

Original Article

ENHANCED APOPTOSIS IN MCF-7 HUMAN BREAST CANCER CELLS BY BIOGENIC GOLD NANOPARTICLES SYNTHESIZED FROM ARGEMONE MEXICANA LEAF EXTRACT

SELVARAJ VARUN, SUDHA SELLAPPA*

Department of Biotechnology, School of Life Sciences, Karpagam University, Eeachanari post, Coimbatore 641 021, Tamil Nadu, India.
Email: sudhasellappa@gmail.com

Received: 10 Jul 2014 Revised and Accepted: 10 Aug 2014

ABSTRACT

Objective: To assess the *in vitro* anticancer activity of *A. mexicana* mediated gold nanoparticles on MCF-7 breast cancer cells.

Methods: The present study reveals the synthesis and characterization of gold nanoparticles from aqueous extract of *Argemone mexicana* L. by biological method. UV-Vis spectroscopy, XRD, FT-IR, SEM and TEM analyses revealed that green synthesized gold nanoparticles were 26 ± 5 nm in size and spherical in shape. MCF- 7 Breast cancer cells were treated with different concentrations of green synthesised gold nanoparticles at different time intervals. Cytotoxicity activity and the mechanism of apoptosis were determined by morphological and chromatin cleavage assays.

Results: Green synthesised gold nanoparticles showed dose-dependent cytotoxic and apoptotic effect in MCF-7 breast cancer cells at concentration of 100 $\mu\text{g}/\text{mL}$.

Conclusion: The present investigation showed that *Argemone* mediated gold nanoparticles may be an alternative chemotherapeutic agent for treatment of breast cancer.

Keywords: *A. mexicana*, Breast cancer, Gold nanoparticles, MCF-7 Cells, MTT assay.

INTRODUCTION

Gold nanoparticles play a vital role in nano biotechnology as biomedicine for convenient surface bio conjugation with bio molecular probes and remarkable plasmon resonant optical properties [1-3]. Gold nanoparticles have a significant function in the delivery of proteins, nucleic acids, *in vivo* delivery, gene therapy, targeting, etc. [4]. Green synthesis of gold nanoparticles using diverse plant extracts have been reported [5-7]. In biological methods, it is found that the extracts of living organisms act both as reducing and stabilizing agent in the synthesizing process of the nanoparticles [8]. *A. mexicana* L. belongs to Papaveraceae family. Phyto constituents such as alkaloids (chelerytherine, sanguinarine, protopine, optisine and berberine) and oil are predominantly present *A. mexicana* leaves. Roots and seeds are traditionally used for skin-diseases, leprosy, bilious fever and inflammations [9]. The aim of present work is to investigate the possibility of synthesizing gold nanoparticles by reduction method using *A. mexicana* leaves as capping agent and to assess the *in vitro* anticancer activity of *A. mexicana* mediated gold nanoparticles on MCF-7 breast cancer cells.

MATERIALS AND METHODS

Materials

Fresh and healthy *Argemone mexicana* L. leaves were collected from Coimbatore district, which belongs to Tamil Nadu, India. It was identified (code number is 1399) by Botanical Survey of India, Coimbatore. All glass wares were washed with distilled water and dried in oven before use. All the chemicals and solvents used in this experiment were of analytical grade and were purchased from Sigma-Aldrich Chemicals, India.

Maintenance of cell cultures

Human breast MCF-7 cell culture was procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were maintained and propagated in Eagles Minimum Essential Medium (EMEM) supplemented with Fetal Bovine Serum (10%, v/v) at 37°C in a CO₂ incubator (5% CO₂, 95% air and 100% relative humidity). MCF-7 cells were harvested after brief trypsinization.

Synthesis and characterization of *Argemone* mediated gold nanoparticles

Gold nanoparticles were synthesized through reduction of gold chloride by aqueous extract of *Argemone* leaf extract. The optical properties of green synthesized gold nanoparticles were analyzed by Ultra Violet-visible spectroscopy at 200–800 nm wave length range. *Argemone* mediated gold nanoparticles were characterized with the help of X-ray diffraction, FT-IR, scanning electron microscopy and transmission electron microscopy.

Trypan blue dye exclusion assay

Trypan blue dye exclusion assay was analysed for to evaluate the influence of *Argemone* mediated gold nanoparticles on the viability of MCF-7 cells. The cells (0.5×10^5 cells/ml) were seeded in six well plates in complete medium. The MCF-7 cells were mixed with different (6.25, 12.5, 25, 50 and 100 $\mu\text{g}/\text{mL}$) concentrations of *Argemone* mediated gold nanoparticles and incubated for 24 and 48 h. After incubation period, the cultures were harvested and washed. Then it was resuspended with PBS (0.4% trypan blue) and cell were counted using hemocytometer. Each experiment was carried out with three replications.

MTT assay

In order to evaluate cytotoxicity of *Argemone* mediated gold nanoparticles, MCF-7 cells were collected in the exponential phase of growth, seeded into 96-well tissue culture plates (15,000 per well) and allowed to adhere for 48 h. Then, 6.25, 12.5, 25, 50, 100 $\mu\text{g}/\text{mL}$ concentration of *Argemone* mediated gold nanoparticles were added to the desired wells and incubated for 48 h. After incubation, 20 μl of EMEM medium having MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. Subsequently, the medium was changed with 100 μl of DMSO, and optical densities were measured at 570 nm. All investigations were performed in triplicates. All the measurements were made in triplicate and expressed as the mean \pm standard error.

Colony-forming assay

MCF -7 cells were plated at a density of 7×10^2 cells per well in six well plates and treated with *Argemone* mediated gold nanoparticles

at various concentrations (6.25, 12.5, 25, 50 and 100 µg/ml). The control cells have been also maintained. Media was altered after 48 h of incubation and the colonies were observed further for 7 days. Cells were stained using crystal violet. Colony number was determined manually.

Morphological assay

The apoptotic effects of *Argemone* mediated gold nanoparticles on MCF-7 cells were determined using nuclear DNA staining assay. MCF-7 cells (*Argemone* gold nanoparticles-treated and untreated) were fixed in 4% of paraformaldehyde in PBS (phosphate-buffered saline) for 20 min. the cells were washed with PBS twice and stained using Propidium Iodide (1 µg/mL) in PBS for 15 min. Stained cells were washed with PBS twice. The stained cells and its changes in nuclei were detected under UV fluorescent microscope.

Caspase activities

MCF-7 cells were seeded in 6-well plates and treated with different concentrations of *Argemone* mediated gold nanoparticles for 48 h. After incubation, the MCF-7 cells were collected and caspase -3 & -9 activity was analysed using a colorimetric assay kits. In this assay, colorimetric detection was performed by chromophore p-nitroaniline (pNA), labelled substrate DEVD-pNA (caspase 3), IETD-pNA (caspase 8) and LEHD-pNA (caspase 9). The free pNA was measured by spectrophotometer at 405 nm.

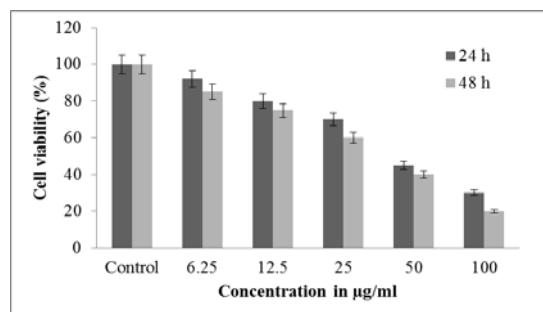


Fig. 1: MCF-7 cells were treated with various concentrations of green synthesised gold nanoparticles for 24 h and 48 h, and the viable cells were determined by the trypan blue-dye exclusion method.

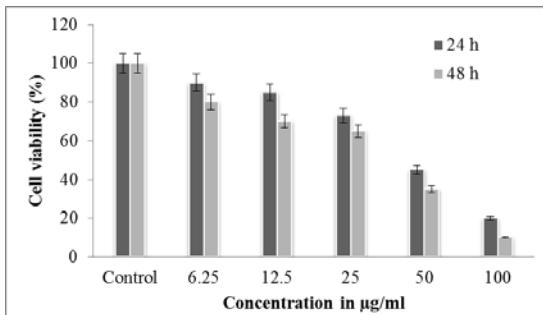


Fig. 2: Effect of various concentrations of green synthesised gold nanoparticles for 24 h and 48 h on viability of MCF-7 cells as measured by MTT assay

DNA Fragmentation assay

DNA fragmentation assay was carried out using agarose gel electrophoresis. After 48 h, *Argemone* mediated gold nanoparticles treated cells were harvested through centrifugation. The cell pellets were mixed with 100 ml lysis buffer and incubated at 50° C for 1 h. Then, 10 µl of Proteinase K was added and incubated at 50° C for 30 min. 3µl of RNase was then added and incubated at 50° C for 2 h. The DNA was extracted by phenol: chloroform: isoamyl alcohol (25:24:1) and 2.5 volume of absolute ethanol. 2.0% of agarose gel was used

and stained with ethidium bromide. The fragmented DNA was visualized under UV light transilluminator.

Statistical Analysis

All experiments were carried out three times. Results were represented as means ± standard deviation (S.D). Student t-test was performed and p<0.05 was considered as statistically significant.

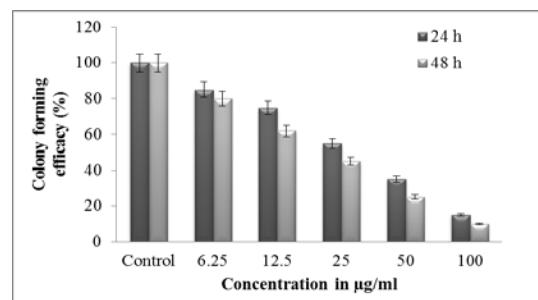


Fig. 3: Effect of various concentrations of green synthesised gold nanoparticles for 24 h and 48 h on viability of MCF-7 cells as measured Colony-forming assay

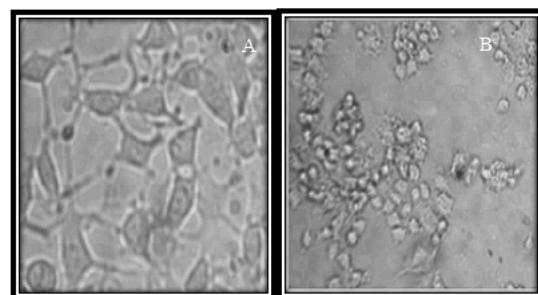


Fig. 4: Apoptotic assay A. Control and B. 100 µg/ml gold nanoparticles treated MCF-7 cells

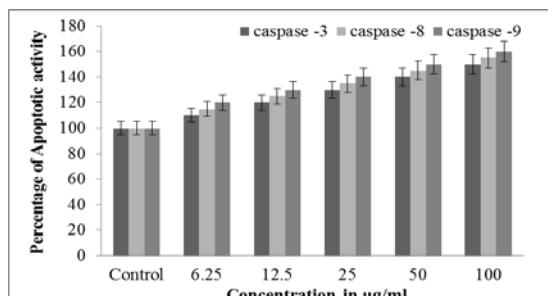


Fig. 5: Dose-dependent increase of caspase -3,-8 and -9 activity in MCF-7 cells treated with different concentrations of green synthesised gold nanoparticles for 48 h.



Fig. 6: DNA fragmentation in MCF-7 cells treated with gold nanoparticles after 48 h (Lane 1: Marker, Lane 2: control MCF-7 cells DNA, Lane 3: gold nanoparticles treated MCF-7 cells DNA)

RESULTS AND DISCUSSION

Characterization of gold nanoparticles

UV- Visible absorption spectrum of gold nanoparticles reveals that gold nanoparticles are mono dispersed. Green synthesized nanoparticles show a broad absorption peak at 545 nm. The band gap of gold nanoparticles was calculated by using formula $E = hc/\lambda$, where h = plank's constant, c = velocity of light and λ = wavelength. The band gap of gold nanoparticle has been stated earlier [10]. X-ray diffraction pattern was done to confirm the phase of gold nanoparticles. The peaks at 2 θ values of 38.2°, 44.5°, 64.7° and 77.6° correspond to crystal planes of (111), (200), (220) and (311) of gold nanoparticles. The diffraction peaks denote as crystalline phase, which was assessed with the data from JCPDS card No. 89-7102. The narrow and strong peak represents that the particle has well crystalline nature. The particle average size was calculated by the Scherrer formula and found to be in the range of 22-26 nm. Similar report of XRD for gold nanoparticles synthesized using plant extract was found by Shankar et al. [11]. FT-IR, SEM and TEM analyses revealed that green synthesized gold nanoparticles were 26 ± 5 nm in size and spherical in shape.

Trypan blue dye exclusion assay

The cytotoxic effect of *Argemone* mediated gold nanoparticles on human breast cancer cell line MCF-7 was analysed for their viability using the trypan blue dye exclusion method. The cells were counted at 24 and 48 h of treatment with gold nanoparticles after staining with trypan blue. After 48 h, gold nanoparticles exhibited a significant increase in cell death than 24 h after treatment. At the concentrations of 50 and 100 $\mu\text{g}/\text{ml}$ resulted in increased cell death whereas at concentration of 6.5 $\mu\text{g}/\text{ml}$ has lowest cell death. Figure 1 clearly reveals that induces cytotoxicity in human breast cancer cells in a dose and time dependent manner, which reflecting the loss of viability.

MTT assay

The anticancer activity of the *Argemone* mediated gold nanoparticles was evaluated against MCF-7 breast cancer cell lines at a various concentrations (6.5- 100 $\mu\text{g}/\text{ml}$). Figure 2 shows the anticancer activity of *Argemone* mediated gold nanoparticles and IC₅₀ value for gold nanoparticles was found to be 54.25 $\mu\text{g}/\text{ml}$. Maximum concentration of gold nanoparticles (100 $\mu\text{g}/\text{ml}$) effectively inhibits the growth of cell by more than 97%. Selim and Hendi [12] reported the anticancer activity of gold nanoparticles and cytotoxic effects of gold nanoparticles against MCF-7 human breast cancer cells.

Colony-forming assay

Colony forming assays showed that, at 50 and 100 $\mu\text{g}/\text{ml}$, green synthesised gold nanoparticles could drastically suppress the MCF- 7 cells, with a 75% reduction compared to control. A minimum (30% inhibition) effect on colony formation was observed in 6.5 $\mu\text{g}/\text{ml}$ gold nanoparticles treated MCF-7 cells (Figure 3). Many investigations were conducted on tumors exhibit resistance to radiation [13] and chemotherapeutic drugs and enhance the colony-forming efficiency [14].

Morphological assay

The stimulation of green synthesised gold nanoparticles mediated cell apoptosis was observed by nuclear DNA staining assay. The survival rate of the cells after treatment of gold nanoparticles was plotted in graphically against the time and dose dependent manner (Figure 4). After 24 h, gold nanoparticles treatment MCF-7 cells showed the characteristic of apoptosis such as chromatin condensation, membrane blebbing and cell shrinkage. 85% of apoptotic changes were detected after 72 h. The data showed and confirmed that the apoptosis was induced by the green synthesised gold nanoparticles.

Caspase activities

Figure 5 shows that the activity of Caspase-3- protease was increased. However, the activity of Caspase -8 and -9 was significantly high in the gold nanoparticles treated MCF-7 breast

cancer cells. The results determined that gold nanoparticles exhibit cytotoxic effects via blocking the multiplying of MCF-7 cells and inducing apoptosis by controlling the expression of caspase-3, -8, and -9 levels. Conversely, despite the lack of Caspase-3 expression, MCF-7 cells undergo morphological apoptosis in response to a variety of agents [15].

DNA fragmentation assay

Figure 6 shows the DNA fragmentation of green synthesised gold nanoparticles treated and untreated MCF-7 breast cancer cells. DNA marker (1 kb ladder) (Lane 1) was used to find the molecular weight of cleaved DNA fragments. The MCF-7 breast cancer cells (untreated gold nanoparticles- control) DNA was showed in Figure 8 (Lane 2). Extensive double strand DNA breaks was observed in gold nanoparticles treated MCF-7 breast cancer cells (Figure 8-Lane 3). The DNA fragmentation assay was subsequently evidenced for induction of apoptosis. DNA fragmentation was induced by silver nanoparticles on MCF-7 cells [16] which is good agreement for this investigation.

CONCLUSION

We have demonstrated the synthesis of gold nanoparticles using green chemistry approach. *A. mexicana* leaf aqueous extract has been used as a reducing and stabilizing agent for the synthesis of gold nanoparticles. The present investigation was determined that the potential anti-cancer activity of *A. mexicana* gold nanoparticles by using MCF-7 cells through induction of apoptosis and by inhibits growth or metastasis or cell proliferation. Our data support the hypothesis that green synthesised gold nanoparticles suppress the growth of breast cancer cells through extraordinary increase in cell apoptosis. Gold nanoparticles would be a wonderful innovation in the field of nano medicine.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENTS

We thank to the Management of Karpagam University, Coimbatore, Tamil Nadu, India for providing necessary facilities to carry out this work.

REFERENCES

- Wu CC, Chen DH. Facile green synthesis of gold nanoparticles with gum arabic as a stabilizing agent and reducing agent. *J Gold Bull* 2010;43:234-9.
- Daniel MC, Astruc D. Gold nanoparticles:assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *J Chem Rev* 2004;104:293-346.
- Kreibig U, Vollmer M. Optical properties of metal clusters. Springer-Verlag, Berlin;1995.
- Tiwari PM, Vig K, Dennis VA, Singh SR. Functionalized gold nanoparticles and their biomedical applications. *J Nanomaterials* 2011;1:31-63.
- Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videau J, Troiani HE, Santiago P, Jose-Yacaman M. Formation and growth of Au nanoparticles inside live *Alfalfa* Plants. *J Nano Letters* 2002;2:397-401.
- Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of gold and silver nanoparticles using *Emblica Officinalis* fruit extract and their phase transfer and transmetallation in an organic solution. *J Nanosci Nanotechnol* 2005;5:1665-71.
- Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinna-mommum camphora* leaf. *J Nanotechnology* 2007;18:105104-15.
- Parashar V, Parashar R, Sharma B, Avinash Pandey C. *Parthenium* leaf extract mediated synthesis of silver nanoparticles:a novel approach towards weed utilization. *Dig J Nanomater Bios* 2000;4:45-50.
- Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research:New Delhi;1986.

10. Noruzi M, Zare D, Khoshnevisan K, Davoodi D. Rapid green synthesis of gold nanoparticles using Rosa hybrida petal extract at room temperature. *J Spectrochim Acta Part A* 2011;79:1461-65.
11. Shankar SS, Rai A, Ankamwar B, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci* 2004;15:496-502.
12. Selim ME, Hendi AA. Gold nanoparticles induce apoptosis in MCF-7 human breast cancer cells. *Asian Pacific J Cancer Prev* 2012;13:1617-20.
13. Prathyumnan S, Sellappa S, Subhadra Keyan K, Joseph S. Enhanced calreticulin expression triggers apoptosis in the MCF-7 cell line. *Asian Pacific J Cancer Prev* 2010;11:1133-36.
14. Hongo T, Mizun Y, Haraguchi S, Yoshida TO. A new anticancer drug sensitivity test using the microplate culture and surviving tumor cell staining method. *J Gan To Kagaku Ryoho* 1986;13:247-54.
15. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. *J Nat Rev Mol Cell Biol* 2004;5:897-907.
16. Yadav S, Seer H, Murthy RR. Cytotoxic, apoptotic and anti angiogenic efficacy of silver nanoparticles, synthesized using tannic acid as reducing agent under controlled temperature. *Int J Pharm Bio Sci* 2012;1:160-77.