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Original Article

ANTICANCER ACTIVITY OF SILVER NANOPARTICLE OF PRODIGIOSIN ON LUNG CANCER

SHILPA KARNA, KAVITHA R.*, DAMODHARAN N.

Department of Pharmaceutics, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India
Email: kavithar@srmist.edu.in

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ABSTRACT

Objective: Nanotechnology is a relatively new branch of science and technology that studies and controls the interactions of synthetic and biological materials. Researchers are becoming more interested in nanoparticles as a result of their vast medicinal potential, particularly against cancer.

Methods: The *Serratia marcescens* culture supernatant containing Prodigiosin was used to synthesize silver nanoparticles in an environmentally benign biogenic manner. The effect of nanoparticles on the growth and proliferation of human lung cancer cell (A549) *in vitro* was investigated in this work. MTT Assay and DNA fragmentation assay were used to characterize the nanoparticles that had been produced.

Results: Cytotoxicity of the Prodigiosin AgNPs was represented as IC50 value of 31.2µg/ml and the viability decrease in the number of nanoparticles-treated cells. DNA fragmentation assay showed the degradation of DNA.

Conclusion: The present study conforms as the synthesized Prodigiosin AgNPs can be a promising anticancer agent regarding its mechanism of action.

Keywords: Prodigiosin, Lung cancer, *Serratia marcenscens,* Silver nanoparticles

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INTRODUCTION

The new field of nanotechnology research finds broad application in the development of nanomedicine that spans healthcare-related areas of nanoscience and technology. In the present scenario the development of hygienic and green technologies for nano synthesis plays a vital role. As a substitute to the physical method, the green synthesis method employing microbes are proved to be more viable and simple. Because of their efficacy and low toxicity, nanocarriers have recently become popular options for medication delivery [1]. Metal nanoparticles, in particular, have piqued researchers' interest because to their material features, availability, capabilities, selective targeting, and long-term release. They are most promising for long-term medical use and have a lot of appeal for possible cancer applications. For the creation of silver nanoparticles, several chemical and biological approaches are being investigated.

Various bacteria have been shown to decrease Ag+ions to generate silver nanoparticles, the majority of which are spherical. Brown coloration proved the development of silver nanoparticles, and the result revealed that silver nanoparticles were present. Silver nanoparticles have special features that aid in molecular diagnostics, therapeutics, and gadgets utilized in a variety of medical operations [2]. In bacterial cells, structural alterations in the cell membrane as well as the presence of tiny electron-dense granules produced with silver and sulfur, have been observed. The procedure is easy, dependable, and offers a number of benefits, including low cost, compatibility, and stability, as well as the medical application as a wound dressing against skin infection pathogens [3].

Cancer is an aberrant type of tissue growth in which cells divide uncontrollably and relatively independently, resulting in a steady rise in the number of dividing cells [4]. It is a huge public health issue around the world and the world's second biggest cause of mortality [5]. There are over 100 different forms of cancer. The combination of nanoparticles and biological molecules has resulted in the development of diagnostic gadgets, contrast agents, and critical cancer treatment techniques. The most important goals of cancer therapy are the identification of novel targets and the development of more selective chemotherapeutic drugs [6].

Prodigiosin the red pigment, is produced by many gram-negative bacteria, such as various strains of the bacterium *Serratia marcescens, Vibrio psychroerythrous, Pseudomonas magneslorubra, Serratia rubidaea* etc. *Serratia marcescens* produces prodigiosin, a bacterial secondary metabolite that is a tripyrrole nitrogen ring pigment [7]. Prodigiosin has a wide range of biological actions, including antimalarial, antifungal, immunosuppressive, and antibiotic properties, as well as the ability to cause malignant cancer cells to die [8]. Prodigiosin's cytotoxic characteristics have been known for several decades [9]. During the stationary phase of bacterial development, *Serratia marcescens* produces it through a bifurcated biosynthetic route in which mono and bipyrrole acquired separately are joined to make linear tripyrrole red pigment [10].

The present work was focused to apply the accurate principles of green methods for the development of biologically synthesized metal nanoparticles by using *Serratia marcescens* in the presence prodigiosin. Cytotoxicity of the prodigiosin AgNPs against human lung cancer cell line A549 was studied and it indicate that this nanoparticle can be further used for the applications of drug delivery.

MATERIALS AND METHODS

Bacterial strain and cultivation

Serratia marcescens KJ was identified by analysing its morphological characteristics, biochemical and physiological properties, and the 16S rDNA sequence [11]. The bacterial strain was grown in nutrient broth and incubated at 35 °C for 72h with agitation at 100rpm [12].

Cell culture

The NCIM in Pune provided lung cancer (A549) cell lines. Monolayer cultures of cells were maintained in RPMI-1640 70 supplemented with 100 g/ml streptomycin, 100 U/ml penicillin, and 10% (v/v) FBS (all from Himedia). All cells were cultivated in a CO2 incubator at 37 °C in a humidified environment of 95% air and 5% CO2 [13].

Biosynthesis of AgNPs

The fermented nutrient broth culture was centrifuged at 12000g for 10 min. The resulting supernatant filtered by using 0.22 µm nitrocellulose membrane filter. The filtrate was mixed with 1 nm to 5nM of HAgCl4 solution as ratio of 9:1 v/v. The formulation noted as F1, F2, F3, F4 and F5 respectively. The preparation incubated at 37 °C in rotary shaker. The formation of silver nanoparticles was determined by colour change and the it confirmed by UV-Vis spectra observed in the range of 500-600 nm [14, 15].

Characterization of AgNPs

Transmission Electron Microscopy was used to examine the produced AgNPs (TEM). The samples were fixed and dehydrated as specified, with the exception of the agar-embedding stage, for TEM investigation. The sample was analyzed by transmission electron microscopy (TEM) using a PHILIPS-CM 200 apparatus with a resolution of 0.23 nm and an accelerating voltage of 200 kV. One of the most essential techniques for determining the structure of crystalline materials is X-ray diffraction. The lynx eye detector on this equipment can be used to evaluate nanoparticles that have been prepared [16, 17]. In a mortar, dried materials were crushed with KBr at a 1:100ratio. The pressed pellet was recovered using a clip and immediately examined with a Nicolet5700 FTIR spectrometer in the range of 4,000–400 cm-1 with a resolution of roughly 2 cm-1 over 1,800 scans [18].

Determination of cytotoxicity by MTT assay

In 96-well plates (0.2 ml/well), cells were seeded at a density of 0.2105 cells/ml and incubated for 24 h. The cells were then treated for 72 h with varied dosages of AgNPs (2–30 µg/ml). MTT was added to the medium and left for an hour to incubate. After carefully removing the medium, 0.1 ml of dimethyl sulfoxide (DMSO) was added to each well and incubated for 5 min while shaking. A microplate reader was used to measure the absorbance at 570 nm. MTT was added to the medium and left for an hour to incubate. After carefully removing the medium, 0.1 ml of dimethyl sulfoxide (DMSO) was added to each well and incubated for 5 min while shaking. A microplate reader was used to measure the absorbance at 570 nm [19, 20].

DNA fermentation assay

The DNA ladder assay was used to investigate the induction of apoptosis. $1X10^6$ cells were lysed in a 250-micro liter lysis solution that contained 50 mmol Tris HCl, pH 8.0, 10 mmol ethylene diamine tetra acetic acid, 0.1 M NaCl, and 0.5 percent sodium dodecyl sulfate. The lysate was treated with 0.5 mg/ml RNase A for 1 h at 37 °C, followed by 0.2 mg/ml proteinase K overnight at 50 °C. This combination was phenol extracted, and DNA was precipitated in the aqueous phase using 25 µl (1/10 volume) of 7.5 M ammonium acetate and 250 µl (1/1 volume) isopropanol. DNA fragments were seen by exposing the gel to UV light followed by photography after electrophoresis on a 1 percent agarose gel containing 1 µg/ml ethidium bromide at 70 V [21].

Statistical analysis

The differences between groups were analyzed using one-way ANOVA in this study. All of the experiments were carried out in threes. Results with a P value of less than 0.05 were deemed statistically significant. The half maximum inhibitory concentration (IC_{50}) was estimated using the Sigma Plot tool and a 4-parameter curve fit (version 12, SPSS, Inc., Chicago) [22, 23].

RESULTS AND DISCUSSION

Characterization of AgNPs by UV-Vis spectroscopy

When aqueous silver nitrate solution was mixed with *Serratia marcescens* culture, the color of the solution changed from green to brown, owing to the reduction of silver metal ions. Excitation of surface plasm on vibrations in silver nanoparticles (AgNPs) results in yellow-brownish coloration of AgNPs in an aqueous solution, as shown. The collective oscillation of free conduction band electrons in AgNPs causes a significant absorption peak in the 420–500 nm range [24, 25]. UV visible spectroscopy was used to study the influence of varying $AgNO_3$ concentrations on the synthesis of *Serratia marcescens* cells-aided AgNPs. The $AgNO_3$ concentration was steadily increased (1 mmol, 2 mmol, 3 mmol, 4 mmol, 5 mmol) by maintaining the cell culture volume constant (10 ml). Constantly increases in intensity as a function of reaction time, with no change in the peak of wavelength [26]. The peak intensity was found to have grown when more silver ions were converted to silver nanoparticles. For 4 mmol $AgNO_3$, however, the absorbance peak is independent. The given result the optimum concentration of $AgNO_3$ is 4 nm (F4) for the biosynthesis of silver nanoparticles.

FTIR analysis

The functional groups present on the surface of bio-synthesized AgNPs were identified using the spectrum. The finger print region exhibited strong broad vibration at 3285 cm^{-1} and was a unique nature observed in the functional group of Prodigiosin. In addition, it also exhibited vibration similar to PGD at 1636 cm^{-1}, 1454 cm^{-1} representing the C=C and N-H bending, respectively. The characteristic vibrations observed in the PGD carbon skeleton was observed at 2928 cm^{-1},2115 cm^{-1} and 1074 cm^{-1} representing the carbon bonds of CH2, C=O, N-H stretching vibrations of mine and carboxylic acids. This was well matched with the literature values [27]. Hence, FTIR study showed that bioactive compounds of *Serratia marcescens* ie., Prodigiosin formed a strong coating on biosynthesized silver nanoparticles. When comparing these FTIR spectra to those from a previous work, a shift on the alkynes stretching 2115 and 2928 cm-1 was found, which could be ascribed to the presence of NAg/AgClPs. These findings support prior observations that protein can interact strongly with Ag to reduce the size of nanoparticles [28].

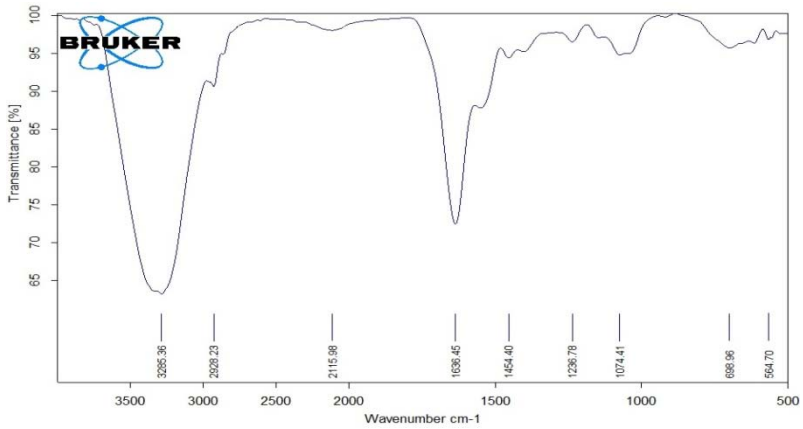

Fig. 1: FTIR spectrum of prodigiosin silver nanoparticles

TEM analysis of silver nanoparticles

The appearance and size of the bio-reduced AgNPs were determined using transmission electron microscopy. The nanoparticles had a restricted size distribution and were virtually spherical in form. In addition, TEM images revealed well-dispersed spherical AgNPs with particle sizes ranging from 10 to 60 nm (fig. 2). Due to solvent evaporation or particle aggregation during TEM measurement, a few particles were agglomerated. The similar result was reported by Rajashekar *et al.* as silver nanoparticles formed mostly spherical and cubical structure with high aggregation when they used algal extract of *Pandinatetrastromatica* [29].

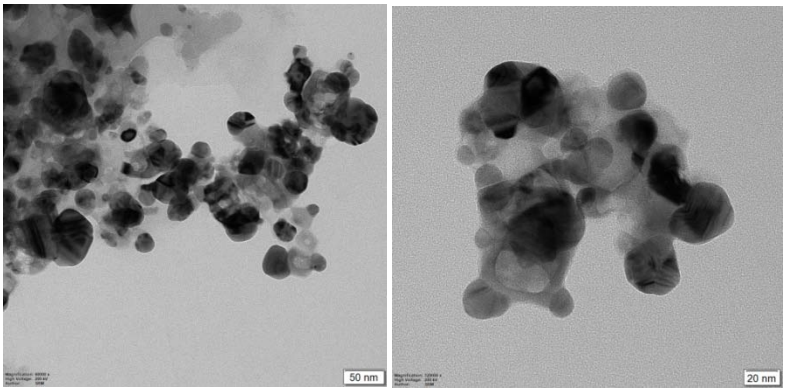

Fig. 2: TEM image of AgNPs with spherical-shaped particles

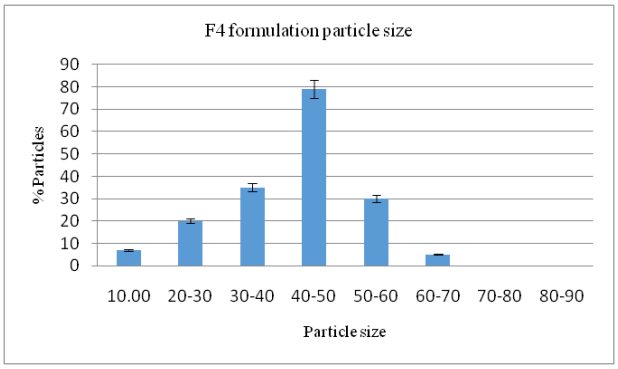

Fig. 3: Showed that the size of most particles ranged from 10-60 nm. Data are expressed as mean±standard error of the mean (n=3)

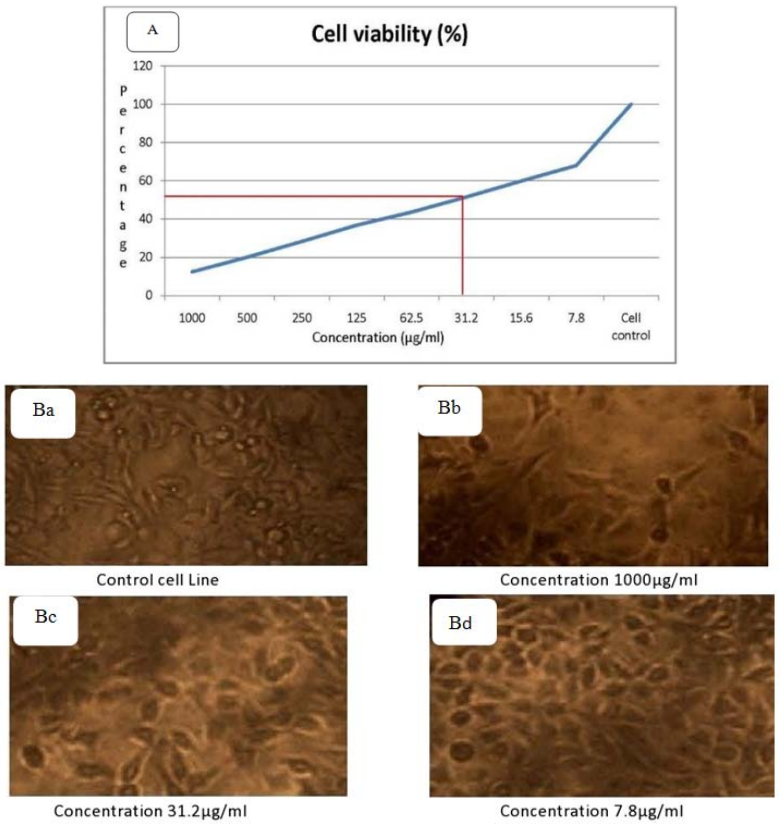

Fig. 4: Cytotoxicity results of synthesized Silver nanoparticles towards A549 cells. A) IC_{50}value of prodigiosin silver nanoparticles at different concentration. B) Morphological study of AgNPs against A549 cells, at different nanoparticles dosage. Control cells (Ba), 1000µg/ml, 13.2 µg/ml, 7.8 µg/ml (Bb-Bd)

Cytotoxicity analysis of AgNPs

MTT viability assay

The cytotoxicity of produced AgNPs against cancer cells ofA549 was assessed using the colorimetric MTT test. Over seven distinct concentrations of investigated materials ranging from 3.12 to 200 g/ml, cell viability was expressed as a viable cell percentage of control. The amount of MTT solution converted to purple formazan crystal by mitochondrial succinate dehydrogenase was used to measure cell viability. The cytotoxicity of green-produced AgNPs is said to be dependent on their size and form, which interfere with cellular metabolic activities. The untreated control cells were smooth and had a healthy cell wall. The cells that were challenged with their IC50 and the highest dose of 1000g/ml showed a dose-dependent decrease in cell population, reduced cell membrane, and significant breakdown of cell membrane. Based on morphological analysis of AgNPs treated cancer cells showing inhibition of cell growth and change in cells membrane surface in MCF-7 [30].

DNA fragmentation assay

The DNA ladder approach was also used to confirm apoptotic induction. The AgNPs caused chromosomal DNA to degrade into smaller fragments (fig. 5), which is a biological marker of cells undergoing apoptosis. Because electrophoresis of necrotic cells' DNA produces a smear rather than a ladder, induction of apoptosis, rather than necrosis, by AgNPs was proven once more. Kora and Sashidhar established that AgNPs are able to exert toxicity by inducing apoptosis, increasing the production of reactive oxygen species (ROS), leading to oxidative stress and causing DNA damage on human cancer cell lines.

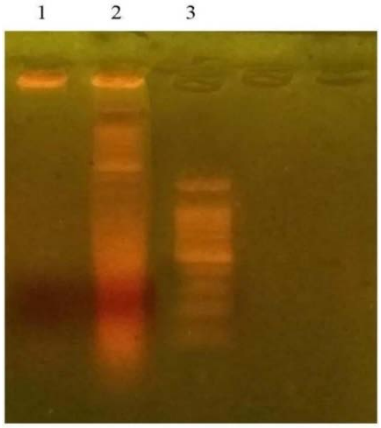

Fig. 5: DNA fragmentation assay for the detection of the mode of the cell death with the treatment of the Prodigiosin AgNPs on Lung cancer cell line A549. Lane 1: Control DNA (without fragment), Lane 2: Indicating the fragmented DNA, Lane 3: DNA ladder

CONCLUSION

We have successfully generated AgNPs from the bacteria *Serratia marcescens* using the prodigiosin and demonstrated their efficacy against medically important infections and cancer cell lines in this study. The nanoparticles are tested for biological properties such as anticancer activity against a lung cancer cell line (A549) and antibacterial activity against four pathogenic bacteria. As a result of our findings, we conclude that these nanomaterials are biocompatible and effective antibacterial and cancer therapeutic agents. These prodigious silver nanoparticles may also prove to be beneficial in the therapy of cancer, particularly lung cancer. Green chemistry is important because it is non-toxic, inexpensive, and environmentally benign. In the field of cancer medication development, green manufacturing of silver nanoparticles has gotten a lot of attention. In the future, we intend to conduct *in vivo* investigations in order to develop novel cancer detection and treatment options.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

There are no conflicts is declare

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