

RESEARCH ARTICLE

ANTICANCER ACTIVITY OF SILVER NANOPARTICLES AGAINST HUMAN BREAST CANCER CELL LINE

Dinesh, B¹., R. Ranjithkumar², C. Sharmila³, K. Selvam⁴ and
B. Chandar Shekar^{5*}

¹Biology Division, Department of Applied Sciences, Higher College of Technology, Muscat-133, Sultanate of Oman.

²Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India.

³Department of Physics, PSGR Krishnammal College for Women, Coimbatore-641004, Tamil Nadu, India

⁴Department of Botany, Periyar University, Salem, Tamil Nadu, India.

⁵Department of Physics, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India.

ABSTRACT

The present study demonstrated the effectiveness of bioinspired synthesized AgNPs against MCF-7 breast cancer cell line, we found a dramatic decrease in cell viability when the concentration of the bioinspired synthesized AgNPs was increased and there was a dose-dependent reduction in cell viability. This study further indicates the significance of green technology for nanoparticle fabrication and future application in control of several human diseases.

Keywords: Anticancer activity, silver nanoparticles, human breast cancer cell line.

1. INTRODUCTION

Cancer is considered as one of the most deadly disease in the world with high mortality. Subsequently, there are several cancer therapies available, chemotherapy has become an important component of cancer treatment for most cancers. In the area on oncology drug discovery, conventional chemotherapeutic agents still exhibit poor specificity in reaching tumor tissue and are often restricted by dose-limiting toxicity (1). The combination of developing controlled-release technology and targeted drug delivery many provides a more efficient and less harmful solution to overcome the limitations in conventional chemotherapy. Recent interest has been focused on developing nanoscale delivery vehicles, which are capable of controlling the release of chemotherapeutic agents directly inside cancer cell (2).

Nanomaterials are expected hopefully to modernise the cancer diagnosis and therapy. Nanoscale particles decorated with multiple functionalities are able to target and visualize tumor site via an imaging technology, thereby allowing of the early detection of cancer at begging stage onwards. Moreover, intelligent nanosystems can be constructed as controlled delivery vehicles which are capable of delivering anticancer drugs to a predetermined site and then releasing them with programmed rate, which can improve therapeutic efficacy (3). The advent of nanotechnology is considered to be the biggest engineering innovation since the industrial revolution. Proponents of the new technology promise to re-engineer the mam-made world, molecule by molecule sparking a wave

of novel revolutionary biomedical products from machines to medicine (4). In inorganic nanoparticles, metal nanoparticles have received considerable attention in recent years because of their unique properties and potential applications in catalysis, photonics, optoelectronics, biological tagging and pharmaceutical application (1,5,6). The discovery and identification of new antitumor drug with low side on immune system has become an essential goal in many studies of immno-therapies (7).

The most effectively studied nanoparticles today are those made from Noble metals, in specifically Ag (8), Au (9) Pt and Palladium (10). The metal nanoparticles find vast applications in various fields ranging from medical to physical fields (6,11-13). Among these metallic nanopartilces, silver nanoparticles play a significant role in the field of biological system, living organisms and medicine (14). Nowadays the silver nanoparticles are one of the most commonly nanomaterials both in everyday life and in research laboratories. Silver nanoparticles are incorporated into many commercial products including clothing/textiles, furniture, household appliances such as refrigerators, cosmetics and even children toys (15). Manikandan *et al.*, (16) demonstrated that the biosynthesis of silver nanopartilces using ethanolic petals extract of *Rosa indica* exhibited potent antimicrobial property against human pathogenic bacteria, anticancer activity against human colon cancer cell and shows potent anti-inflammatory activity. This high degree of AgNPs commercialization has been achieved due to their significant antimicrobial and antifungal and

*Correspondence: Chandar Shekar, B., Nanotechnology Research Lab, Department of Physics, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: chandar.bellan@gmail.com

anticancer activity (17). Hence, the aim of this present study deals with the *invitro* anticancer potential of silver nanoparticles (AgNPs) synthesized from the aqueous extract betel nut and betel leaf on MCF-7 human breast cancer cell line.

2. MATERIALS AND METHODS

2.1. Cell culture

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity (18).

2.2. Synthesis and characterization of nanoparticles

Betel nut (BN) and betel leaf (BL) aqueous extract mediated green synthesized silver nanoparticles preparation methodology, characterization and antibacterial properties were already reported.

2.3. *In vitro* cytotoxic of AgNPs

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10⁵ cells/mL. About 100µl per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 h 100µl of medium containing the treated with various concentrations (25, 50, 100, 150 and 250 µg/ml) bioinspired synthesized AgNPs from betel nut and betel leaf aqueous extract. The treated cells were then incubated at 37°C, 5% CO₂, 95% Air and 100% relative humidity for 48 h. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.4. MTT assay

After cell treatment process, the cells were then subjected for MTT assay. The stock concentration (5mg/ml) of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, a yellow tetrazole) in phosphate buffered saline (PBS) was prepared and 15µl of MTT was added in each AgNPs treated well and incubated at 37°C for 4 h. The medium with MTT was then flicked off and the formed purple color formazan

crystals were solubilized in 100µl of Dimethyl sulphoxide (DMSO), and read at 570 nm in a multi well ELISA plate reader. Each experiment was performed in triplicate for each experiment. *In vitro* Cytotoxicity was calculated at the percentage of viable cells at different concentration of sample relative to untreated (Control) cell. Optical density (OD) value was subjected to sort out percentage of cell inhibition by using the following formula (19).

$$\text{Percentage of viability} = \frac{\text{Mean OD value to experimental sample (AgNPs)}}{\text{Mean OD value to experimental control (Untreated)}} \times 100$$

Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population IC₅₀(Incubation Concentration) was determined.

2.5. Morphological observation

MCF-7 cells were grown and incubated with AgNPs at their IC₅₀ concentration, the AgNPs treated and untreated plates were observed under amicroscope to detect morphological changes and photographed.

3. RESULTS AND DISCUSSION

Silver nanoparticles as a potent antimicrobial agent, is gaining greater demand in medical applications (14). At the same time, there are only limited studies in the cytotoxic effects of biological route synthesized silver nanoparticles, against human cancer cell lines. In this present experiment, MTT assay was used to assess the effects of AgNPs on proliferation of MCF-7 cells. Best of our knowledge, this is the first study to report the anticancer activity of silver AgNPs synthesized using aqueous extract betel nut and betel leaf against breast cancer cell lines (MCF-7). The obtained results of the present experiment revealed that the dose dependent cytotoxicity in AgNPs treated MCF-7 cells.

3.1. *In vitro* assessment of BN-AgNPs cytotoxicity

The aqueous extract of betel nut mediated bioinspired synthesized silver nanoparticles(BN-AgNPs) demonstrated a considerable cytotoxicity against MCF-7 human breast cancer cell lines at different concentration (25, 50, 100, 150 and 250 µg/ml). Figure 1 depict 87 % of cell death was observed in maximum concentration (250 µg/ml) of BN-AgNPs. More than 60 % of cell death was observed in concentration of 150µg/ml. Consequently, fifty percentage of cell death, which determines the inhibitory concentration (IC₅₀) value of bioinspired BN-AgNPs against human

breast cancer MCF-7 cells holds at around 108µg/ml.

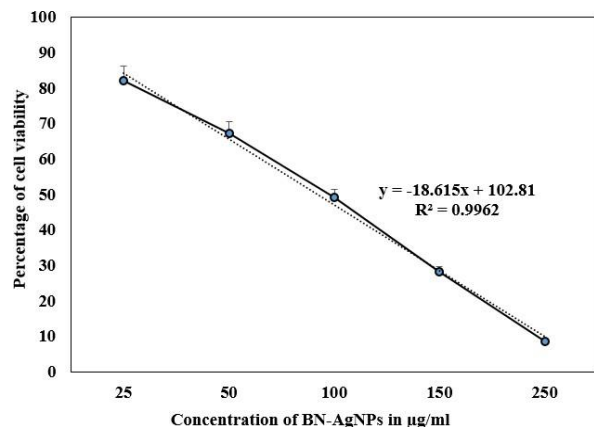


Fig. 1. Cytotoxicity effect of BN-AgNPs on cancer cell line MCF-7

Previously cytotoxicity effect of biological route synthesized silver nanoparticles has been reported against different cancer cell lines including, human lung cancer (A549) cell line (20), human epithelium cells (HEP G2) of liver cancer (21), human acute promyelocytic leukemia (HL-60) cell line (22) and human cervical carcinoma (Hela) cell line(23). Recently it was reported by Palaniappan *et al.* (24) that green synthesized colloids nano silver particles using *Cymodocea serrulata* leaf aqueous extract triggers cellular toxicity in treated potential against human lung cancer A549 cell lines. Hence, in this report we found that 150 µg/ml of bioinspired synthesized BN-AgNPs inhibits more than 60% of breast cancer cells. In addition, the microscopic observation of untreated and different concentration of BN-AgNPs treated cell lines showed in Figure 2. The improved cytotoxicity is mainly due to its easy permeability to the cellular barriers and its strong affinity towards biological macromolecules, addition it release reactive oxygen species that cause damages to cellular components via intercellular oxidative stress (25).

The aqueous extract of betel leaf mediated bioinspired synthesized silver nanoparticles (BL-AgNPs) revealed a significant cytotoxicity against human breast cancer MCF-7 cell lines at different concentration (25-250 µg/ml). The obtain results of the present experiment indicated more than 90 % of cell death was observed in maximum concentration at 250 µg/ml of BN-AgNPs. At the BL-AgNPs concentration of 150µg/ml showed around 71 % of cell death. Thus, fifty percentage of cell death, which determines the inhibitory concentration (IC₅₀) value of bioinspired BL-AgNPs

against human breast cancer MCF-7 cells holds at around 96 µg/ml. Cytotoxicity assays of BL-AgNPs achieved more than 50 % of cell death was observed in concentration of 100 µg/ml (Fig. 3).

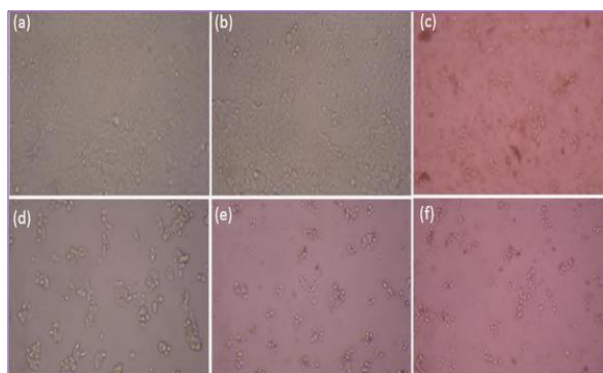


Fig. 2. Microscopic observation of MCF cell treated with BN-AgNPs (a) Control (Untreated) (b) 25µg/ml, (c) 50 µg/ml, (d) 100 µg/ml, (e) 150 µg/ml and (f) 250 µg/ml. (More than 60 % of cell death observed at the concentration of 150 µg/ml).

3.2. In vitro assessment of BL-AgNPs cytotoxicity

Similar report of cytotoxicity was discussed by Sukirtha *et al.* (19) against Hela cell lines by using *Melia azedarach* mediated green synthesized AgNPs. Another report by Vivek *et al.* (18) noticeably discussed cytotoxicity activity of *Annona squamosa* extract reduced green synthesized AgNPs are exerting effect on human breast cancer MCF-7 cells in vitro at lower concentration level and did not affect the normal HBL (100) at lower concentration. Conversely, increased concentration of AgNPs produced significant toxicity against the normal HBL 100 cell. Similarly, our present experimental study revealed the dose dependent cytotoxicity was observed in AgNPs treated MCF-7 cells.

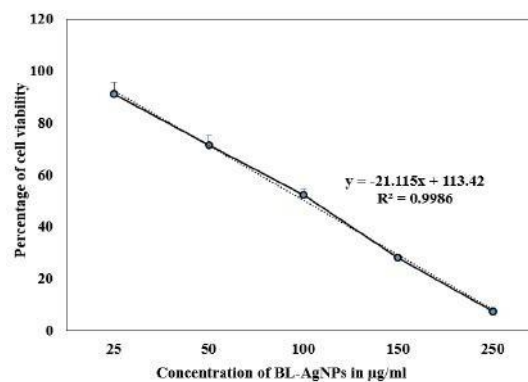


Fig. 3. Cytotoxicity effect of BL-AgNPs on cancer cell line MCF-7

A few in vitro studies have previously shown translocation of AgNPs in cancer cell with an IC₅₀ value of 300 µg/ml (26) 30 µg/ml (16). In fact, inside cancer cells AgNPs may induce reactive oxygen species and cause damage to cellular components leading to cell death (27). Likewise, our present study exposed the presence of 100 µg/ml of betel nut aqueous extract assets bioinspired silver nanoparticles (BL-AgNPs) is sufficient to inhibit the more than 50% of MCF-7 breast cancer cells (Fig. 4).

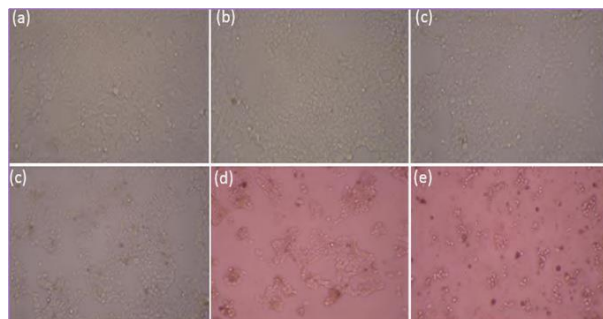


Fig. 4. Microscopic observation of MCF cell treated with BL-AgNPs (a) Control (Untreated) (b) 25 µg/ml, (c) 50 µg/ml, (d) 100 µg/ml, (e) 150 µg/ml and (f) 250 µg/ml. (More than 70 % of cell death observed at the concentration of 150 µg/ml).

4. CONCLUSION

The present study demonstrated the effectiveness of bioinspired synthesized AgNPs against MCF-7 breast cancer cell line, we found a dramatic decrease in cell viability when the concentration of the bioinspired synthesized AgNPs was increased and there was a dose-dependent reduction in cell viability. The decrease in cell viability with increase in AgNPs concentration, suggests that more number of AgNPs could accumulate inside cells resulting in enhanced stress, ultimately leading to cell death. The obtained results from the present study clearly show the enhanced effectiveness of the biologically synthesized AgNPs against MCF-7 breast cancer cells. This study further indicates the significance of green technology for nanoparticle fabrication and future application in control of several human diseases.

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