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ROLE OF SILVER NANOPARTICLES IN PROSTATE CANCER CELL PROLIFERATION AND MIGRATION

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Abstract

Men's prostate cancer death and incidence rates are rising, making it a critical public health concern. The goal of the current study was to examine the cytotoxic effects of silver nanoparticles (AgNPs) on the in vitro PC-3 human prostate cancer cell line. By applying different concentrations of AgNPs (5-80 g/mL), the antiproliferative impact of AgNPs on PC-3 cells was evaluated. Using a wound-healing assay, we have also looked into how AgNPs affect cancer cells' ability to migrate. Then, ANOVA and the Student's t-test were used to examine the obtained results. AgNPs drastically reduced PC-3 cell viability in a dose- and time-dependent manner. The IC50 (50% inhibitory concentration) at 48 h was calculated to be 65.7 g/mL based on the dose-dependent viability curve. The antimetastatic activity of the AgNPs

against PC-3 cells was further demonstrated by a migration assay. Following 24 hours, cells treated with AgNPs migrated at a rate that was 2.5 times slower than control cells. According to our research, AgNPs are a suitable treatment option for prostate cancer treatment in the future.

Keywords: Silver nanoparticles, Viability, Metastasis, Prostate cancer.

INTRODUCTION

Prostate cancer was the second most common malignancy and the fifth leading cause of cancer-related mortality in males in 2020, with an expected 1.4 million new cases and 375,000 deaths worldwide 1. According to GLOBOCAN 2020 estimates, prostate cancer accounts for 14.1% and 6.8%, respectively, of all new cases and deaths from cancer in men1. Depending on its severity, it may remain localized or spread to the lymph nodes and bones2. Although the precise process is yet unknown3, a number of variables, including age, genetics, ethnicity, hypertension, obesity, environmental pollutants, chemical hazards, and radiation, appear to be implicated in the etiology of prostate cancer3. Chemotherapy, radiotherapy, cryosurgery, and hormone replacement therapy are some of the current treatment options for prostate cancer4. Because the effectiveness of therapy ultimately succumbs to the resistance pathways set up by cancer cells5, resistance to treatment is still the major hurdle in cancer today. These medications' severe adverse effects have also been linked to toxicity on healthy cells, vomiting, nausea, and hair loss. 5. Several studies have sought to find novel drugs for the treatment of cancer in order to prevent these unwanted effects.

In this regard, nanotechnology, which is regarded as the leading technology of the twenty-first century7, can significantly contribute to overcoming the constraints of conventional treatment approaches. Due to their unique physical, chemical, and biological characteristics, nanoparticles—which have a particle size range of 1-100 nm—have recently attracted increased interest. Silver nanoparticles (AgNPs), one of the most widely used nanoparticles, are utilized in a wide range of commercial sectors, including the medical and pharmaceutical industries, agriculture, wound dressings, food packaging, and electronics8–10. AgNPs also demonstrate anti-biofilm, antibacterial, antioxidant, anti-inflammatory, anti-angiogenic, and anticancer actions (Figure 1)11,12 and have a wide range of medicinal uses. AgNPs have a significant impact on contemporary anticancer therapy, particularly in the detection and identification of malignant tumors and in the regulation and external stimulation of drug delivery systems 13. The type of application, the properties of the particles, and the organisms have an impact on how silver nanoparticles are used. AgNPs are well suited for interacting with cancer cells because of their size, shape, and surface features, which also have an impact on cell physiology14. AgNPs' enormous promise for treating cancer is related to their ability to cause oxidative stress, membrane disruption, mitochondrial malfunction, and DNA damage that leads to cell death15.

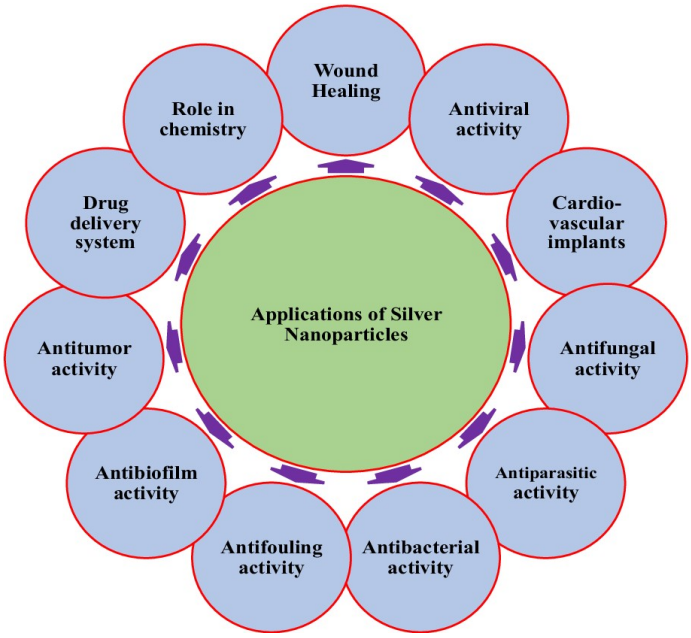

Fig. 1. Biomedical application of silver nanoparticles

Evidence suggests that particle size, shape, concentration, and level of agglomeration or aggregation all influence the cytotoxicity of AgNPs. In addition, regardless of AgNPs' physical and chemical characteristics, the type of organism they interact with will determine their cytotoxicity. In fact, not all cell lines exhibit the same toxic reaction. As a result, the current study examined the cytotoxic impact of AgNPs on the PC-3 prostate cancer cell line's in vitro viability and migration rate.

MATERIALS AND METHODS

Cell Culture

PC-3 human prostate cancer cell lines were obtained. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) to confluence at 37°C and 5% CO2 atmosphere, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Sigma-Aldrich; St. Louis, MO, USA).

Preparation and Characterizationof AgNPs

In this assay, silver nanoparticles (AgNPs) with 99.99% purity were used. Morphology and microstructure of AgNPs were investigated with transmission electron microscopy (TEM) 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).

Preparation of Different Concentrationsof AgNPs Solution

A 200 g/mL stoke solution was created by weighing 2 mg of AgNPs powder with 10 mL of complete culture medium (DMEM containing 10% FBS and 1% penicillin/streptomycin). The AgNPs mixture was then filtered and purified after being sonicate

for 15 minutes. The base solution was diluted to create the various concentrations (5, 10, 20, 50, and 100 g/mL), which were then kept 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. Lou- is, 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 hoursof 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 readat 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 threeindependent experiments. GraphPad Prism (LaJolla, 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'st-test or one-way analysis of variance (ANOVA),followed by Tukey's test for multiple comparisonsusing 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

AgNPs were characterized using TEM, which revealed that they had a spherical form and smooth surfaces. They are roughly 20 nm in size and have an agglomeration tendency (Figure 2a). The peak location correlates to the peak in the JCPDS 04- 0783 reference card, according to the data collected from XRD, and there are also some other peaks in the graph, which are likely caused by stabilizers that were added during the nanoparticles process (Figure 2b).

Cytotoxicity Effect of AgNPs in PC-3 Cells

The cell viability test is a crucial technique for a toxicological inquiry that can reveal details about cell survival, metabolism, and death as well as explain how cells react to medications and toxic substances 16. Using an MTT assay, the produced AgNPs' in vitro lethal effect on prostate cancer PC-3 cells was assessed at various doses. Findings showed that AgNPs may be cytotoxic (have an anticancer impact) on the tested cell line. As seen in Figure 3, there was a dosage and time-dependent decrease in cell viability when PC-3 cells were exposed to AgNPs. Following 24, 48, and 72 hours of treatment, the IC50 values of AgNPs for PC-3 cells were calculated to be 73, 65.7, and 32 g/mL, respectively. Only 22% of the prostate cancer cells were still viable at 80 g/mL after being treated for 72 hours (Figure 3a), indicating that the cells are no longer able to multiply at these concentrations.

The Effect of AgNPs on the Migration of PC3 Cells

We used an in vitro scratch wound-healing model to explore the impact of AgNPs on the migratory ability of PC-3 cells because cancer cell migration is crucial to the course of the disease (17). AgNPs treatment for 24 hours significantly (p0.001) decreased PC-3 cells' migration rate from 60% to 24% when compared to untreated control cells (Figure 4).

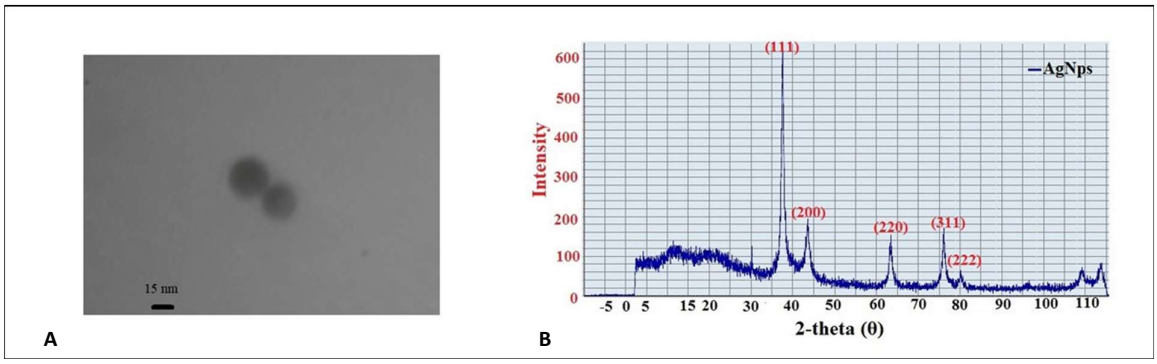

Fig. 2. Characterization of AgNPs by TEM and XRD. A, Smooth surfaces of the tested silver nanoparticles (TEM image). Seve- ral fields were photographed and were used to determine the diameter of nanoparticles. The average range of observed diameterwas 20 nm. B, The X-ray powder diffraction (XRD) peaks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

DISCUSSION

Currently, prostate cancer is regarded as one of the leading causes of cancer-related morbidity and mortality in men globally. New therapeutic techniques are required as alternatives to or in conjunction with existing medicines due to the ineffectiveness and side effects of standard cancer treatments. Recently, nanoparticles have shown a revolutionary

method to defeat cancer, particularly prostate cancer. In anti-microbial research, nanotechnology, biotechnology medication delivery, cancer treatment, and the biomedical field3,9,18, silver nanoparticles are frequently used. The most recent studies described how AgNPs cause cytotoxicity, including the inhibition of cell viability, the induction of oxidative stress, and the involvement of gene expression in the AgNPs-mediated apoptosis of tumor cells15. It has been demonstrated that the toxicity of nanoparticles is affected by their size, surface charge, and shape19. AgNPs in our work had particles with a diameter of about 20 nm. For nanoparticles to aggregate in tumor tissues and pass through the vascular gap of tumor capillaries, the particle size is crucial. So, for nanoparticles to collect in the tumor, they must be smaller than the vascular gap of the tumor capillary (up to 400 nm) 20. According to research, increasing the surface area of the particles while reducing their size aids in particle diffusion across cells18,20.

In this regard, Avalos et al.21 discovered that small AgNPs are significantly more cytotoxic than large AgNPs when examining the effects of different sizes of AgNPs (4.7 and 42 nm) interacting with HepG2 and leukemia cells. Our findings also showed that nanoparticle surfaces had a tendency to aggregate and were spherical, homogeneous, and smooth. The form of nanoparticles is one of the most crucial elements in cellular internalization22. For instance, exocytosis of nanoparticles with sharp forms is less efficient than that of spherical particles23 because of their entrance into the endosome membrane. Moreover, compared to spherical NPs22, ellipsoidal nanoparticles demonstrated reduced cell uptake.

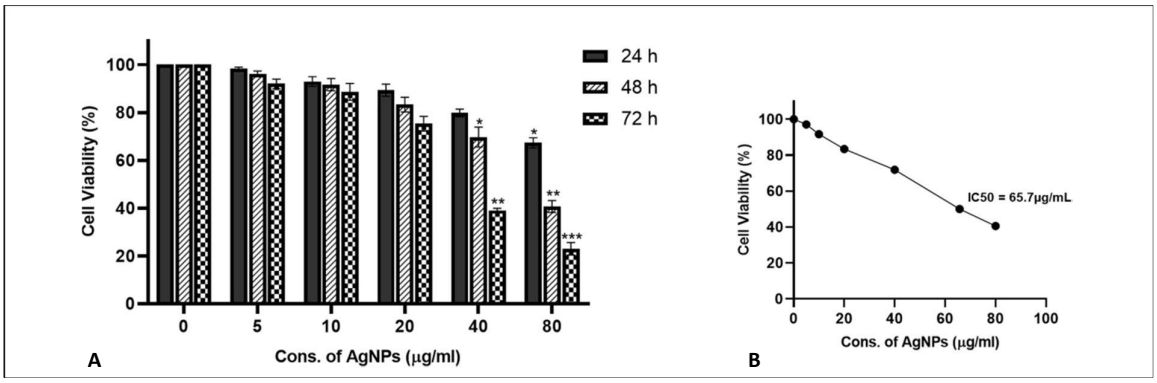

Fig. 3. The anti-proliferative effect of AgNPs induced PC3 cell line. After AgNPs treatment, the MTT data revealed a decrease in cell viability ratio. A, The AgNPs induction in PC3 cells significantly decreased the number of viable cells compared with the control group in a time a concentration manner.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

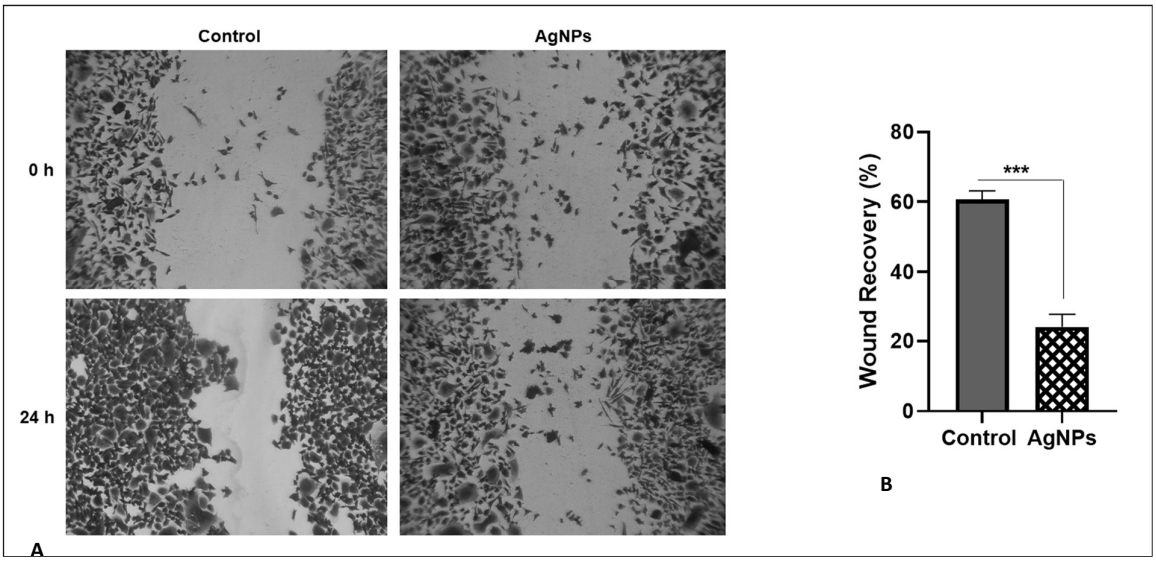

Fig. 4. Effect of AgNPs on PC3 cells migration. A, Cell migration measurement with scratch wound assay in AgNPs (65.7 µg/mL). B, Percentage of wound healing was measured and presented on a histogram using ImageJ software (***$p < 0.001$).

In our investigation, the MTT test was used to determine the half-maximal inhibitory concentration (IC50) of AgNPs, which was found to be 65.7 g/mL after 48 hours. The findings imply that AgNPs have a dose- and time-dependent ability to lower PC-3 cells' viability. AgNPs have been shown to have an impact on MCF-7 human breast cancer cells by Jeyaraj et al24. They reported an IC50 value of 20 g/ mL for 22 nm-sized AgNPs that were slightly agglomerated24. Another study discovered that the IC50 values of manufactured AgNPs against PC-3 were 173.21 g/mL25. According to Krishnaraj et al.26, AgNPs showed comparatively more harmful effects (40%) on MDA-MB-231 cells at a dosage of 100 g/ml than the other treated cells. We observed the lowest viability (22%) of PC-3 cells after a 72-hour treatment with AgNPs at an 80 g/ml concentration.

Inhibiting unchecked and uncontrolled cell proliferation, a key characteristic of cancer, would greatly advance the development of anticancer medications. Other research teams have reported, in accordance with our findings, that AgNPs have a cytotoxic effect on the viability of a variety of cancer cells, including breast cancer MDA-MB-231 cells, breast cancer MCF-7 cells, cervical cancer HeLa cells, human lung cancer H1299 cells, human colorectal adenocarcinoma HT29 cells, human prostate DU145 cells, and prostate cancer LN- CaP cell lines in vitro and in vivo31. Chen et al.32 recently demonstrated that AgNPs could trigger autophagy in PC-3 cells by stimulating the AMPK/mTOR signaling pathway in response to lysosome damage and hypoxia.

We assess AgNPs' impact on prostate cancer cells' rate of migration as well. It is well-known that tumor cell migration is an important stage in the development and spread of tumors33. The most common reason for prostate cancer death is tumor spread to the lungs, bones, and lymph nodes. Interestingly, the PC-3 cells showed a substantial inhibitory efficacy of AgNPs on migration, which was consistent with recent research showing that AgNPs may have a potential role in lowering the migration feature of cancer cells12. The current findings showed how, at inhibitory concentrations, AgNPs can prevent prostate cancer cells from

spreading throughout the body. Other key mechanisms implicated in the anticancer efficacy of silver nanoparticles include stimulation of morphological changes34, loss of cell membrane integrity24, induction of oxidative stress35, increased lactate dehydrogenase leakage, DNA fragmentation and chromosomal aberrations15, uncontrolled cel- lular transport34, mitochondrial dysfunction and loss of ATP synthesis34, caspase-3 activity, and induction of apoptosis24.

CONCLUSIONS

AgNPs may have the ability to have cytotoxic and antiproliferative effects on the PC-3 prostate cancer cell line, according to the findings of the current study. Additionally, one of the ways that AgNPs exert their anticancer effects on cancer cells is likely through inhibiting cell migration. AgNPs may therefore be a candidate for use as a chemotherapeutic agent or in combination with anticancer medications to treat cancer. For the development of AgNPs as novel anticancer therapeutics in the future, additional in vitro and in vivo investigations must be conducted.

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