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Silver Nanoparticles: Cytotoxic and Apoptotic Activity on HT-29 and A549 Cell Lines

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Abstract

Silver nanoparticles (Ag-NPs) are versatile materials with a broad range of applications in various fields such as cancer therapy, drug delivery. In this work, cytotoxic and apoptotic activities of silver nanoparticles was evaluation against lung (A549) and colon (HT-29) cell lines. The cytotoxic activity of nanoparticles was performed by MTT assay, while their apoptotic activity was tested through TUNEL method. The results of MTT of A549 have illustrate that fifty percent of cells destruction in concentrations more than 250 µg/ml of Ag-NPs. Apoptotic results of nanoparticles have shown more than fifty percent of apoptosis on A549 cell line. HT-29 display full apoptosis at concentrations more than 500 µg/ml. It seems that synthesized Ag-NPs by using *P. farcta* extract can be candidate as anti-cancer agent in treatment many cancers through creating or discovering new drug forms

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Introduction

In recent years, nanoparticles have developed as diagnostic and therapeutic agents for the treatment of many diseases such as diabetes, asthma, allergies and cancer [1]. Ag-NPs have special physicochemical properties, which apply such as solar cells, catalyst in chemistry reaction, electric batteries etc. [1]. Also, they have contain antimicrobial [2], antiviral [3], antifungal [4, 5], anti-inflammatory [6], and antioxidant effects [7]. Studies have showed the usage of nanoparticles in drug delivery and molecular imaging [8], while researches have reported their capability in eliminating cancer cells [9, 10]. Some cancer cells have been reported to show resistance toward many drugs, specially platin derivatives such as cis-platin, as well as showing toxic effects. Thus, it is vital to discover new drugs for this particular disease in which Ag-NPs seem to fit as a suitable suggestion [11]. Recent studies on Ag-NPs have shown significant anti-cancer activity on breast (MCF-7) [9], mouse fibroblasts (L929) [8], colon (HCT-119) [12], lung (A549) [13], pharynx (Hep-2) [14], rat skeletal muscle (L6) [15], and prostate (MDA-MB-231) [16] cancer cell lines. The aim of this project is to investigate the anticancer activities of synthesized Ag-NPs by using *Prosopis farcta* extract against lung (A549) and colon (HT-29) cancer cell lines. These nanoparticles, which contain spherical shapes with size about 10.8 nm [17], have been already synthesized by our team members. The cytotoxic and apoptotic activities of the mentioned nanoparticles were investigated through the use of MTT assay and TUNEL test, respectively.

Materials and Methods

Synthesis of Ag-NPs

Ag-NPs were synthesized according to the protocol of Miri *et al* [17]. Briefly, 5 ml of *P. farcta* aqueous extract was added to 95 ml of silver nitrate solution (1 mM) in dark, room temperature and shaken with 150 rpm. After 1 hour, the color of solution changed to brown, which indicates the formation of Ag-NPs. The synthesized nanoparticles were centrifuged at 4000 rpm for 10 min, and sediment was used to prepare stock suspension using water as vehicle.

Cell line and Cell Culture

The lung (A549) and colon (HT-29) cell lines were prepared from Pasteur Institute of Iran. Cells were incubated in RPMI media with 10% FBS, 100 µg/ml of Streptomycin, and 100 U/ml of Penicillin, at a temperature of 37 ºC and the atmosphere of CO_2 with 5% moisture.

Evaluation Cytotoxicity Effect by MTT Assay

The cytotoxicity of synthesized nanoparticles was enquired into by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, 200 µl of cell suspension was added to each well ($1x10^4$ Cell/well) and the plates were incubated for 24 h. Afterwards, 50 µl of synthesized nanoparticles (50, 250, 500 and 1000 µg/ml) were separately added to the wells and incubated for certain time periods. 20 µl of MTT solution (5 mg/ml MTT dye in PBS buffer) was added to each well and the plates were incubated for 3 h at 37 ºC. Finally, 100 µl of DMSO was added to the wells. Optical absorbance was measured at 570 nm through the use of a micro-plate reader. Cell viability was expressed as a percent relative to untreated control cells at 24 h treatment time.

Evaluation Apoptotic Effect by TUNEL Stain

Terminal deoxynucleotidyl transferase-mediated dUPT nick end labeling (TUNEL) test was performed according to the manufacturer's instructions (*in situ* cell death detection kit, POD; Roche Diagnostics, USA). In brief, samples were treated by Ag-NPs solution (50, 250, 500 and 1000 µg/ml concentrations, separately) for 24 h, while the cells were fixed in 4% paraformaldehyde at 15-25 ˚C for 60 min; then they were washed twice by PBS. Afterwards, 200 µl of blocking solution (3% H_2O_2 in methanol) was added to each well and the plates were incubated for 10 min at 15-25 ˚C. Cells were washed twice by PBS and then, permeabilization solution (0.1% Triton X-100) was added to them. The results were stored for 2 min at 2-8 ˚C. Cells were washed twice again by PBS, while 50 µl of "TUNEL reaction mix" was added to each sample and incubated in the dark at 37 ˚C for a duration of 1 hour. Later on, they were dried after being washed by PBS and 50 µl of POD-Convertor was added to the samples. The plates were incubated at 37 ˚C for 30 min. After that, 50 µl of DAB substrate was added to the plates and they were stored in 15-25 ˚C for 10 min. At the end, the healthy

and apoptotic cells were calculated by direct view through invert microscope.

Statistical Analysis

Statistical analysis was performed by GraphPad Prism 5 and the statistical comparisons of multi-group data were analyzed through the use of two way ANOVA. Values of $^{*}p < 0.05$ were considered as statistically significant. Each test was performed in triplicates and results were presented as mean ± SD.

Result and Discussion

In our previous studies, Ag-NPs were synthesized by using *P. farcta* aqueous extract as the reducing agent for the reduction of Ag^{+} to Ag^{0} [17]. In this study, we survey the cytotoxic and apoptotic activities of synthesized Ag-NPs using *P. farcta* extract against lung (A549) and colon (HT-29) cancer cell lines. In this regard, the cytotoxic activity of Ag-NPs on A549 and HT-29 cancer cell lines have been tested and performed through the utilization of MTT assay. This method is based on the conversion of tetrazolium salt (solvable, pink) to formazan (unsolvable, purple) by the mitochondria of living cells. The experiment was performed through the application of four concentrations (50, 250, 500 and 1000 µg/ml) of nanoparticles at 24 h treatment time. The achieved results regarding the cytotoxic activity of Ag-NPs against A549 cells has shown a significant decrease in the viability of cells at 24 h in comparison to the of control ($^{*}P<0.05$), which has gone as far as attaining a fifty percent of cells destruction in concentrations more than 250 µg/ml of Ag-NPs. The results of cytotoxic activity of synthesized Ag-NPs on HT-29 cells show no significant differences between the treated cells and control at 24 h (Fig. 1).

Moaddab has reported that the cytotoxic activity of Ag-NPs has increased due to chemical and physical interactions of silver ions with functional groups of intracellular proteins such as nitrogen and phosphate groups [18]. Lately, cytotoxic effect of biosynthesized Ag-NPs has been reported against many cancer cells such as human cervical (HeLa) [19], prostate (MCF-7) [20], lung (A549) [21] and acute promyelocytic leukemia (HL-60) cells [22].

Apoptotic activity of Ag-NPs was done on lung (A549) and colon (HT-29) cancer cell lines, using TUNEL assay. The basis of this method is the connection of Terminal deoxynucleotidy transferase (TdT) enzyme to broken DNA strands (one of the symptoms of apoptosis). Through the mentioned method, apoptotic cells are characterized by labeling them through attaching the fluorescence color of the kit. In this assay, the apoptotic activity of Ag-NPs (50, 250, 500 and 1000 µg/ml) was put under investigation against A549 and HT-29 cell lines. It is illustrated through the results that as the nanoparticles concentration increases, their apoptotic effect significantly increases as well. The concentration of 1000 µg/ml showed 86% apoptosis effect on A549 cells, and concentrations higher than 500 µg/ml demonstrated 100% apoptotic effect on HT-29 (Fig. 2). Previous studies have stated that cellular death, caused by nanoparticles through apoptosis; occur by the changes in cell nuclei such as chromatin agglomerate or nuclear fragmentation [9], while B. Gajendran *et al* have reported that Ag-NPs cause apoptosis through active oxygen species, increasing ROS level, and creating changes in the mitochondrial membrane potential [23].

Conclusion

According to various researches and this study, it can be suggested that Ag-NPs is capable of annihilating cancer cell lines. In this research, the cytotoxic effect of synthesized Ag-NPs, using *P. farcta* extract, has been enquired into through the MTT assay. The results have indicated that the viability of A549 is dependent on the nanoparticles concentration. The apoptotic results of Ag-NPs have shown more than 50% of apoptosis on both cell lines. Also regarding this case, HT-29 has demonstrated full apoptosis at concentrations more than 500 µg/ml. The Size, shape and chemical surface of nanoparticles can be effective on its cytotoxic activity, while on the other hand, the synthesis method can affect these particular factors as well. Therefore, comparisons and supplementary studies on Ag-NPs and *in vitro* studies may provide a pathway for the treatment of cancer by, creating or discovering new drug forms that can deliver the drug to the site of cancer.

Conflicts of Interest

The authors declare no conflict of interest.

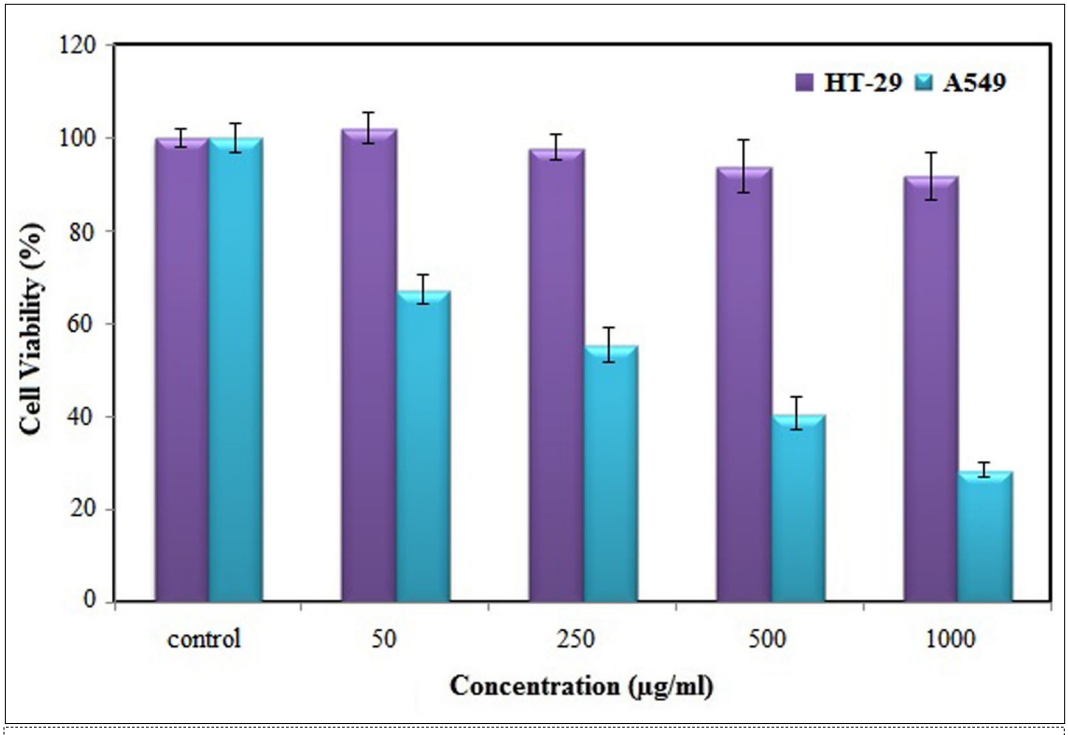

Fig. 1. MTT cell viability assay of synthesized Ag-NPs on A549 and HT-29 cancer cell lines at 24 h. Data were means ± SD of three independent experiments. *p<0.05 compared to the control group, by ANOVA.

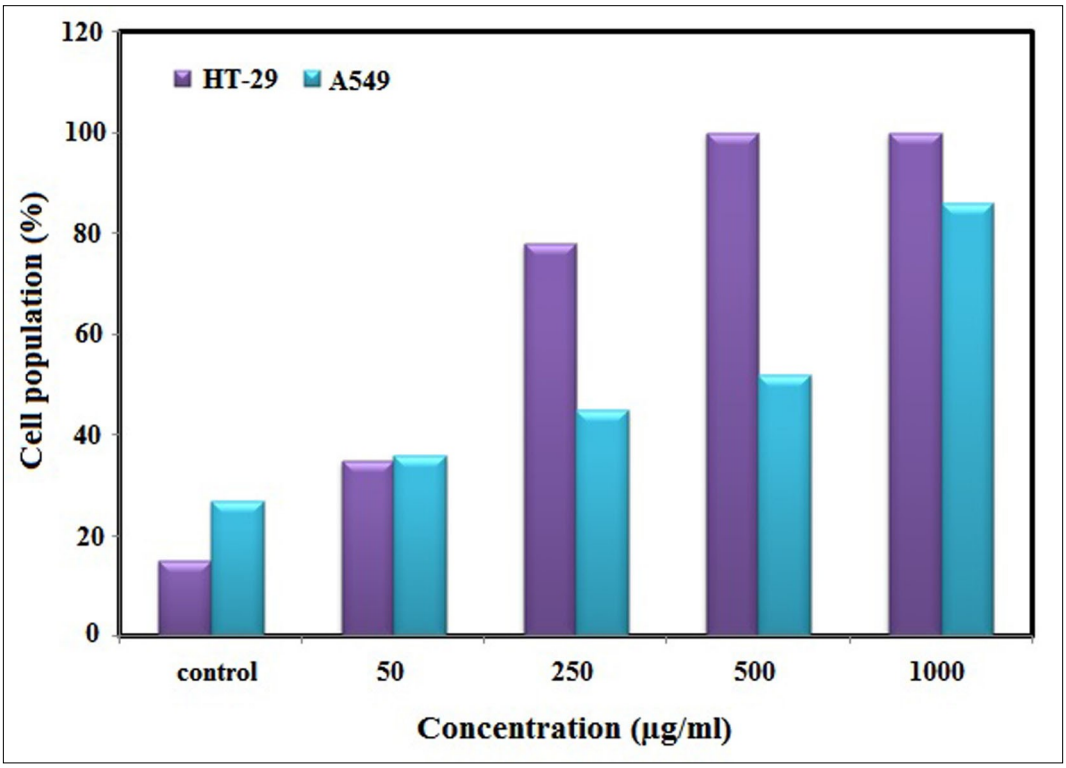

Fig. 2. Apoptosis analysis of synthesized Ag-NPs using TUNEL assay on A549 and HT-29 cancer cell lines for 24 h.

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