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Research Paper

Investigation of the effect of pomegranate extract and monodisperse silver nanoparticle combination on MCF-7 cell line

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ABSTRACT

In this study, we aimed to investigate whether the combination therapy of pomegranate extract and silver nanoparticle is effective on MCF-7 cell culture. The pomegranate extract was mixed and incubated with silver nitrate for the microwave assisted green synthesized of silver nanoparticle. Obtained nanoparticles were investigated using X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), UV–vis, Field Emission Scanning Electron Microscopy (FESEM), and Transmission Electron Microscopy (TEM) methods. The spectroscopic and morphological studies of the monodisperse Ag NPs which have particle size of 15.4 nm indicate the highly crystalline form, well dispersity, and colloidal stable NPs. After fully characterization of prepared nanoparticles, the effectiveness of Ag NPs was determined by evaluating cell viability, nuclear degradation and cell cycle parameters. The results obtained demonstrate that biosynthesized Ag NPs can inhibit the proliferation of human breast cancer cell line MCF-7 in the IC50 at a dose of 12.85 µg/mL and inhibit the proliferation of Ag NPs against anti-growth arresting MCF-7 cell line. This case demonstrates that it may exert its proliferative effect by reducing DNA synthesis and apoptosis-inducing cell cycle stages.

1. Introduction

In recent years, nanoparticles and their usage have paid attention as critical technologies in many industrial applications (Saleh and Gupta, 2014, 2012a; Ahmaruzzaman and Gupta, 2011; Gupta et al., 2015; Kouvaris et al., 2012; Parveen et al., 2012; Lead and Ju-Nam, 2008; Kim et al., 2010; Başkaya et al., 2017; Park and Choi, 2011; Hubenthal, 2011; Jin et al., 2010; Karatepe et al., 2016; Noruzi et al., 2011; Chen et al., 2008; Chen and Schluesener, 2008; Viuda-Martos et al., 2010; Arulvasu et al., 2010; Eroles et al., 2012; Rai et al., 2006; Vijayakumar et al., 2013; Tangkanakul et al., 2009; Bakar et al., 2009; Miller, 1959; Goksu et al., 2016; Esirden et al., 2015; Noguez, 2007; Yang and Li, 2013; Philip, 2009; Piao et al., 2011; Asha Rani et al., 2013; Celik et al., 2016a,b,c; Yildiz et al., 2016a,b; Sen et al., 2013, 2012, 2014; Erken et al., 2015; Pamuk et al., 2015; Dasdelen et al., 2017; Castelli et al., 2013; Cheraghia et al., 2017; Shabani-Nooshabadi et al., 2017; Karimi-Maleh et al., 2017a,b; Cheraghi et al., 2017; Sheikshoae et al., 2017; Karimi-Maleh et al., 2016; Gupta et al., 2012a; Saleh and Gupta, 2012b; Gupta et al., 1998; Mittal et al., 2010a,b, 2009; Gupta et al., 2011a, 2012b,c; Gupta et al., 2011b; Saleh and Gupta, 2012c; Khani et al., 2010; Karthikeyan et al., 2012; Jain et al., 2003; Gupta et al., 2013). Because of their specific electrical, optical, magnetic, chemical and mechanical properties, nanoparticles are currently used in many high-

tech fields such as diagnostics, antimicrobials, medical applications such as drug delivery (Parveen et al., 2012), environmental protection (Lead and Ju-Nam, 2008; Kim et al., 2010) and energy conversion. The synthesis of these types of nanoparticle generally involves the use of a number of physical and chemical methods, such as laser ablation, pyrolysis, chemical or physical vapor deposition, sol-gel and lithography electrostatics, and is generally costly and/or toxic (Park and Choi, 2011). Recently, it has been a great effort to use the biological synthesis of noble metal nanoparticles as an environmentally friendly method (Hubenthal et al., 2011), and these have been mostly obtained using plant or fruit extracts (Jin et al., 2010). These green methods offer crystal nanoparticles at low cost, fast, efficient and often in different sizes. This method not only depends on the nature and concentration of plant solubility but also on the pH, temperature and incubation time of the synthesis reaction (Noruzi et al., 2011; Chen and Schluesener, 2008; Chen et al., 2008). Within the scope of this work, nanoparticles are synthesized in conditions as environmentally friendly, stable, reliable, low cost, ultra-fast and easy to synthesize compared to other conventional methods (Viuda-Martos et al., 2010; Arulvasu et al., 2010). *P. granatum* which is a well-known medical plant belonging to the family Lythraceae have been used for the synthesis of Ag NPs (Eroles et al., 2012). In a recent study, cytotoxic activity of *P. granatum* against human colon adenocarcinoma and laryngeal cancer cell lines has been

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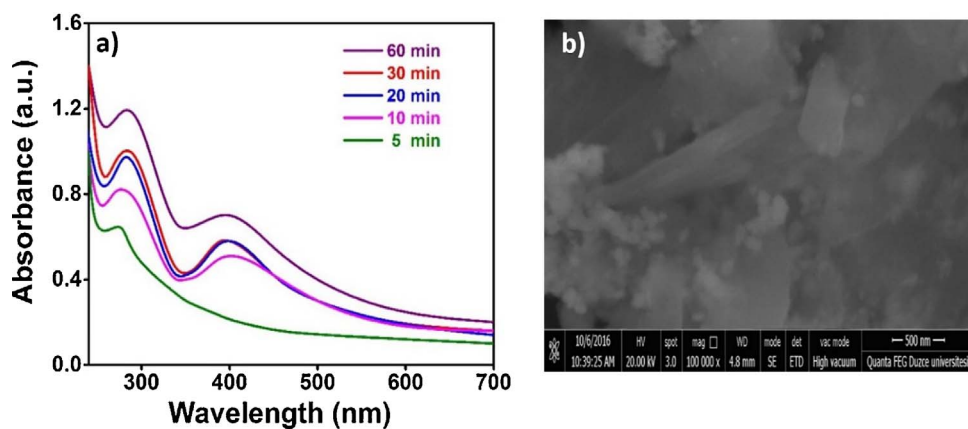


Fig. 1. (a) The UV-vis absorption spectra of Ag NPs synthesized from pomegranate peels (b) SEM image of the produced Ag NPs.

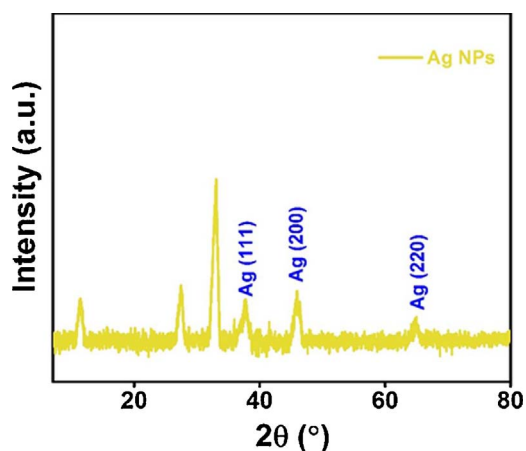


Fig. 2. XRD pattern of Ag NPs exhibiting the diffraction of crystalline silver.

reported (Rai et al., 2006). For this purpose, addressed herein, *P. granatum* was investigated for the microwave assisted green synthesized Ag NPs for antiproliferative effect against MCF-7 cell line.

2. Experimental procedure

2.1. Microwave assisted green synthesis of AgNPs using pomegranate peel extract

For green synthesis of Ag NPs in medium, 10 mM aqueous AgNO_3 solution was added to 220 mL pomegranate extract and mixed in a vial. Then the vial was placed on the turntable of the microwave oven. The mixture was irradiated for a total of 3 min duration. After irradiation, appearance of a dark brown colour indicates the formation of Ag NPs. Ag NPs were then obtained by centrifugation at 12,000 rpm for 15 min. A pellet was washed three times with ethanol, then dried in a vacuum oven.

3. Results and discussion

Mixing the pomegranate extract with the AgNO_3 solution at 1 mM resulted in a yellowish-brown color for approximately three minutes, which was thought to be caused by over-induction of plasma vibrations in the surface (Miller, 1959). The whole reaction took about 3 min in microwave condition. Fig. 1a shows the UV-vis spectroscopy of the liquid component as a function of time variation of the pomegranate extract with 1 mM liquid AgNO_3 solution. Metal nanoparticles have free electrons, which cause a surface plasmon resonance (SPR) absorption

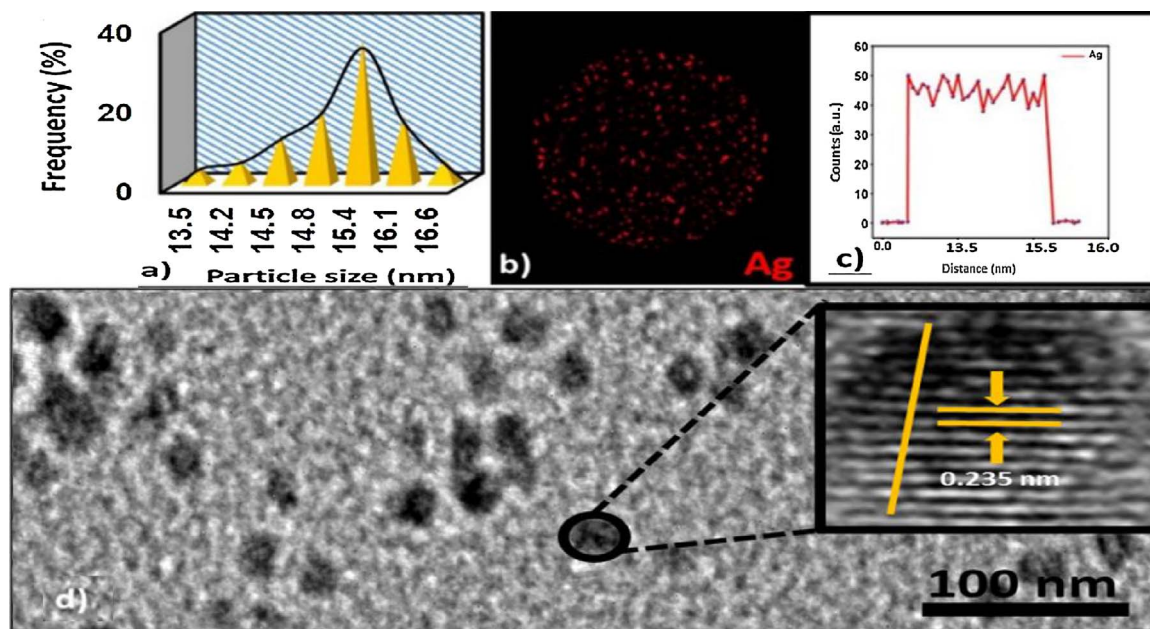


Fig. 3. (a) Particle size histogram (b) EELS elemental color-mapping (c) EELS line profile. (d) TEM and HRTEM images of Ag NPs.

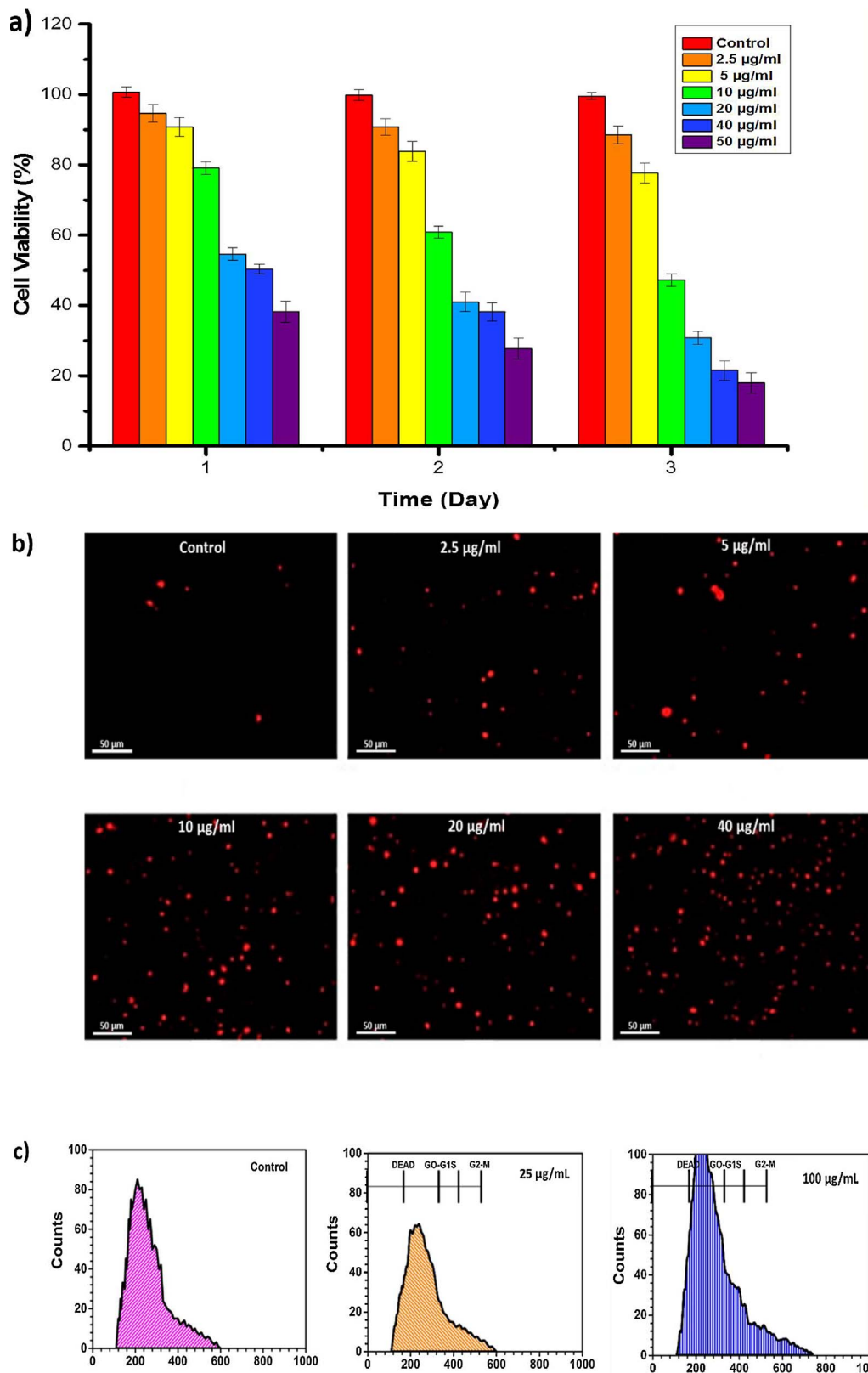


Fig. 4. (a) Cell viability following Ag NPs application on MCF7 cells analyzed using MTT method. (b) Propidium iodide (PI) stain exposed to both untreated (control) and AgNPs treated MCF-7 cells. (c) Cell cycle analysis of both untreated and Ag NPs treated MCF-7 cells.

band attributed to the association of vibrating electrons. The SPR band of the biosynthesized Ag NPs was recorded at 420 nm during the reaction at room temperature. Furthermore, FESEM images of synthesized Ag NPs confirms the formation of nanoparticles as shown in Fig. 1b. Silver nanoparticles usually exhibit SPR-related absorption peak at 3 keV energy (Eroles et al., 2012). Besides, Ag NPs have also been

characterized by XRD. For this purpose, it's shown the distinct peaks corresponding to (111), (2 0 0) and (2 2 0) in the face centered cubic (fcc) structure of Ag NPs during XRD analysis (Fig. 2). These results were good agreement with previous studies (Rai et al., 2006; Vijayakumar et al., 2013).

Besides, Fig. S1 shows the FTIR results demonstrating the

Table 1

The comparison of Ag NPs with the other nanoparticles in literature.

Nanoparticle	IC 50	Cell	Reference
Pomegranate-silver nanoparticle	12.85 µg/mL	MCF-7	This work
Solanum trilobatum-silver nanoparticle	300 µg/mL	MCF-7	Ramar et al. (2015)
Potentilla fulgens Wall. Hook-silver nanoparticle	4.91 µg/mL and 8.23 µg/mL	MCF-7 U-87	Mittal et al. (2015)
Turmeric extract-iron	3.1 ± 0.4 M and 4.9 ± 0.5 M (Photocytotoxicity)	HeLa MCF-7	Sarkar et al. (2016)
Datura-silver nanoparticle	20 µg/mL	MCF-7	Gajendran et al. (2014)

interaction of *P. granatum* biomolecules with nanoparticles with distinct peaks at 2926, 1682, 1542 and 1334 cm^{-1} . As shown in this figure there is a change in the absorption peaks indicating the formation of Ag NPs encapsulated by biocompounds (Tangkanakul et al., 2009; Bakar et al., 2009; Miller, 1959; Bradford, 1976) The distributions determined bonds bound to hydrogen bonded OH stretching (2926 cm^{-1}), C=O stretching (1682 cm^{-1}), NH band (1542 cm^{-1}) and N=O band (1334 cm^{-1}) shows the formation of Ag NPs covered with biocompounds. Lastly, monodisperse Ag NPs has also been characterized by TEM. The morphology and size of the nanoparticles were found to be from 13.5 to 16.6 nm (mostly 15.4 nm) by the help of TEM (Fig. 3). Furthermore, Fig. 3b and c indicates that the EELS mapping and line profile of Ag NPs which also shows the existence of Ag in prepared nanomaterials. Besides, atomic lattice fringes of Ag NPs have also been measured as 0.235 which also indicates the existence of monodisperse Ag NPs.

After the characterization of microwave assisted green synthesized silver nanoparticle, we have observed that the cytotoxic effect of Ag NPs on the MCF7 cell array increased with increasing concentration of nanoparticles. There was a significant difference in cell viability between the control and the treated groups as shown in Fig. 4a and the IC50 level was found to be 12.85 µg/mL Ag NPs for the MCF7 cells. The effect of Ag NPs on the control cell array is lower compared to the MCF7 cell line in applied dilutions (Bradford, 1976; Noguez, 2007; Yang and Li, 2013; Philip, 2009; Piao et al., 2011; Asha Rani et al., 2013). It's observed that the microwave assisted green synthesized Ag NPs in combination with pomegranate extract caused apoptotic changes in cancer cells, and there was a marked difference compared to the control groups in this regard as shown in Fig. 4b. Furthermore, the anti-proliferative effect of Ag NPs biosynthesized from pomegranate extract upon staining with Propidium iodide (PI) showed apoptotic changes and nuclear condensation. In case of control cells, a very negligible number of PI positive cells were noticed. By contrast a progressive increase in the number of PI positive cells was noted in monodisperse Ag NPs treated cells as shown in Fig. 4b. This data suggest that monodisperse Ag NPs scan induce cell death in MCF7 cells through the active oxygen species mediated apoptotic process. The increased ROS levels and subsequent loss of mitochondria membrane potential might be the reason for increased apoptotic morphological changes in Ag NPs treated cells (Castelli et al., 2013; Bilto et al., 2015). Flow cytometry was also used to further investigate whether monodisperse Ag NPs affected the cell cycle as shown in Fig. 4c. By performing of flow cytometry analysis, it's discovered that the treatment with monodisperse Ag NPs for 24 h significantly reduced the DNA content which is good agreement with the literature (Bilto et al., 2015; Guzman et al., 2012). Table 1 also indicates the comparison of Ag NPs with the other nanoparticles in literature for this purpose. As shown in this table current Ag NPs have superior IC 50 values compared to the others in literature.

4. Conclusion

Addressed herein, this work explored the formation of microwave assisted monodisperse Ag NPs from environmentally friendly syntheses using pomegranate extract. The average particle size of monodisperse AgNPs is in the range of 13.5–16.6 nm (mostly 15.4 nm) and is regular

spherical shape. In addition, the prepared monodisperse Ag NPs have possesses anticarcinogenic activity against MCF-7 cells. It can be concluded that the microwave assisted green synthesized monodisperse AgNPs can be used as potential cancer agents in pharmaceutical industry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jbiotec.2017.09.012>.

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