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Research Article

Synthesis of Silver-Doxycycline Complex Nanoparticles and Their Biological Evaluation on MCF-7 Cell Line of the Breast Cancer

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In the current study, we aim to evaluate the effect of the combination of silver and doxycycline (silver-doxycycline complex) on the viability of the MCF-7 cell line of the breast in comparison with each of them. The Ag-doxycycline NPs were synthesized using silver nitrate and doxycycline solutions. The synthesized Ag-doxycycline NPs were characterized with several analyses. Ag-doxycycline NPs with a concentration of 25 μM is significantly more effective in decreasing the viability of MCF-7 cells than Ag with the same concentration ($P < 0.05$). Doxycycline with a concentration of 6.25 μM also has a more potent effect on the viability of MCF-7 cells than Ag with the same concentration ($P < 0.05$). Ag-doxycycline NPs with a 25 μM concentration is more effective than the concentration of 3.125 μM ($P < 0.05$). Ag-doxycycline NPs were found to be more effective than AgNPs alone in inhibiting the growth of the MCF-7 cells. Also, the increasing utility of nanotechnology in multiple aspects of medicine can lead to using this technology in the treatment of different types of cancer in the future.

1. Introduction

Breast cancer is one of the most common types of cancer worldwide and is one of the leading causes of cancer mortality among women [1, 2]. In 2018, 266,120 females were diagnosed with breast cancer across USA, and 41,400 deaths were reported between two genders [3, 4]. An investigation in 2017 revealed a raise of cases by 33% from 2005 to 2015, while the increase in population was the reason for 12.6%. More than 19 million new cancer cases and approximately 10.0 million cancer deaths were diagnosed in 2020 [5, 6]. There are different treatment strategies against breast cancer, including chemotherapy, radiation therapy, surgery, hormone therapy, and targeted therapy [7].

As a conventional treatment method, chemotherapy has less impact on slow-growing tumors; furthermore, its serious side effects such as hematological, dermatological, and gastrointestinal toxicity influence patients' quality of life and compliance [8]. Moreover, chemotherapeutic agents are

considered carcinogens and a leading cause of recurrence [9]. It is demonstrated that cancer precursor cells resist DNA damage caused by radiotherapy due to increased DNA repair pace in cells [10]. Surgery has also some drawbacks such as the inability to kill microscopic disease around the edges of the tumor and damage to near normal tissues.

The nanoparticles of silver (AgNPs) have been employed as an antimicrobial, disinfectant, and anticancer agent. Previous investigations suggested that they might also be a possible alternative tool for the treatment of human breast cancer. Besides, recent studies are in favor of their chemotherapeutic benefit against tumor cells. According to nanotechnology science [11–13], AgNPs could enter the cells and suppress tumor cells through activating P53 enzyme, DNA breakage through an increase in reactive oxygen species amount, and apoptosis in the absence of P53 [14–17].

There are diverse chemical, physical, photochemical, and biological approaches for synthesizing AgNPs [18]. In physical processes, metal nanoparticles are generally synthesized by evaporation condensation, which could be done using a tube furnace at atmospheric pressure [18] or laser ablation. However, these methods contain significant disadvantages such as sizeable space occupation, high energy consumption, prolonged procedure, vacuum condition, and high-cost instruments [19–21]. On the other hand, physical synthesis facilitates producing AgNPs with narrow size distribution and immense quantities of particles in one single process. The photochemical technique comprises several drawbacks, among which expensive equipment and vacuum state could be mentioned [18]. Biosynthetic methods are carried out using naturally reducing agents. The nanoparticles acquired from biological microorganisms such as bacteria, fungi, algae, plants, and their components seem to be environment-friendly because poisonous materials are not employed in their production. It is considered an affordable and green route. However, the small amount of AgNPs obtained in the biosynthetic procedures is a noticeable obstacle [18].

The most common approach for the synthesis of AgNPs is chemical reduction of metal ions due to its convenient operation and simple supplies required. In chemical synthesis, a restricted control over metal nanoparticles' growth is required to achieve AgNPs of small size, spherical shape, and narrow diameter distribution. The chemical routes permit the production of nanoparticles in high yield with three major components: (a) metal precursors, (b) reducing agents, and (c) stabilizing agents. To achieve colloidal solutions out of silver salts, two stages, namely, nucleation and subsequent growth, by which the size and the shape of synthesized AgNPs are strongly dependent, should be considered [22–24].

Doxycycline, a semisynthetic tetracycline, prohibits matrix metalloproteinase (MMP) activation and cellular proliferation [25]. It interferes with protein synthesis in cancer cells [26–28]. Selective permeability of different cell lines from different cancers to doxycycline led to the emergence of a hypothesis that asserts its influence in cancer treatment. Furthermore, the combination of doxycycline with targeted drugs has been applied in clinical tests of cases with advanced cancer [29]. Affordability, accessibility, and selective impact on cancer cells could also be mentioned as some advantages of doxycycline [30–34].

In this study, we aim to combine AgNPs with doxycycline and compare its effect with each of doxycycline-hydrochloride and AgNPs on MCF-7 breast cancer cell line.

2. Materials and Methods

2.1. Materials. Silver nitrate and sodium citrate were purchased from Sigma-Aldrich (Missouri, United States). Doxycycline monohydrate was supplied by the RAZAK laboratory (Tehran, Iran). Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD) were used for the characterization of Ag-doxycycline complex nanoparticles.

2.2. Synthesis of Ag-Doxycyclin Complex. In order to reach the optimized condition for the synthesis of Ag-doxycycline complex, different concentrations of doxycycline monohydrate and silver nitrate were tested. The most effective value of these factors which led to the most efficient synthesis was found and used for the subsequent experiments. For this, 1 mM of $AgNO_3$ in the solution of 0.176 g doxycycline in 250 mL deionized stilled water was stirred with 400 rpm at 40°C and pH = 6.4 for 1 h. The homogenous color change of solution into gray-black was the indicator of forming particles with nearly same size and shape throughout the sample. Then, the solution was centrifuged, and deposited particles were dehumidified and weighted.

2.3. Synthesis of Control Groups. 0.176 g of doxycycline monohydrate in 250 mL deionized stilled water and 1 mM of $AgNO_3$ with 1% sodium citrate solution with continuous stirring and boiling for 10 min were considered as group 1 and 2 of control, respectively.

2.4. Synthesis of Ag Nanoparticles. A solution of $AgNO_3$ with a concentration of 2 mM was provided. A solution of sodium citrate with a concentration of 7 mM was then provided which was fixed at pH 9. In continuation, the first solution (20 mL) was added dropwise to the second solution (100 mL) to form a yellow solution, indicating the formation of Ag nanoparticles. Finally, Ag nanoparticles were collected *via* centrifugation and washed with water several times [35].

2.5. Determining the Physicochemical Properties of the Nanoparticles

2.5.1. Transmission Electron Microscopy. Transmission electron microscopy (TEM) was used to determine the morphology and distribution of the sizes of the nanoparticles. The lower magnifications were utilized to determine the distribution of the sizes of the nanoparticles. In contrast, higher magnifications were used to determine the morphology and structure of the nanoparticles.

2.6. Fourier Transform Infrared Spectroscopy (FTIR). The Fourier transform infrared spectroscopy (FTIR) was used to obtain the infrared spectrum of transmission or absorption of materials. In this study, FTIR was used to determine the structure and molecular components of the nanoparticles.

2.7. X-Ray Powder Diffraction (XRD). The X-ray powder diffraction (XRD) was used to determine the crystallization and the crystal shape of the nanoparticles.

2.7.1. Effect of AgNPs and Ag-Doxycycline NPs on the Viability of MCF-7 Cell Line. Cells were cultured using RPMI-1640 medium with 10% fetal bovine serum (FBS). Penicillin and streptomycin were also added in a concentration of 100 unit/ml and 100 μM, respectively. Trypsin was added up to monolayer cells to make single-cell suspension, and subsequently, the number of viable cells was counted using a hemocytometer. Then, the cell suspensions were seeded in 96-well plates, and cellular density was 10,000 cells/well. Plates were then incubated for 48 h at 37°C with 100% humidity and 5% CO_2 to make the cells attach to the bottom of the wells. Afterwards, wells were classified into four groups, and the attached cells within them were treated with a serial concentration of Ag-doxycycline NPs (group no. 1), AgNPs (group no. 2), and doxycycline monohydrate water-based solution (group no. 3). Serial dilution was done to obtain NPs and doxycycline in six different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 μM). Six wells without any treatment were considered as a control group (group no. 4). The cells were incubated for another 24 h at 37°C, 100% humidity, and 5% CO_2. Next, the solution of 20 μL from MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) was joined in each well, in which 5 mg/mL of PBS (phosphate-buffered saline) was previously added. Another period of 4 h of incubation at 37°C was also applied to achieve MTT reduction. The resulting purple-colored formazan crystals were solubilized using 100 μL of DMSO (dimethyl sulfoxide). Finally, the colorimetric study was conducted at 570 nm using a plate reader (EXL 800, USA).

2.8. Statistical Analysis. A chi-squared test was applied to compare cell viability among three groups. Data analysis was carried out using SPSS software (v. 25.0). P value <0.05 was considered as statistically significant.

3. Results and Discussion

3.1. Synthesis of Ag-Doxycycline Complex. First, doxycycline monohydrate in the form of oral capsules was prepared and weighed for determining the optimized conditions of synthesis. The amounts of 0.176, 0.088, and 0.441 g of doxycycline monohydrate in 250 mL stilled water were prepared. Then, $AgNO_3$ solution with the concentration of 0.5 and 1 mM was added to each amount of doxycycline. The homogenous color change of solution from white to gray-black was considered as forming particles into approximately similar shape and size. According to our observation, 0.176 g of doxycycline in 250 mL distilled water with 1 mM of silver nitrate was stirred with 400 rpm at pH = 6.4 and 40°C changed color from white into gray-black, indicating the formation of nanoparticles.

3.2. Characterization. The size of the Ag-doxycycline complex was estimated by observing the SEM images. The Ag-doxycycline complex was nanoparticles with sizes ranging below 500 nm in diameter size. According to TEM images, most of the nanoparticles are of spherical shape (Figure 1).

The size and shape of drugs are great factors in improving the primary and secondary properties of medicinal materials. During the last few decades, interdisciplinary exploring has focused on expanding biocompatible substances with different shapes to exhibit high selectivity for disorder sites [36].

It was proved that the spherical shape of nanomaterials can efficiently improve the activity of drugs. For instance, An and coworkers presented that the spherical nanodrug efficiently reduced cell viability with NIR irradiations [37]. In another study, the in vitro toxicity assay confirmed that spherical nanodrug shows a more therapeutic benefit than the helical one [38]. Chan and coworkers also described that spherical gold nanoparticles had a greater tendency to be taken up by HeLa cells in comparison with rod-shaped gold nanoparticles [39, 40] In addition, Fouda et al. reported the larvicidal activity of spherical AgNPs against the 3rd instar larvae of the dengue vector *Aedes aegypti* as well [41].

Spherical AgNPs were prepared via aqueous leaf extract of *Euphorbia prostrata*. These AgNPs displayed parasitic property against *Leishmania donovani* in the amastigotes and promastigotes stage [42, 43].

The spherical nanoparticles of silver were also extracted from *Moringa oleifera* which led to a considerable reduction in the average size of leishmaniasis cutaneous lesions in comparison with untreated mice [43, 44].

We found two peak absorptions in the FTIR—one at 3437.26 cm^{-1} and the other at 1619.69 cm^{-1} (Figure 2). The peak in 1619.69 cm^{-1} may be related to doxycycline's aromatic ring carbonyl group stretch vibration [45]. On the other hand, the peak at 3437.26 cm^{-1} is related to OH stretch vibration in alcohol and phenol [46].

The XDR of Ag-doxycycline NPs is shown in Figure 3, which reveals 12 peaks. The diffraction peaks at $\theta = 38.268$, 44.38, 64.61, and 77.46 are compatible with the XDR of silver nanoparticles pattern [47]. The XDR pattern of doxycycline has been reported by Thangadurai et al. in 2005 [48]. The X-ray diffraction data for doxycycline was mostly in less than 30°. Due to the higher count times of the silver nanoparticles, the diffraction peaks of doxycycline were overshadowed in XDR of the Ag-doxycycline NPs.

The effect of different concentrations of Ag-doxycycline NPs, Ag, and doxycycline on MCF-7 cells is shown in Figure 4. The Ag-doxycycline NPs with a concentration of 25 μM was significantly more effective in decreasing the viability of MCF-7 cells than Ag with the same concentration ($P < 0.05$). The doxycycline with a concentration of 6.25 μM

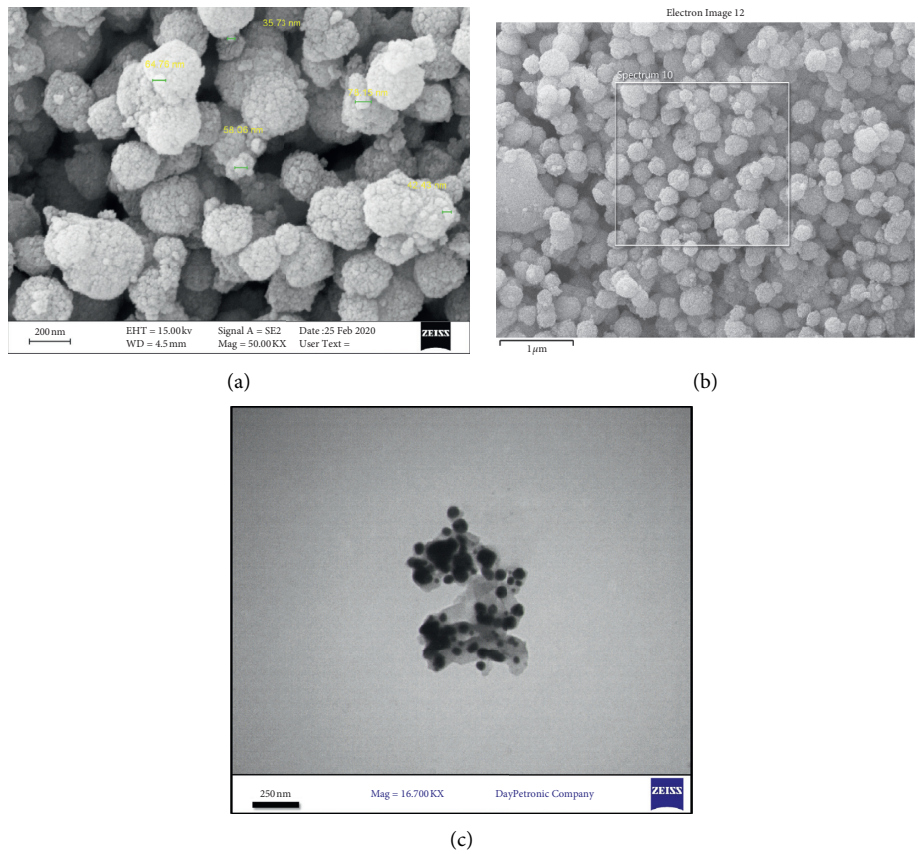

Figure 1: (a, b) SEM images of silver-doxycycline complex. (c) TEM image of silver-doxycycline complex.

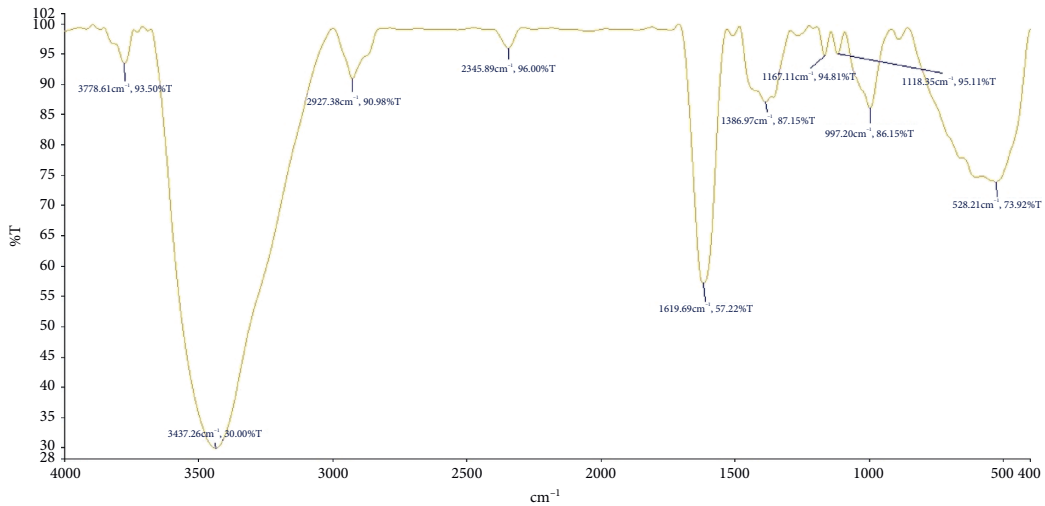

Figure 2: Recorded FTIR spectrum of silver-doxycycline complex.

also displayed a more potent effect on the viability of MCF-7 cells than Ag with the same concentration ($P < 0.05$). Finally, the Ag-doxycycline NPs with a concentration of 25 μM have been found to be more effective than the concentration of 3.125 μM ($P < 0.05$).

Although several studies have been conducted on the effects of doxycycline and silver on tumoral cells, the current study is the first one evaluating the efficacy of Ag-doxycycline NPs on breast cancer cells. The selective destruction of tumor cells while showing a minimum effect on the normal human cells is one of the advantages of AgNPs [49–52].

Jeyaraj et al. found that AgNPs with a concentration of 50 μM can inhibit all MCF-7 tumoral cells [53]. Direct interaction with the DNA of tumoral cells is one

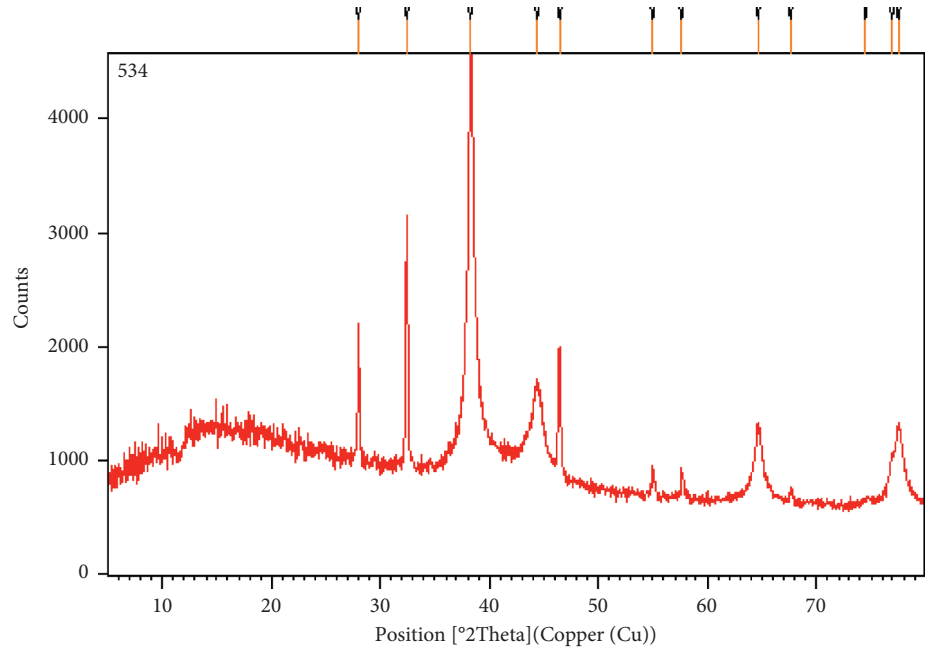

Figure 3: XDR pattern of Ag-doxycycline nanoparticles.

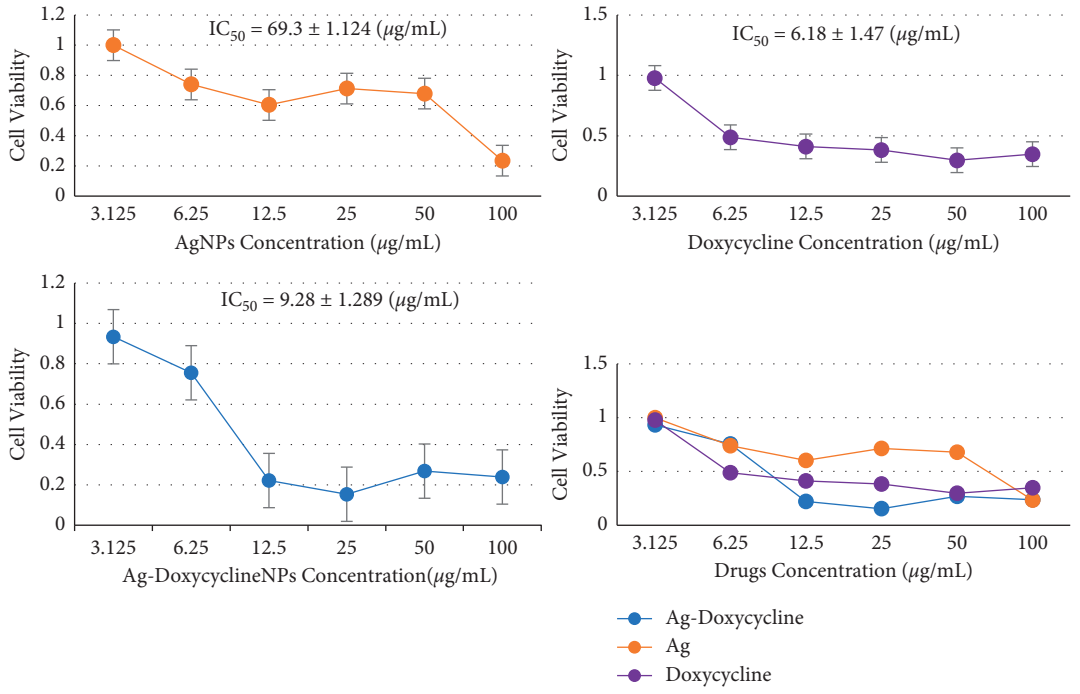

Figure 4: Effects of different concentrations of Ag-doxycycline NPs, Ag, and doxycycline on the viability of breast cancer cells.

mechanism that AgNPs deliver their antitumoral effect, considering their small size and their potential to cross the nucleus [54]. Inducing apoptosis is another mechanism of action by AgNPs that leads to a decrease in the viability of tumor cells [53–55]. According to the previously reported study, AgNPs increase radiotherapy efficacy [56]. Therefore, we believe concurrent radiotherapy may enhance the efficacy of Ag-doxycycline NPs. The concurrent treatment with both radiotherapy and Ag-doxycycline NPs leads to a further reduction in breast cancer cells' viability. Similarly, it was shown that the administration of doxycycline and cyclophosphamide in mice with breast cancer may lead to a more potent response to cyclophosphamide [57]. Administration of cyclophosphamide with Ag-doxycycline NPs may also enhance the response to this treatment.

4. Conclusion

To sum up, the Ag-doxyxcycline NPs were prepared using silver nitrate and doxycycline solutions. The synthesized Ag-doxyxcycline NPs were characterized by different analyses including scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD). In continuation, the synthesized Ag-doxyxcycline NPs were evaluated on the MCF-7 cell line of breast cancer. The Ag-doxycycline NPs displayed good results in inhibiting the growth of the MCF-7 cells.

Data Availability

The data used to support the findings of the current article are included in the text.

Conflicts of Interest

All authors state that there are no conflicts of interest.

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