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Pure silver nanoparticles showed potential anticancer effect on colon and breast cancer cell lines

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ABSTRACT

Present study was designed to investigate the anticancer potential of silver nanoparticles (AgNPs) on Human cancer cell lines (MCF-7 & HCT-15). AgNPs showed a dose-dependent toxicity towards both the cell lines. AgNPs decreased the viability of tested cells to 50% at 24.44ppm for MCF-7 & 12.89ppm for HCT-15 cells respectively. Percent cytotoxicity of AgNPs was determined by Lactate dehydrogenase (LDH) Assay. LDH activities in medium of AgNPs treated cells were found to be significantly elevated on both the cell lines as compared to the non treated cells. This study revealed that AgNPs demonstrate strong anticancer potential on MCF-7 and HCT-15, however more effect was observed on MCF-7 than HCT-15 cell.

INTRODUCTION

Cancer is the third leading cause of death in developed countries and the second leading cause of death in the United States (Jemal et al., 2011). Many challenges remain in treating cancer patients, including treatment-related adverse events, poor outcomes and the lack of a therapeutic target and balancing treatment toxicity with quality of life in patients with metastatic cancer who have already received extensive therapy. Therefore, there is still an urgent need for new therapeutic options for cancer.

To overcome these problems, nanotechnology has been introduced by researchers for the detection, treatment and prevention of cancer at its early stages (Yezhelyev et al., 2006). Among the noble metals, silver is the most widely used and studied due to its unique properties and its use in the biomedical field. Silver is non-toxic, under the suitable dosage it can be an antibiotic (Alt et al., 2004). Silver powder is effective in treatment for ulcers and until the dawn of antibiotics, silver compounds were a major weapon against wound infection in World War I (Chen and Schluesener, 2008). Nanosilver exhibits remarkable physical, chemical and biological properties. It is widely used as an antibiotic against wound infections and serious burn injuries (Silver et al., 2006). Colloidal silver disables oxygen metabolism enzyme of virus, fungus, bacterium or any other single cell pathogen (Panacek et al., 2006; Rhee et al., 2008). Colloidal silver has been used to treat different infections or parasitic diseases. Intravesical administration of nanocrystalline silver (1%) decreased urine histamine, bladder tumor necrosis factor-alpha and mast cell activation without any toxic effect. This action may be useful for interstitial cystitis (Boucher et al., 2008). Silver nanoparticles exhibit improved properties depending upon their size, morphology and distribution (Awwad et al., 2013). Silver nanoparticles have potent, antiangiogenic, anticancer, antibacterial, antifungal and larvicidal properties (Gnanadesigan et al., 2012).

To the best of our knowledge this is the first study to demonstrate the anticancer potential of pure AgNPs MCF-7 and HCT-15 cell lines. AgNPs were produced with physical vapor deposition (PVD) process which maintains 99.99% purity, and the unique technology was applied to allow our AgNPs to be evenly dispersed in sterilized water. Therefore, unlike AgNPs made with chemical reduction which involves the addition of dispersing agent to avoid the aggregation of nanoparticles, the AgNPs used in this study are evenly suspended in water without the addition of dispersing agent which further increases the purity of AgNPs

MATERIALS AND METHODS

Reagents

RPMI-1640, DMEM, FBS and Sulphorhodamine Blue were procured from Sigma-Aldrich. All the other chemical compounds used in this study were of analytical grade and received from (Sigma-Aldrich, E-Merck and SRL chemical substances Pvt. Ltd. and so on.). LDH kit was procured from coral clinical systems.

Preparation of Silver nanoparticles

Silver nanoparticles (3-5nm) were manufactured by Gold NanoTech, Inc., Taipei, Taiwan, by physical vapor deposition (PVD). Nanoparticels were suspended in sterilized water to maintain 99.99% purity.

Cell maintenance and culture procedures

The human Colon (HCT-15) and Breast (MCF-7) cancer cell lines were obtained from National Center for Cell Science Pune. Cell lines were grown and maintained in RPMI-1640 and DMEM medium, pH 7.4. The media were supplemented with complete medium containing fetal bovine serum (FBS) (10%), penicillin (100 U/ml) and streptomycin (100 mg/ml). The cells were grown in CO2 incubator at 37°C with 80% humidity and 5% CO2, changing media at least twice a week (Devi and Bhimba, 2012) .

Cytotoxicity assay

When the culture reached at 90% confluent, cells were detached by trypsinised Hank's buffer to make single cell suspensions. The number of cells/ml of suspension was counted with the help of haemocytometer and adjusted to 40,000 cells/ml of cell suspension by diluting with complete medium. The cell suspensions (100 µL/well) were seeded to each well of 96-well plates. The plates were then incubated at 37˚C in an atmosphere of 5% CO2 and 90% relative humidity for 24 hrs. After 24h, cells were treated with different concentration of silver nanoparticles (25, 12.5, 6.25, 3.125ppm) and incubated for 24h at 37 °C, 5 % CO2, 90% relative humidity. Cell viability was assessed by SRB assay.

Sulphorhodamine Blue (SRB) Assay

SRB is a pink aminoxanthine, water soluble dye is used to assess cell growth. It binds to the basic amino acid residues of cellular proteins in the plasma membrane. The adsorbed dye is dissolved in alkaline medium and solubilized stain is measured spectrophotometrically to determine viability in treated and untreated cells. Thus colorimetric measurement of the bound dye gives an estimate of the total protein mass that is directly related to the cell number. SRB (100 µl) was added to each well and plate was kept at room temperature for 30 min. The plate was then washed with 1% acetic acid for four times.

Plate was dried and 100 µl Tris-buffer was added to each well to soluble the dye. The plates were shaken gently for 10 min on a shaker and the optical density was recorded on ELISA reader 540 nm. percent Cell growth and percent Growth inhibition was calculated as follows

Calculation

$$\% \text{ Cell growth} = \frac{\text{Cell growth in presence of test material}}{\text{Cell growth in absence of test material}} \times 100$$

% Growth inhibition = 100 – % Cell growth

LDH leakage assay

Intracellular lactate dehydrogenase (LDH) leakage is a well known indicator of cellular membrane integrity. In present study LDH leakage assay was performed by using commercial kit manufactured by coral clinical systems. Cells were treated with AgNPs for 24h and LDH activity in media was estimated to confirm the cellular damage. For the measurement of total LDH another aliquot of cells were treated with 0.25 ml of Triton X–100. LDH in the media was determined according to the manufacturer's protocol. Briefly working reagent was prepared by mixing buffer reagent with starter reagent (4:1ratio). 0.02ml of sample (media) was added with 1ml of working reagent, mixed well and absorbance was read at 340nm. Mean absorbance change was calculated per minute (ΔOD/min.). Then percent cytotoxicity was determined as follows.

$$\% \text{ cytotoxicity} = \frac{\Delta\text{OD of treated cells} - \Delta\text{OD of blank}}{\Delta\text{OD of lysed cells} - \Delta\text{OD of blank}}$$

RESULTS

The cytotoxic activity of the AuNPs on cancer cells

The antiproliferative effect of the AgNPs was evaluated in vitro against cells at four different concentrations (25, 12.5, 6.25, 3.125ppm). Table 1 depicts that AgNPs were able to decrease viability of the HCT-15 and MCF-7 cells in a dose-dependent manner as. AgNPs at a concentration of 25ppm showed (61.7%) and (51.4%) inhibition in HCT-15 and MCF-7 cell lines respectively. AgNPs showed 12.4%, 4.3% and 1.0% growth inhibition of HCT-15 cells and 38.3%, 35.6% and 34.3% growth inhibition of MCF-7 cells at 12.5ppm, 6.25ppm, 3.125ppm respectively.

Inhibitory concentration 50 (IC50)

Figure 1A and 1B demonstrates the inhibitory concentration 50 (IC50) of AgNPs on HCT-15 and MCF-7 cells. The calculated IC50 values of AgNPs are 12.89ppm in HCT-15 cells and 24.44ppm in MCF-7 cells respectively.

LDH Leakage Assay

Figure 2 depicts the percent cytotoxicity of AgNPs on HCT-15 and MCF-7 cells which was confirmed by LDH assay. After 24h exposure of AgNPs at 25ppm, significantly raised activities of LDH was found. AgNPs showed 50.2% and 59.9% cytotoxicity on MCF-7and HCT-15 respectively.

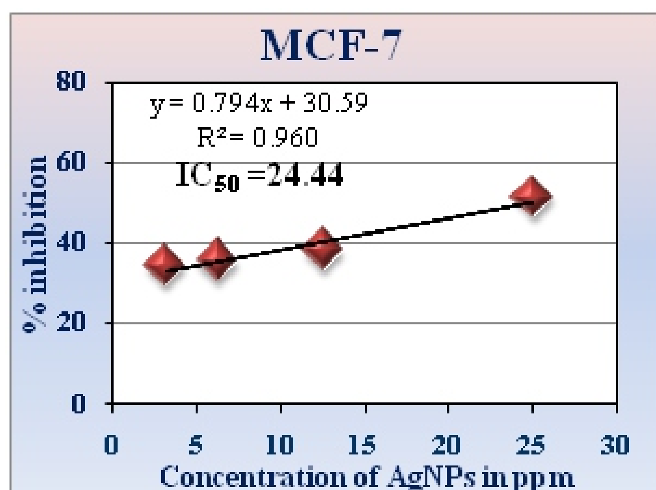
DISCUSSION

The biomedical applications of silver nanoparticles are promising with their tremendous effects in the fields of medicine, drug delivery and anti angiogenic property of cancer (Sahoo et al., 2004; Gurunathan et al., 2009). In the present study in vitro antiprolifirative effect of AgNPs was screened against two cancer cell lines (HCT-15 and MCF-7) and their viabilities were evaluated using SRB assay.

Table 1: Antiproliferative effect of AgNPs on HCT-15 and MCF-7cancer cells

Concentration	HCT-15	MCF-7
AgNPs 25 ppm	61.7± 3.41	51.4±2.84
AgNPs 12.5 ppm	12.4±0.69	38.3±2.12
AgNPs 6.25 ppm	4.30±0.24	35.6±1.97
AgNPs 3.12 ppm	1.00±0.06	34.3±1.90

Values are the mean± S.E. of three wells in each group of three repeated experiments



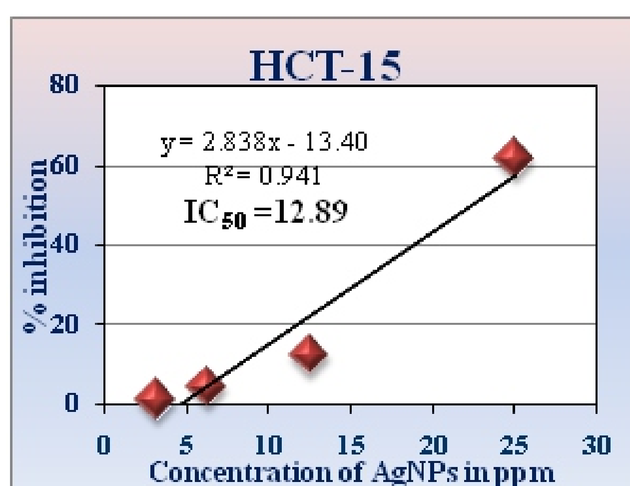


Figure 1. IC50 of AgNPs on HCT-15 and MCF-7 cancer cell lines

Values are the mean± S.E. of three wells in each group of three repeated experiments. IC50 determined by Graph-pad instat software

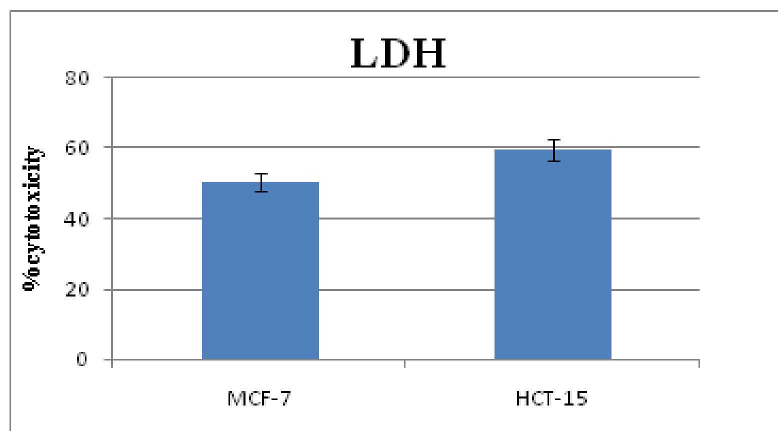


Figure 2: Determination of toxicity of AgNPs on HCT-15 and MCF-7 cell lines by LDH leakage assay

Values are the mean± S.E. of three wells in each group of three repeated experiments

Our results demonstrated that AgNPs inhibited the viability of HCT-15 and MCF-7 cells in a concentration dependent manner. Concentration of 25ppm was found more effective on HCT-15 cells as compared to MCF-7 cells, however lower doses were more effective on MCF-7 cells than HCT-15 cells. Inhibitory concentration 50 (IC50) was calculated and was found 18.63ppm for HCT-15 cells and 15.83ppm for MCF-7 cells which confirmed that AgNPs are more effective on MCF-7 than HCT-15 cells. Antiproliferative nature of AgNPs in present investigation correlates with the findings of Nagajyothi et al., (2014) in which they reported antiproliferative activity of AgNPs against A549 human lung cancer cell line and MCF-7 human breast cancer (HTB-22) cell line. Likewise, Verma et al., (2013) reported anticancer activity of silver nanoparticles biosynthesized by Penicillium spp against human colon adenocarcinoma (HT 29) cell lines.

In the present study, estimation of LDH leakage assay was performed to confirm the marked cell membrane damage in cancer cells. LDH is a cytosolic enzyme and is released during the damage of cell membrane. Our results demonstrated that LDH activities were found to be significantly enhanced in the medium of cells treated with AgNPs, which signified that AgNPs induced cellular damage in both cell lines. Our results are in consistant with the investigation of Satyavani et al., (2011). More effect was observed on HCT-15 cells than MCF-7 cells.

CONCLUSION

In conclusions, AgNPs showed potential anticancerous potential towards both the cancer cell lines however more effect was found on HCT-15 cell line than MCF-7 cells. Use of AgNPs should emerge as one of the novel approaches in cancer therapy. However more study is needed to understand the molecular mechanism of action.

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Disclosure statement

No potential conflict of interest was reported by the author.

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