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Research Article

Gold Nanoparticles of *Argemone mexicana* Induces Oxidative Stress and Inhibits Cell Growth in MCF-7 Cells

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ABSTRACT

Gold nanoparticles (AuNPs), the mainly accepted nano-compounds, have unique characteristics. *Argemone mexicana* is a plant of the *Papaveraceae* family that is rich in alkaloids. The cytotoxic properties of a new AuNPs, synthesized from an aqueous leaf extract of *A. mexicana*, were examined on MCF-7 breast cancer cells. The cells were cultured for 48 h in 96 well culture plates, and then exposed to the AuNPs. Indicators of oxidative stress and cytotoxicity, including glutathione, malondialdehyde, and lactate dehydrogenase, were quantitatively assessed. Cell viability decreased significantly as a function of different nanoparticle dosage (1.25 to 640 μg/mL) and exposure time 72 h. Exposure to AuNPs increased malondialdehyde and lactate dehydrogenase levels with reduced glutathione levels ($p < 0.05$). The increased production of malondialdehyde and lactate dehydrogenase release from the cells indicated lipid peroxidation and membrane damage. In conclusion, exposure to AuNPs results in a dose-dependent cytotoxicity in MCF-7cells that is closely correlated to increased oxidative stress. Additional research is necessary to apply the use of this novel compound in cancer therapy experiments.

Keywords: *Argemone mexicana*, AuNPs, MCF-7, Oxidative stress, Cytotoxicity.

INTRODUCTION

Nanotechnology is a most effective area of research, making an impact in human life[1]. As nanoparticles (NPs) are more biocompatible than the conventional therapeutics, they are exploited for drug encapsulation and delivery[2]. The size reduction of NPs plays an important role in improving their bio availability and compatibility for therapeutical applications in diseases like cancer[3].

In general, metal nanoparticles are synthesized and stabilized by chemical reduction, electrochemical techniques and photochemical reactions. Recently, green nanotechnology is an area with significant goal of facilitating the production of nanotechnology-based products that are eco-friendly and safer[4]. Employing green chemistry to the synthesis of nanomaterials has an essential value in medicine[5]. Furthermore prior studies relating the synthesis of gold nanoparticles by plants has described their potential biomedical applications[6-8]. *Argemone mexicana* L. belongs to family Papaveraceae and is used as therapeutic herb. It contains alkaloids, flavonoids, tannins, sterols and terpenes. Phytoconstituents such as chelerytherine, sarguinarine, protopine, optisine and berberine are predominantly present in *A. mexicana* leaves; moreover, roots and seeds are conventionally used for skin-diseases, leprosy, bilious fever and inflammations[9]. *A. mexicana* is identified to have antibacterial, antifungal and anti-malarial activities. Leaves are useful in the treatment of ulcer, warts, cold sores, cough, wounds, cutaneous infections, skin diseases and itches[10]. In the present study, *A. mexicana* AuNPs exhibited *in vitro* cytotoxic effect against MCF-7 human breast cancer cells and it is directly associated with increased oxidative stress.

MATERIALS AND METHODS

Plant collection and extract preparation

Fresh and healthy *Argemone mexicana* L. plants were collected from Coimbatore district, Tamil Nadu, India. It was identified (No.BSI/SRC/5/23/201314/Tech./1399) by Botanical Survey of India, Coimbatore. All the chemicals and solvents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemicals, India. Fresh plant materials were washed under running tap water, air dried for seven days in shadow and then homogenized to fine powder and stored in airtight bottles. 10 g of fine powder was taken in clean sterile soxhlet apparatus and extracted with 150 mL of water. After extraction the extracts were collected for further analysis[11,12].

Green synthesis of AuNPs using Argemone mexicana leaves

Gold nanoparticles were synthesized through reduction of gold chloride by aqueous extract of *A.mexicana* leaf . The optical properties of green synthesized gold nanoparticles were analyzed by Ultra Violet-visible spectroscopy at 200-800 nm wave length range. Biosynthesized gold nanoparticles were characterized with the help of X-ray

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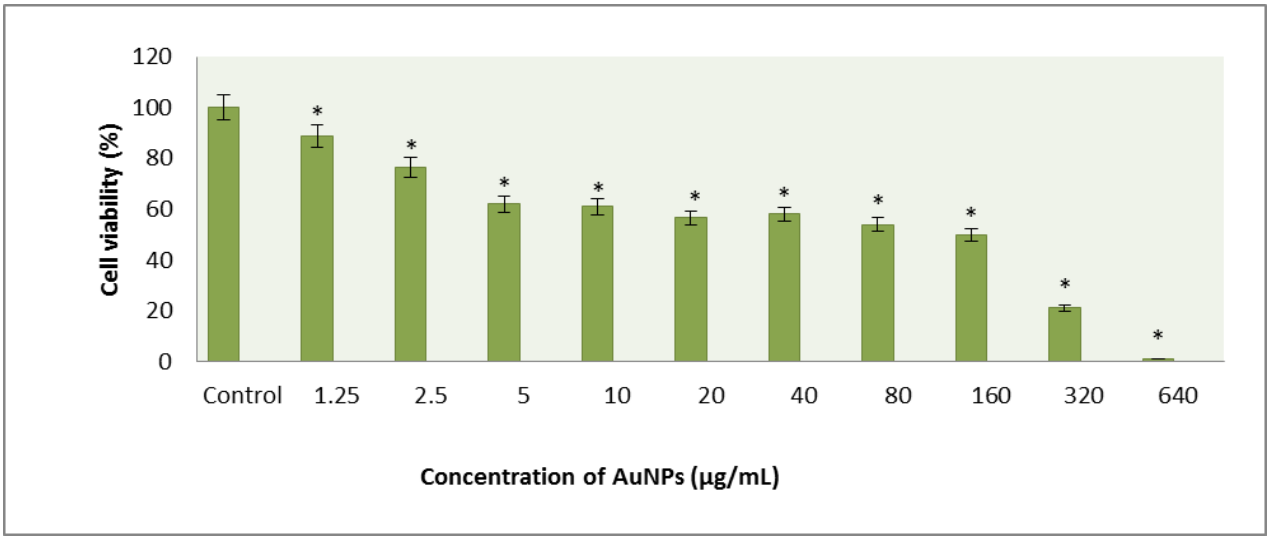

Figure: 1 Trypan blue exclusion assay: MCF-7 cells after 72 h incubation with biosynthesized AuNPs (1.25 to 640 µg/mL) from aqueous extract *Argemone mexicana* leaf. Each value represents the mean ± SD of three experiments. *p < 0.05.

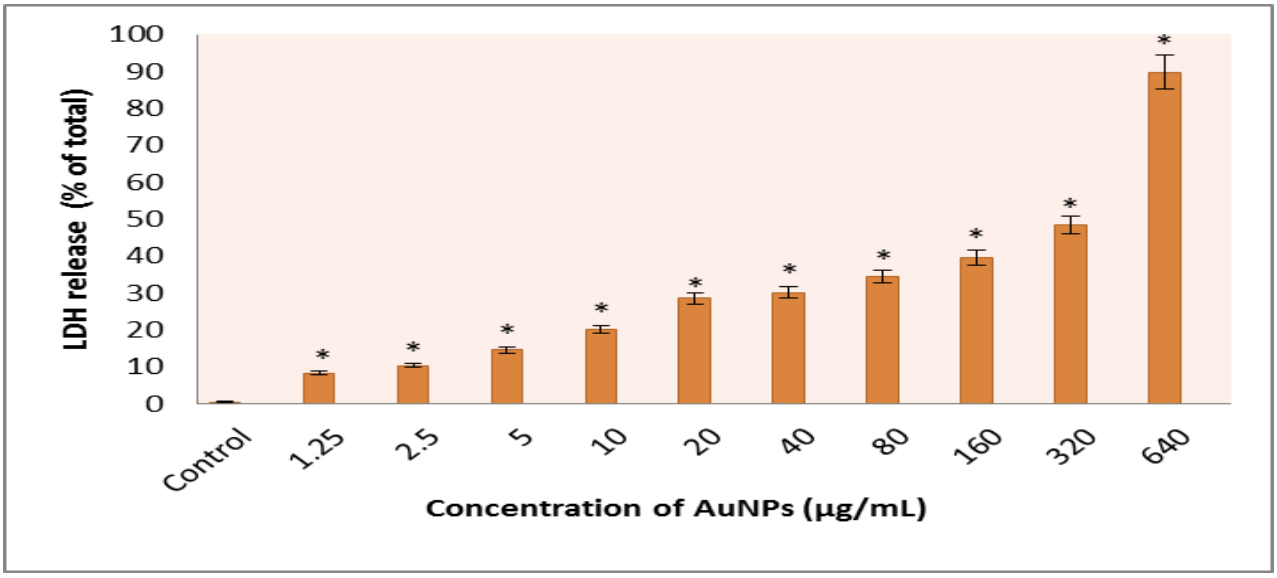

Figure: 2 LDH Activity: LDH activity was measured in the supernatants of MCF-7 cell culture after 72 h incubation with) biosynthesized AuNPs (1.25 to 640 µg/mL). Reported values are the mean ± SD of three independent experiments carried out in triplicate. *p < 0.05.

diffraction, FT-IR, scanning electron microscopy and transmission electron microscopy[11,12].

Cell viability

For cytotoxicity assessment, trypan blue exclusion assay was performed[13]. Briefly, the cells were seeded, at a density of 1 × 10^6 cells /mL in DMEM medium. Following 24 h of cell growth, different concentrations of AuNPs (1.25 to 640 µg/mL) were added to the cells. After 72 h of incubation, cells were trypsinized, washed and resuspended in PBS containing 0.4 % trypan blue and 100 cells were counted at various fields in haemocytometer for each experiment. The counting was done in triplicate. Concentration that inhibits 50 % of the cell growth (IC_{50}) compared to the untreated sample was determined by plotting the graph of percentage of cell viability versus concentration of the test compound. Results are given as the mean ± SD of three independent experiments.

LDH leakage assay

Cytotoxicity induced by AuNPs was assessed by lactate dehydrogenase (LDH) leakage into the culture medium. The cells were seeded in 96-well plates in 100 µL of media, and then treated with different concentrations of AuNPs (1.25 to 640 µg/mL). The treated cells then were incubated for 18 h, after which 40 µL of the medium was transferred to a new 96-well plate and further incubated for 72 h to determine LDH release. Forty microliter of 6 % triton X-100 was added to the original 96-well plates to determine the total LDH concentration. An aliquot of 100 µL of 4.6 mM pyruvic acid in 0.1 M potassium phosphate buffer (pH 7.5) was added to each well of the plate containing the medium followed by 100 µL of 0.4 mg/mL reduced β-NADH in 0.1 M potassium phosphate buffer

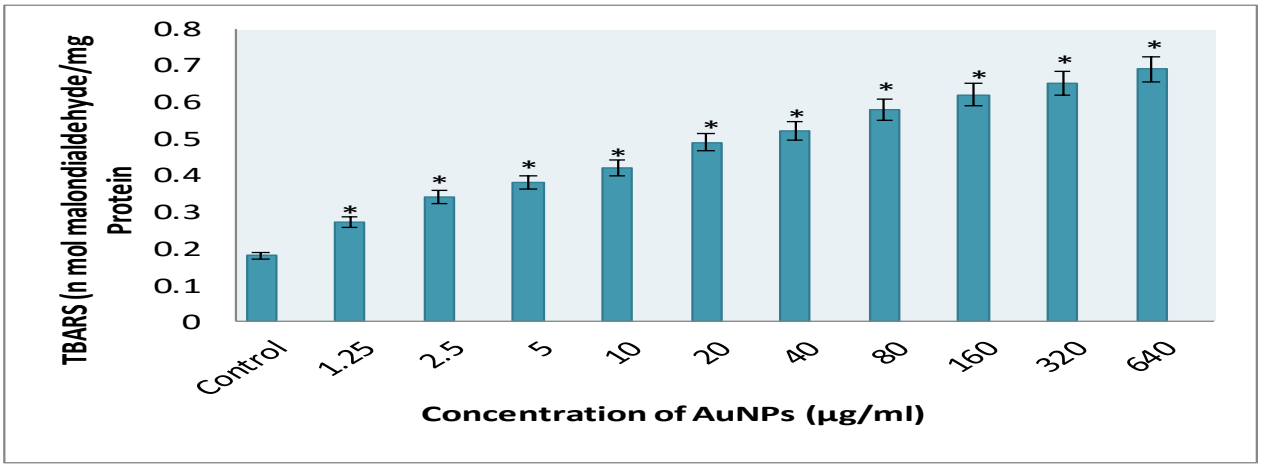

Figure: 3 Effects of AuNPs on Lipid: Mean levels of Lipid peroxidation was measured in the MCF-7 cell culture after 72 h incubation with biosynthesized AuNPs (1.25 to 640 µg/mL). Reported values are the mean ± SD of three independent experiments carried out in triplicate. *p < 0.05.

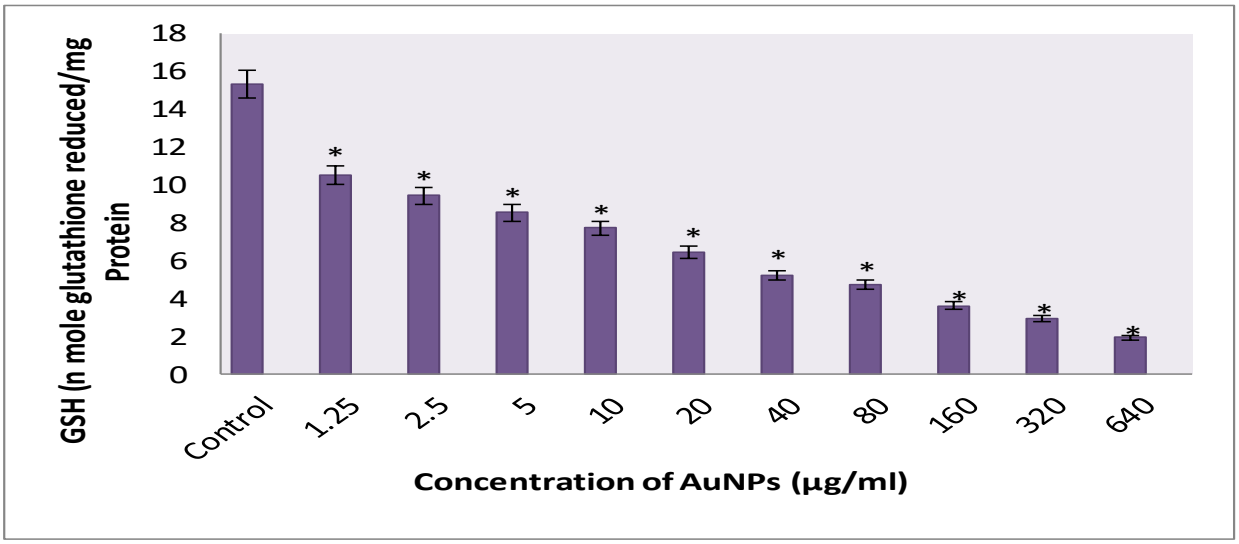

Figure: 4 Effects of AuNPs on intracellular Glutathione: Mean levels of Glutathione were measured in the MCF-7 cell culture after 72 h incubation with biosynthesized AuNPs (1.25 to 640 µg/mL). Reported values are the mean ± SD of three independent experiments carried out in triplicate. *p < 0.05.

(pH 7.5). The kinetic change in absorbance at 340 nm was read for 1 min in an ELISA microplate reader[14]. Results are given as the mean ± SD of three independent experiments.

Lipid peroxidation and Glutathione assay

To determine the lipid peroxidation and glutathione levels, MCF-7 cells were incubated with different concentrations of AuNPs (1.25 to 640 µg/mL) for 5 h. Cells were trypsinized, centrifuged and washed with PBS and sonicated on ice in a ultra sonicador for 4 min; the solution was then centrifuged at 1500 g for 5 min at 4°C. The obtained supernatants were used. Malondialdehyde a product of lipid peroxidation was estimated spectrophotometrically at 535 nm after reaction with thiobarbituric acid to obtain an index for lipid peroxidation. The results were reported as nmol of TBARS per mg of protein[15]. GSH content in the supernatant was determined by the Griffith method[16]. Cellular GSH content is expressed as µg of GSH mg-1 of protein. Protein estimation of each sample was done following the method of Lowry *et al.* (1951) using bovine serum albumin as a reference standard[17].

Statistical analysis

All experiments were performed three times in duplicate and data was statistically analyzed with the unpaired student's t-test using the Prism Software.

RESULTS AND DISCUSSION

Cell viability of the breast cancer cells to AuNPs was assed by trypan blue exclusion assay. Breast cancer cells were treated with different concentrations of AuNPs (1.25 to 640 µg/mL) for 72 h incubation period. AuNPs inhibited the cell growth and decreased cell survival through induction of cell death. As observed in (Figure 1) AuNPs induced dose dependent cytotoxic effect on MCF-7 breast cancer cells; the median lethal dose was (LD50) 160 µg/mL (*P < 0.05). The cells treated with higher concentrations of AuNPs showed a significant decrease in

the number of viable cells. The LDH leakage assay is the most common employed method for the detection of cell death following exposure to toxic substances. The LDH leakage assay is based on the measurement of lactate dehydrogenase activity in the extracellular medium. Consistency, rapidity and easy assessment are some of the characteristics of LDH assay[18]. It has been employed as an indicator of cytotoxicity in MCF-7cells following exposure to metals and other toxic substances. The loss of intracellular LDH and its release into the culture medium is an indicator of permanent cell death due to cell membrane damage[19]. Hence, the anticancer activity of the biosynthesized AuNPs was confirmed by LDH leakage assay. Effect of AuNPs on LDH release into the culture medium after 24 h treatment with 160, 320 and 640 µg/mL concentrations of AuNPs were 45.3, 50.5 and 89.3 % respectively (Figure 2). Involvement of intracellular redox status as a key mediator of apoptosis has been well established in may cancer cell lines. Reduced glutathione (GSH) is a major determinant of cellular redox equilibrium and contributes to an extraordinary range of metabolic processes and plays a role in detoxification of intracellular H_2O_2, also depletion of intacellular glutathione renders the cancer cells more sensitive to apoptosis and inhibits cell proliferation[18,19]. In MCF-7 cells GSH was depleted in following exposure to AuNPs and effect was on dose dependent manner (Figure 3) and it was associated with increase lipid peroxidation. AuNPs treated MCF-7 cells showed a dose dependent increase in lipid peroxidation products in all concentrations studied (Figure 4). Increased lipid peroxidation was associated with apoptosis and cell death[20]. Breast cancer is the most important cause of mortality in women[21]. Nowadays, some cytotoxic agents are used for its treatment. However, they are costly and known to induce several side effects such as myelosuppression, anemia, and generation of cellular resistance[22,23]. Hence, it is important to find alternative therapies or drugs to overcome these drawbacks. Our results showed that biosynthesized AuNPs induced a dose-dependent cell death in MCF-7 breast cancer cell and the present study might contribute to the comprehension of this compound on cancer therapy.

CONCLUSION

Our study showed the induction of apoptosis in MCF-7 cells by gold nanoparticles deserves further research to determine if *in vivo* exposure consequences may exist for gold nanoparticles application.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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