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Original paper

AgCl Nanoparticles *in vitro* Anticancer Effects Against Human Cervical Cancer Cells

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Abstract

Background and aim: Nanoparticles have potential anticancer effects on cancer cells. In vitro and in vivo studies have reported the cytotoxic effects of silver nanoparticles on cancer cells. Despite a number of studies carried out on the anticancer effects of silver nanoparticles on cancer cells, there are few report on the anticancer effects of green synthesized silver nanoparticles on cervical cancer cells. The aim of this study was to determine the cytotoxic effects of green synthesized AgCl on cervical cancer (Hela) cells in vitro.

Materials and methods: AgCl nanoparticles were synthesized using Onopordum acanthium extract. HeLa cells were divided into control (no- treated) group and groups treated with 1.5625, 3.125, 6.25, and 12.5µg/ml of AgCl nanoparticles. Cell viability was evaluated using MTT assay method.

Results: The results of MTT showed that viability of Hela cells did not significantly change in groups treated with 1.5625 and 3.125 µg/ml of AgCl nanoparticles. However, the Hela cells viability significantly decreased compared to the control group at concentrations ≥ 6.25 µg/ml of AgCl nanoparticles.

Conclusion: Our findings revealed that despite lower concentrations of AgCl nanoparticles, higher concentrations had cytotoxic effects on cervical cancer cells in vitro.

Keywords: *AgCl nanoparticle, Green synthesis, Cytotoxic effect, HeLa*

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Introduction

Silver nanoparticles (AgNPs) are materials having one of the dimensions in the range of 1-100 nm. Their novel cytotoxic characteristics against mammalian cancer cells in vitro makes them more applicable in tumor therapy as anti-cancer agents [1]. In recent years, many studies have shown that various nanoparticles can have cytotoxic effects on different cancer cells in vitro [2]. Both gold and silver nanoparticles are used to destroy the cancer cells. Gold nanoparticles can have anticancer effects against lung and liver [3], bladder [4], breast [5] and even cervical [6] cancer cells in vitro. In addition, silver nanoparticles can also have anti-cancer effects on many cancer cells, but all of these studies are only in vitro and they are still not on practical and clinical levels [7]. On the other hand, cancer is currently one of the most important public health issues, especially in the third world countries where there are economic and health problems. Moreover, cervical cancer is the 4th most common cancer in women worldwide and because of the high prevalence of this specific cancer among the least developing and developing countries, it requires remarkable attention and further researches [8]. Some previous studies have given us information about the cytotoxic effects of green synthesized silver nanoparticles using different plants extract. The aim of this study was to determine the cytotoxic effects of green synthesized AgCl on cervical cancer (Hela) cells *in vitro*.

Material and Methods

AgCl Nanoparticles Preparation

AgCl nanoparticles were synthesized using *Onopordum acanthium* extract and characterized using X-ray diffraction (XRD) (X' Pert Pro, Panalytical, UK) and field emission scanning electron microscopy (FESEM) (Philips, Amsterdam, Netherlands, XL30).

Cell Preparation

HeLa cancer cells were purchased from Pasteur Institute of Iran. These cells were delivered frozen in vials placed in a nitrogen tank to protect the cells.

Cell Culture

Hela cells were placed in a Falcon tube containing 10 ml of DMEM medium and were centrifuged at 300 rpm for 5 min. After centrifugation, the supernatant was discarded. 10 ml of fresh medium was added to cell pellet and mixed. The solution was poured in a flask and placed in incubator. Proliferating cells were attached to the bottom of the flask. Supernatant was discarded and cells were washed with PBS to remove the waste. PBS was then removed. To separate cells from each other and from the bottom of the flask, 0.5 ml of trypsin was used. Cells were incubated at 37 °C for 3 to 5 minutes. Reveres microscope was used to examine if the cells were detaching from each other and from the bottom of the flask. DMEM was added to stop trypsinization. The solution was aspirated and poured to a 15 ml sterile Falcon tube and centrifuged at 1500 rpm for 5 min. After centrifugation, the cells were placed on the bottom of the flask. The medium was discarded, add fresh medium was poured into culture flask. After cell proliferation and reaching the confluencey 80 to 90%, they were used for treatment [9].

Treatment

For this aim, the proliferated cells were divided into control (untreated) group and groups treated with 1.5625, 3.125, 6.25 and 12.5 µg/ml of AgCl nanoparticles for 24 hours.

MTT Assay

After 24 hours of treatment, MTT assay was used to evaluate the cytotoxic effect of green synthesized AgCl nanoparticles on Hela cells. In this regard, considering sufficient culture medium for the cells, as well as considering at least 6 repetitions, different doses of AgCl nanoparticles

were added to the wells containing the cells and the plates were kept in the incubator for 24 hours. After the desired time had elapsed, the liquid was drained from the plate and MTT dye was added. Subsequently, 4-6 hours after dye addition, the MTT solution was drained and DMSO was added. After complete dissolution, the amount of light absorption of the solutions was read using 570 nm wavelengths and cell viability was calculated [10]. The half-maximal inhibitory concentration (IC50) of AgCl nanoparticles was calculated by linear approximation regression of the percentage survival versus the AgCl nanoparticles concentration.

Statistical Analysis

SPSS20 software was used for statistical analysis. Kolmogorov-Smirnov test was performed to ensure the normal distribution of data, which was followed by one-way analysis of variance (ANOVA) and Tukey post hoc test. P values less than 0.05 were considered statistically significant.

Results

The results of MTT showed that viability of Hela cells did not significantly change in groups treated with 1.5625 and 3.125 µg/ml of AgCl nanoparticles. However, the Hela cells viability significantly decreased when treated with 6.25 and 12.5 µg/ml of AgCl nanoparticles compared to control group ($p<0.05$ and $p<0.001$, respectively) (Figure 1).

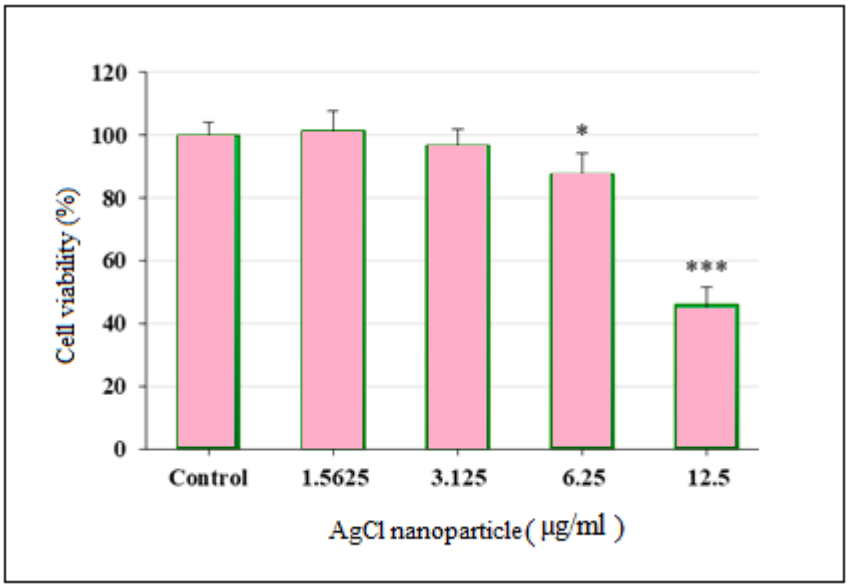

Figure 1. Viability of HeLa cells treated with different concentrations of AgCl nanoparticles(µg/ml). ***** indicates significant difference compared with control group (*:p<0.05 , ***:p<0.001).

Discussion

Previous studies have shown that silver nanoparticles can exert anti-cancer effects on various cancer cells *in vitro*. As an example, silver nanoparticles synthesized from *Padina tetrastromatica* can have anti-cancer effects on breast and lung cancer cells [11], [12]. Silver nanoparticles also have anticancer effects on colon cancer cells *in vitro* [13]. Also, according to the information obtained from previous studies, silver nanoparticles can also affect reproductive system cancers and be effective in curing these types of cancer. For instance, silver nanoparticles can have cytotoxic effects on ovarian cancer [14] and prostate cancer cells [15]. In line with our finding, the

anti-cancer effects of green synthesized silver nanoparticles using extract of *Beauveria bassiana* [16], *Sargassum wightii* [17], *Nepeta deflersiana* [18] and *Detarium microcarpum* plant [19] have been observed on cervical cancer cells. It seems that in most of these cancers, the green synthesized silver nanoparticles exert their cytotoxic effects on cancer cells by activating the apoptosis pathways [20], [21].

Conclusion

Our findings indicated that AgCl nanoparticles did not show anticancer effects against cervical cancer cells at lower concentrations, however, higher concentrations had cytotoxic effects on cervical cancer cells *in vitro*.

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Conflict of interests

The authors have no conflicts of interests to declare.

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