reported the cytotoxic effect of AgNPs on the viability of various cancer cells such as breast cancer MDA-MB-231 cells, breast cancer MCF-7 cells[27], cervical cancer HeLa cells[28], human lung cancer H1299 cells[12], human colorectal adenocarcinoma HT29 cells[29], human prostate DU145 Cells[30], and prostate cancer LNCaP cell lines *in vitro* and *in vivo*[31]. Recently, Chen *et al*[32] revealed that AgNPs could induce autophagy through activating the AMPK/mTOR signaling pathway resulting from lysosome injury and cell hypoxia in PC-3 cells.

We also evaluate the effect of AgNPs on the migration rate of prostate cancer cells. Tumor cell migration is well-known to be a crucial stage in tumor progression and metastasis[33]. Prostate cancer death is generally caused by tumor spread to the lungs, bones, and lymph nodes.

Herein, the strong inhibition efficacy of AgNPs on migration was observed in PC-3 cells, which were in line with other studies demonstrating that AgNPs may have a potential function in reducing the migration property of cancer cells[12]. Present results depicted the role of AgNPs in suppressing the metastasis of prostate cancer cells in the body at inhibitory concentrations.

Stimulation of morphological changes[34], loss of cell membrane integrity[24], induction of oxidative stress[35], increased leakage of lactate dehydrogenase, DNA fragmentation and

chromosomal aberrations[15], uncontrolled cellular transport[34], mitochondrial dysfunction and loss of ATP synthesis[34], caspase-3 activity, and induction of apoptosis[24] have been suggested as other main mechanisms responsible for anticancer efficacy of silver nanoparticles.

CONCLUSIONS

Based on the results obtained in the present study, AgNPs have the potential to exert cytotoxic and antiproliferative effects in PC-3 prostate cancer cell line. Furthermore, inhibition of cell migration probably is one of the mechanisms used by AgNPs to exert anticancer actives in cancer cells. Therefore, AgNPs could be a possible candidate as a chemotherapeutic agent or in combination with anticancer drugs in the treatment of cancer. Further *in vitro* and *in vivo* studies need to be carried out for developing AgNPs as new anticancer therapies in the future.

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Ethical Committee:

The study was conducted according to the Institutional requirements and Helsinki Declaration.

Informed Consent:

Informed Consent is not required for this study.

Conflict of Interest:

The Authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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