Original Article

UNVEILING THE APOPTOTIC POTENTIAL OF SILVER NANOPARTICLES ON HUMAN COLON CARCINOMA HT29 CELLS

C. RANJITH KUMAR[1,2], DR. SUDHA RAMAIAH[1], DR. ANANDANBARASU[1]

[1]Medical and Biological Computing Laboratory, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamilnadu, India.

[2]Nanobiotechnology Division, Dr Yellapragada Lifesciences, Chennai-600077, Tamilnadu, India.

Email: aanand@vit.ac.in

DOI: 10.47750/pnr.2022.13.S06.338

Abstract

The synthesis of nanoparticles is an essential element of nanotechnology, because of its potential for use in chemotherapies, the interest in this area of research is expanding. For the synthesis of silver nanoparticles, the chemical reduction technique was used. According to these investigations, the particles have an average size of 16 nm and are mainly spherical in shape. Additionally, A dose-dependent cytotoxicity was also shown by the produced AgNPs against a human colon cancer cell line (HT29). At 72 hours of incubation, it was discovered that the inhibitory concentration (IC50) values for HT29 cells were 48.12 g/ml. The use of AO/EtBr labelling demonstrated an apoptotic induction. The current findings clearly suggested that HT29 cell lines treated with AgNPs would exhibit anticancer activity.

Keywords: Silver nanoparticle, Cytotoxicity, HT29 cells, Apoptosis, AO/EtBr .

1. INTRODUCTION

Rapid advancements of nanoparticles over conventional materials in recent decades, the use of nanotechnology in several scientific domains has raised significant concerns. Silver nanoparticles are synthesized by chemically converting silver salt precursors to silver nanoparticles using a number of widely known methods. Recent research emphasises the relevance of synthesizing silver nanoparticles and analysing their size and characteristics [1, 2, 3]. The top-down method in nanoscience involves mechanically grinding bulk metals and stabilising the resulting nano-sized metal particle by adding colloidal protective chemicals [4], whereas the bottom-up method starts with molecules or atoms to produce nanoparticles [5]. The present study was aimed for the synthesis of silver nanoparticles by chemical route [6, 7] which is an easy, simple and easy method for preparing metal particles in nano regime. A simple reaction to produce metal nanoparticles is reduction of the matching metal cation. Co-precipitation, a frequent term for the general process, refers to the co-occurrence of many phenomena, including reduction, nucleation, growth, coarsening, and/or agglomeration [8, 9].

The present study represents the synthesis of silver nanoparticles by chemical reduction method and their characterization using UV spec, X-ray Diffraction, Thermogravimetric analysis and HR-TEM. Furthermore, current studies on the cytotoxic responses of the colon cancer cells exposed to silver NPs are very limited. Therefore, we selected HT29 cell line as a model in this study to confirm the anticancer activity of silver nanoparticles through cytotoxicity and morphological evidences.

2. MATERIALS AND METHODS

2.1 Chemicals

Silver nitrate, 3- [4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT), Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), Dulbecco's Modified Eagles Medium (DMEM), antibiotics and ethidium bromide were purchased from sigma, USA. Other chemicals and consumables were purchased from Hi-Media Laboratories, Mumbai, India.

2.2 Synthesis of silver nano particles:

0.0169g of silver nitrate was dissolved in 100 ml of ethanol and heated in an ambient atmosphere to create the silver nitrate solution and 1g of trisodium citrate was dissolved in 100 ml of distilled water to create the 1 percent tri sodium citrate solution. 20ml of silver nitrate solution was kept in hot plate at 900C for 5 minutes and then add 2.5 ml of trisodium citrate drop by drop once the reduction process begins colour change appears and the solution turn into pale yellow. After the changes in colour, solution was stirred in magnetic stirrer for 15mins [10].

2.3 Characterization of silver nano particles

The synthesized silver nano particles were characterized by UV Vis spectroscopy; size and morphology by employing TEM, crystalline structure from X-ray diffraction (XRD) technique and stability by employing Thermogravimetric analysis.

2.4 Cell lines Maintenance

Human colon Cancer cell lines (HT29) was procured from National Centre for Cell Science (NCCS), Pune, India. These cell lines were cultured in a culture dish containing DMEM media with 10% FBS. The culture dishes were incubated at 37oC in a humidified 5% CO2 incubator until the experiments start.

2.5 Treatment of Cancer cell line with Plant extract

Different concentration (25, 50, 75 and 100 µg/ml) of AgNPs were prepared in 100 µl of 5% DMEM. In short, after 24 h, the growth medium was removed and each concentration of 100 µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO2 incubator for 72 hours.

2.6 Cytotoxicity test for plant extracts (MTT Assay)

The determination of cell cytotoxicity of AgNPs on colon cancer cell lines (HT29) was carried out, using a colorimetric assay that measures the reduction of yellow-3-(4,5Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) by mitochondrial succinate dehydrogenase enzyme described by Mosmann in 1983. The number of living cells and % of inhibition is directly proportional to the level of the formazan. The intensity of colour was quantified using a simple colorimetric assay. The results can be found out on a multi-well scanning spectrophotometer (ELISA reader).

2.7 Acridine orange/ethidium bromide (AO/EB) staining

The control and treated cells were seeded in a 6 well plate (3 × 104 /well) and they were treated with different concentrations of AgNPs and incubated in CO2 incubator for 72 h. The cells were fixed in methanol: glacial acetic acid (3:1) for 30 min at room temperature washed in PBS and stained with 1:1 ratio of AO/EtBr. Stained cells were immediately washed with PBS and viewed under a fluorescence microscope with a magnification of ×40.

3. RESULTS & DISCUSSION

Silver nanoparticles were formed after the reduction of aqueous silver salts with tri sodium citrate (1%) within 10-20 minutes of the reaction, the change in pale yellow colour appeared after the completion of reaction. The growth of silver “seed

nucleation" was observed when the reducing agent is added into aqueous silver salt in drop wise manner. This led to the localized surface plasmon due to the optical property of (Pale yellow colour) synthesized silver nanoparticle.

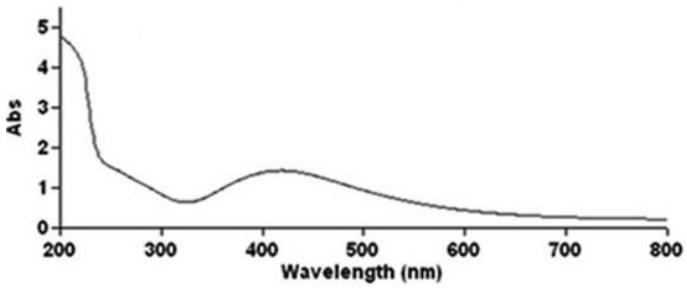

Figure 1: UV Visible spectra analysis of the silver nanoparticles.

The UV visible spectra analysis of the silver nanoparticles (Figure. 1) synthesized by chemical reduction show the characteristic absorption peak at 420 nm and the peak broadening indicates that the silver nanoparticles are poly dispersed in nature and the size uniformity [11].

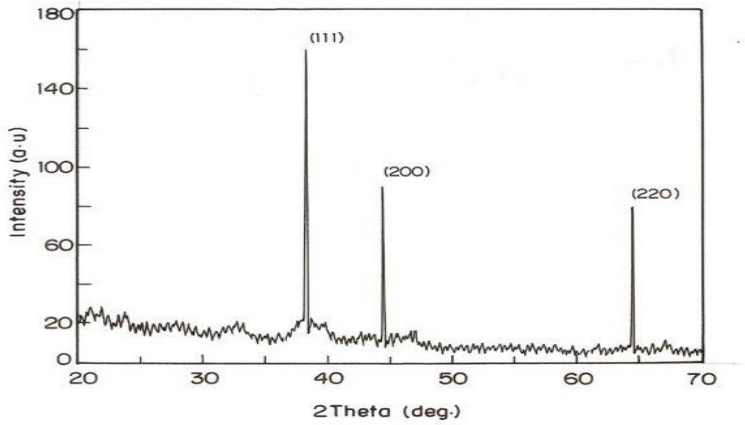

Figure 2: XRD pattern of the synthesis of silver nanoparticles.

In accordance with Wang's (2000) [12] description, Figure 2 displays the X-ray Diffraction patterns of silver nanoparticles that were captured. As part of the XRD examination, samples were air dried, powdered, and utilised. The XRD patterns made from chemically reduced silver nanoparticles display typical peaks at the (2 = 1) position, denoted by the number 111. Based on the face-centered cubic structure of silver, it is possible to index the Bragg reflections that are detected to correspond to the 111 sets of lattice planes. The silver nanoparticles' crystalline nature is therefore amply demonstrated by the XRD pattern. There are documented peaks at 2 =1 in the XRD pattern of pure silver ions. A value of = 4.081 has been calculated as the lattice constant for pure silver, which is compatible with the value of = 4.0862 A0 published by the JCPDS file number 4-0783. This calculation verified the particle polycrystallinity hypothesis. Sharper peaks show that the particles are in the nano regime, which is obvious. Scherrer's formula was used to calculate the silver nano crystallites' sizes, which were then reported. This silver peak has an FWHM of 111.

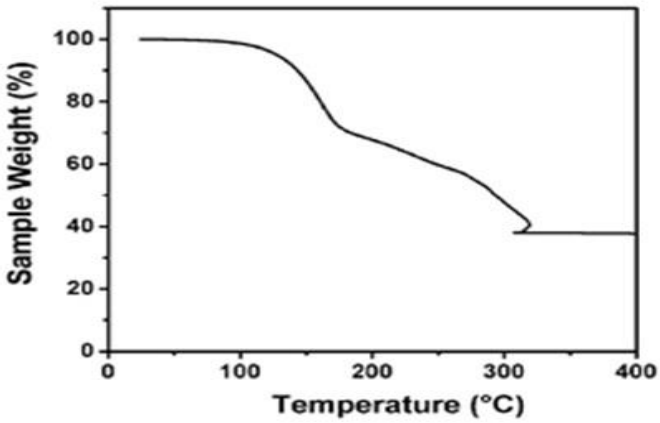

Figure 3: Thermogravimetric analysis of silver nanoparticles

To determine the quality of the silver nano particles, a thermo gravimetric study up to 4000c was performed on them (Figure. 3). At 2600C, the silver nanoparticles were found to be stable, and 14% of impurities were added to their surfaces to decorate them [13], resulting in a monolayer coating that is insignificant compared to the impurities in the sample.

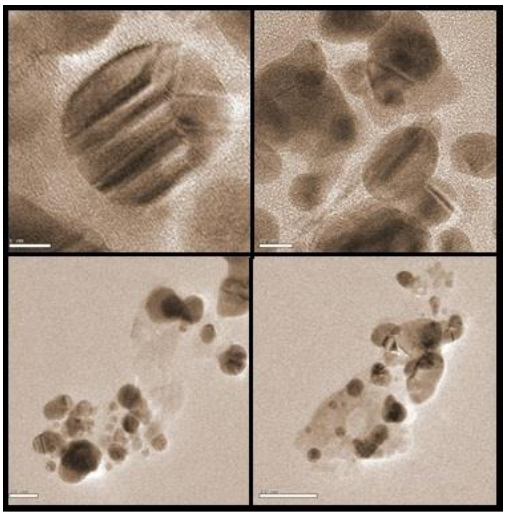

Plate 1: HR-TEM Images of the silver nanoparticles

According to Plate 1, the TEM samples were obtained at 5 nm, 10 nm, 20 nm, and 50 nm. The morphology of silver nanoparticles is evident in transmission electron microscopy pictures, and it is highly varied, with well-formed cubical and spherical shapes in aggregates [14] and a size range of 5 to 50 nm.

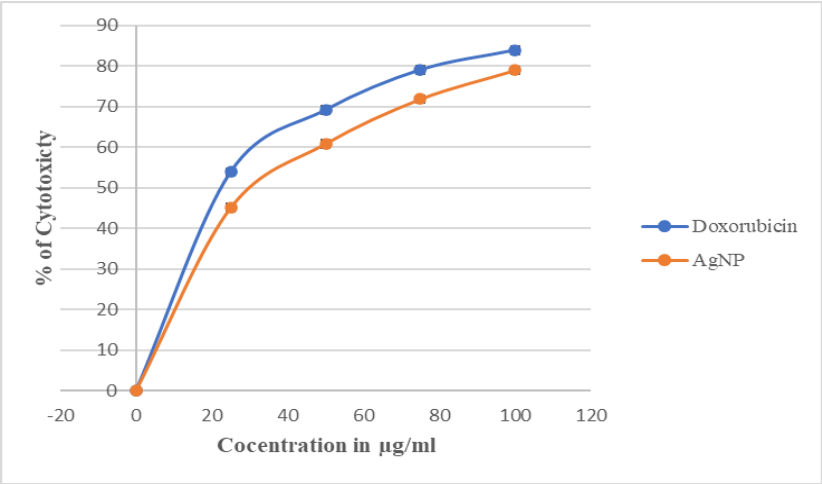

Figure 4: MTT assay-based cytotoxicity effect using Ag NPs against HT29 Cell lines

Figure 4 shows the percentages of cell viability attained after a continuous 72-hour exposure. It was discovered that NPs' cytotoxicity was concentration-dependent. Additionally, it was discovered that cell viability declined with concentration. The MTT assay was used to determine the IC50. For the positive controls doxorubicin and AgNPs, the inhibitory concentration (IC50) values for the HT29 cells were 40.62 g/ml and 48.12 g/ml, respectively.

HT29 cells treated with drugs and AgNPs showed morphological differences from untreated cells. Plate 2. bright field and fluorescence photos taken after silver nanoparticles were applied to HT29 cells and cultured at 37 C for 72 hours. Bright field microscopy used to detect cytotoxicity. AO/EtBr-stained HT29 cells that had been treated and left untreated were examined using fluorescence microscopy.

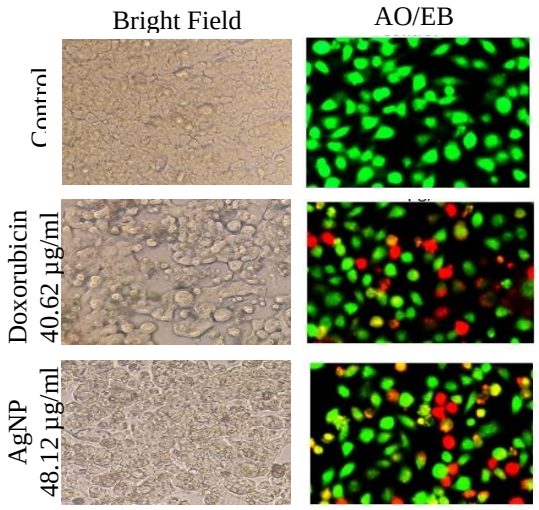

Plate 2: Bright field and fluorescence microscopy images of IC50 concentration of silver nanoparticles treated on HT29 cell lines

Live cells are represented by cell lines that are green, while apoptotic cells are represented by orange, and necrotic cells are represented by red. In contrast to HT29 cells, the AgNPs treatment caused the most obvious morphological changes, including cytoplasmic condensation, cell shrinkage, the production of numerous cells with surface bulges at the plasma membrane, and the accumulation of nuclear chromatin into dense masses beneath the nuclear membrane. The membranes of these cells also distorted and took the form of vesicles. Both cells underwent AgNPs treatment and had rounder morphologies and condensed chromatin. We also saw a decline in the populations of HT29 cells and progressive structural alteration [15, 16].

CONCLUSION

The current study may present a chance to concentrate on the therapeutic active principles of AgNPs against human colon cancer cell lines in order to expand the potential for clinical applicability and to clarify the precise molecular pathways involved in the cell growth suppression.

ACKNOWLEDGEMENT

Authors would like to acknowledge the support for the management of Vellore institute of Technology to carryout this research work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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