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CYTOTOXIC EFFECT OF GREEN SYNTHESIZED SILVER NANOPARTICLES WITH *SALVIA OFFICINALIS* ON MCF-7 HUMAN BREAST CANCER CELLS

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ABSTRACT

Alternative in vitro nanotechnological methods have been developed to conventional methods in the treatment strategies of breast cancer. Silver nanoparticles (AgNPs) from metallic nanoparticles, especially those amplified using the green synthesis method, show promise as a suitable anticancer candidate in the field of nanomedicine. The purposed of the investigation was to green synthesize and characterize of silver nanoparticles (So-AgNPs) by *Salvia officinalis* aqueous extract and evaluate their anticancer effect on metastatic breast cancer cell line MCF-7. The formation of So-AgNPs were characterized by scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX) and UV-visible spectroscopy. The surface absorption peak of So-AgNPs was observed at 417 nm by the UV-Vis analysis. MCF-7 and CRL-4010 cells were treated with different concentrations (0-0.05 µg/mL) of AgNPs for 24 h. The cytotoxic effect of green synthesized AgNPs against MCF-7 and CRL-4010 cell lines was approved by MTT [3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl tetrazolium bromide] assay. MTT results showed that So-AgNPs have higher cytotoxic activity in MCF-7 breast cancer cell lines than in CRL-4010 human breast epithelial cells (IC50 values 8.49 and 9.69 µg/mL, respectively). AgNPs synthesized via *Salvia officinalis* extract exhibited a significant cytotoxic effect on MCF-7 cell lines, demonstrating that they could be a potential antitumor agent in the treatment of metastatic breast cancer. However, further research is required to elucidate the mechanisms of action.

INTRODUCTION

Breast cancer, one of the cancers with the highest incidence in women worldwide, is characterized by the uncontrolled growth and spread of abnormal cells (1, 2). Breast cancer is a metastatic cancer and can usually pass to distant organs such as liver, bone, brain, and lung. Despite various treatment options such as surgery, radiotherapy and chemotherapy, there was no significant increase in the survival rate of patients with metastatic breast cancer (3,4). New strategies for treatment are being developed due to the complex nature of the manifestation of cancer in each patient (5).

Nanoscience and nanotechnology represent a field of research that includes the chemical composition, shapes, controlled distribution, and synthesis of nanoparticles (NPs) formed by the arrangement of atoms on the scale of 1-100 nm and their use for human benefit (6, 7). Nanomedicine is one of the application areas of nanotechnology having the potential to develop solutions for the diagnosis, prevention or treatment of diseases at the cellular level. It also include different multidisciplinary medicine fields like regenerative medicine and drug delivery system (8). The metallic nanoparticles are playing imperative role in pharmaceutical and medicinal sciences (9-12). Green synthesis is preferred method compared to chemical and physical methods due to having a number of advantages such as being cost-effective, being eco-friendly approach, and being easily available (13,14). Plant-mediated biosynthesis of NPs has attracted great attention among researchers because of plant act as reducing and stabilizing agents for NPs. It is a very important factor that plants have different amounts of reducing content in terms of the size and shape of the NPs to be biosynthesized. (15-18).

Salvia officinalis is rich in polyphenols consisting of

flavone glycosides and a series of rosmarinic acid derivatives and has antioxidant properties (19). Pharmacological findings from studies on include antimutagenic, anticancerogenic, hypoglycemic, antimicrobial, anti-inflammatory, hypolipidemic, antinociceptive, antioxidant, and antidementia effects (20). In addition, many researchers have investigated the pharmacological effects of green synthesized silver nanoparticles mediated by plant extracts (21-23). The use of metallic nanoparticles, especially silver nanoparticles (AgNPs) synthesized from plants using the green synthesis method, as anti-cancer agents in the field of nanomedicine is promising (24). The possible mechanism for the biosynthesis, plant extracts interact with metal ions and reduce metal ions to nanoparticles. (25). Chemical compounds of easily provide the reduction of Ag^{+} ions to Ag0 for the formation of AgNPs (26,27).
The aimed of this study was to synthesize, characterize aqueous extract-based silver nanoparticles (So-AgNPs) and analyze to cytotoxic effect via MTT assay on MCF-7 metastatic breast cancer cells and CRL-4010 human mammary epithelial cells.

EXPERIMENTAL

Preparation of Aqueous Extract and Biosynthesis of Silver Nanoparticles

The dried leaves were obtained from the local herbal market in Burdur (Turkey). The extract was prepared by boiling 0.2 g of leaves in 100 mL of deionized water. The aqueous *Salvia officinalis* extract was filtered with whatman no.1 filter paper and stored at 4˚C until following usage.

For the green synthesis of silver nanoparticles (AgNPs); 95 mL of 5mM $AgNO_3$ solution was mixed with 5 mL of *Salvia officinalis* aqueous extract. The mixture was microwaved (1200 W, 50 Hz) for 1 min. Time-dependent color change indicating the formation of *Salvia officinalis*-AgNPs (So-AgNPs) was followed by observing the progress of the reaction.

Characterization Techniques

The formation of So-AgNPs shows the yellow to dark brown color change that takes place during the reaction. Shimadzu-UV-1801 spectrophotometer was used to monitor the spectra of the So-AgNPs. The absorption spectra of the So-AgNP solution were recorded in the wavelength range of 300-700 nm. The morphology of the green synthesized AgNPs were observed SEM with EDX.

Cell Culture

The human breast cancer cell line MCF-7 and human mammary epithelial cell line CRL-4010 were purchased commercially from American Type Culture Collection. All of the cells are treated in DMEM (Dulbecco's modified eagle medium) (PAN-biotech) medium with Streptomycin/Penicillin containing 10% fetal bovine serum (FBS), sodium pyruvate, non-essential amino acids, and L-glutamine at 37 °C temperature with 5% CO_2 in humidified atmosphere and grown as monolayer cultures in the incubator.

MTT Assay

The cytotoxic properties of biosynthesized So-AgNPs against metastatic breast MCF-7 and normal breast epithelium CRL-4010 cells were assessed by MTT assay. For experiments, the cells (at a density of 3000 cells per well) were seeded into 96-well plates with the medium containing 10% FBS and incubated for 24 h with 5% CO_2 at 37 ˚C in humidified atmosphere. After overnight culturing, the medium was replaced with DMEM containing 1% FBS and the So-AgNPs (0-0.05 mM). The cells not treated with So -AgNPs were used as controls. After the applied treatments, the plates were incubated for 24 h. Then 10 µL of MTT (5 mg/mL) was transferred to each well and incubated for 4 h. After incubation, the purple-colored formazan crystals formed were dissolved in 100 µL dimethyl sulfoxide (DMSO). Plate reader (Multiscan GO, Thermo Fisher Sci.) was used to evaluate Optical density (OD) at 570 nm. Results were expressed as average values of three independent experiments. The following formula was used to calculate cell viability.

The IC_{50} values of So-AgNPs for MCF-7 and CRL-4010 cells were estimated separately from the data of generated dose–response curve. According to the results, the IC_{50} values of applied So-AgNPs to each cell line were calculated with the GraphPad Prism8 software.

Statistical Analysis

GraphPad Prism (v8) software was used to evaluate the experiment data. Analyzes were two-tailed and a p-value less than 0.05 was considered significant.

RESULTS

Synthesis and Characterization of So-AgNPs

The biosynthesis of So-AgNPs were performed with the *Salvia officinalis* aqueous extract. Changing of

Figure 1. Biosynthesis of So-AgNPs (time dependent color change)

color from yellow to dark brown was shown the formation of nanoparticles (Figure 1). Absorption screening of So-AgNPs was performed using UV-visible spectrophotometer in the 300-700 nm range. The UV-vis absorption spectrum for silver nanoparticle is revealed in the wavelength range of 400-500 nm. The absorption peak related to So-

$$\text{Cell viability (\%)} = \frac{\text{the OD of treated well}}{\text{the OD of control well}} \times 100$$

AgNPs was observed at 417 nm (Figure 2). The morphology of the So-AgNPs were recorded via SEM (Figure 3). According to the EDX result, a strong peak at 3 keV in the plotted graph confirms the Ag element (Figure 4).

In vitro Anticancer Activity of So-AgNPs

The MCF-7 and CRL-4010 cells were exposed to various concentrations of So-AgNPs for 24 h, then the cytotoxicity assessment was performed using the MTT assay. MTT results showed that So-AgNPs decreased cell viability in a dose and time-dependent manner compared to the control (Figure 5A-B). The inhibitory concentration (IC50 value) of So-AgNPs was calculated to be 8.49 and 9.69 µg/mL respectively, on MCF-7 and CRL-4010 after 24 h of cell treatment.

DISCUSSION

Nowadays, nanotechnology has found an innovative usage in application areas such as industry, health, scientific research, and medicine (28,29). The use of metallic nanoparticles attracts utmost attention because of their unique properties that make them practicable in dissimilar areas fields of nanotechnology (30,31). Especially silver nanoparticles have a great potential with antibacterial, antiviral, antifungal and antimicrobial properties in the field of nanomedicine because they can readily cross cell membranes (32). The physicochemical properties of AgNPs are crucial to understanding their cellular uptake, infiltration into biological membranes or barriers, particle distribution, and therapeutic effects (33). The conventional procedures to synthesize metal nanoparticles require complex and costly tools or high-priced chemicals. Although there are different methods such as chemical and physical methods for the synthesis of AgNPs, green approaches of

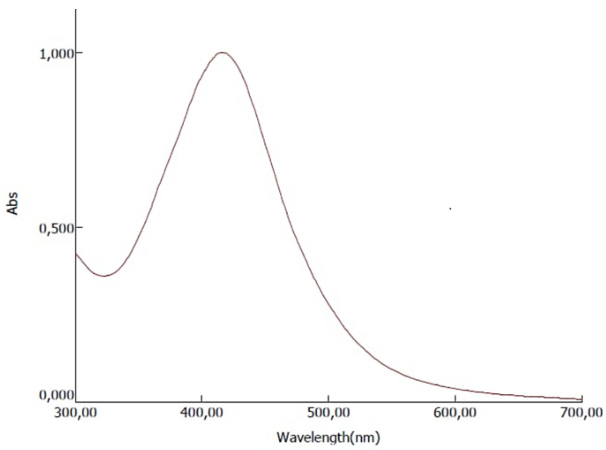

Figure 2. UV-vis spectrum of synthesized So-AgNPs

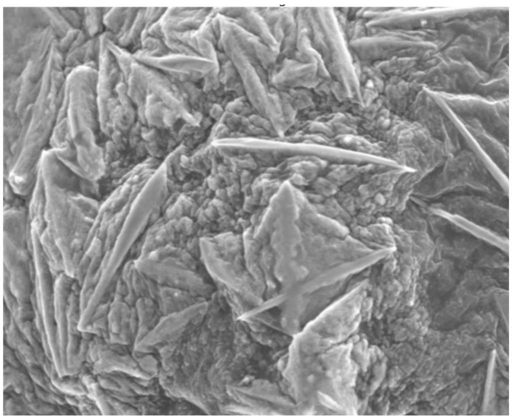

Figure 3. SEM image of synthesized So-AgNPs

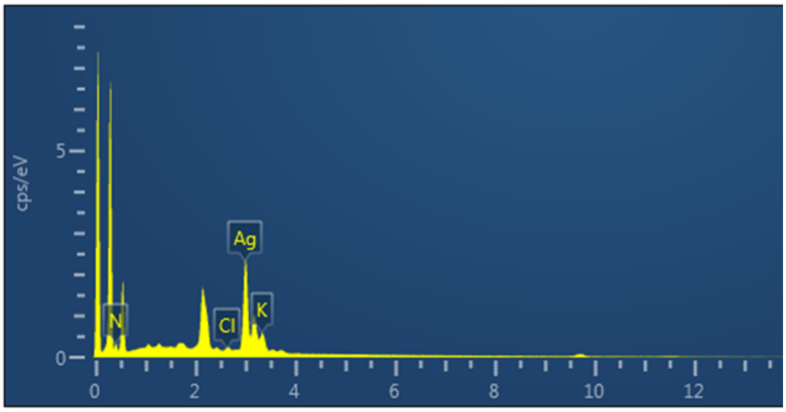

Figure 4. EDX spectrum of So-AgNPs

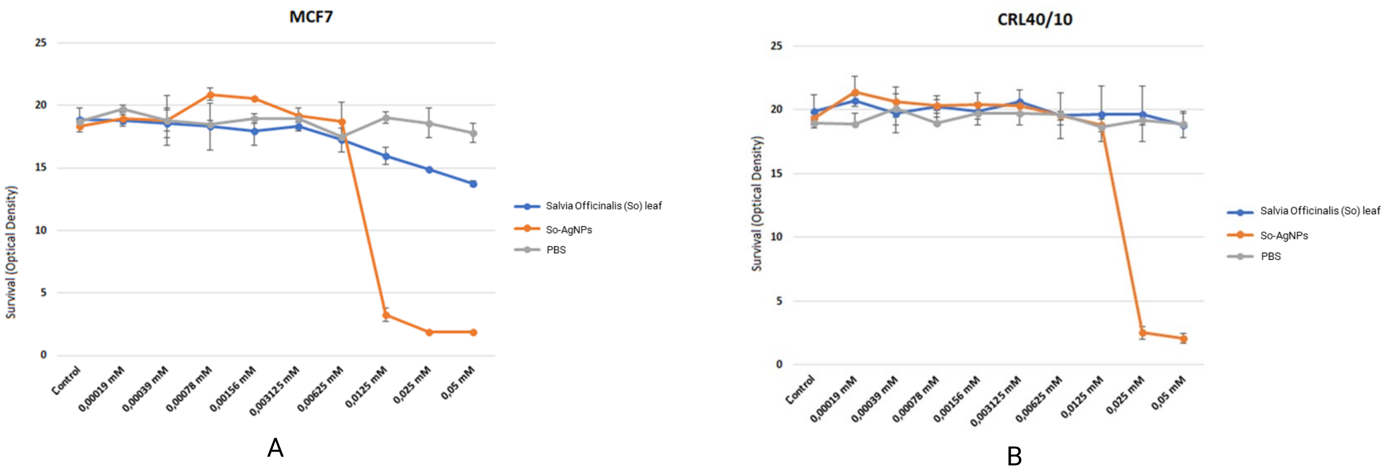

Figure 5. The results of the MTT assay in MCF-7 (A) and CRL-4010 (B) cells treated with So-AgNPs for 24 h

synthesis of AgNPs are preferred owing to its simplicity, cost-effectiveness, and eco-friendliness (34,35). Biogenic sources such as yeasts, fungi, algae, plants, and bacteria are used in the green synthesis of AgNPs in different shape and size (36,37).

Herbal extracts used in green approaches have the potential to reduce metal ions to metallic nanoparticles as a consequence of their interaction and herbals act like bioreactors for metal nanoparticle synthesis (25,38). It is known that *Salvia officinalis* extract has therapeutic and pharmacological effects because of its antimicrobial, anti-inflammatory and antioxidant activities (39-41). The most important reason for using *Salvia officinalis* extract to synthesize AgNPs in this study is its content and these known properties of *Salvia officinalis*.

In the present study, biosynthesis of So-AgNPs with *Salvia officinalis* extract was achieved to evaluate the cytotoxic effects of So-AgNPs on the MCF-7 and CRL-4010 cell lines. UV-vis spectroscopic analysis, SEM-EDX was used to characterize the biosynthesized So-AgNPs. As shown in Figure 2, a peak at 417 nm was appeared related to biosynthesized silver nanoparticles. Similar findings were obtained in the study when compared with the studies in the literature on green synthesized silver nanoparticles. (42,43). According to SEM image, So-AgNPs have nonuniformly distributed AgNPs with a degree of aggregation. Their shape showed a cluster of relatively spherical and rod-like morphology. SEM -EDX images were similar to the study conducted by Vishwanatha et al. (44,45).

Studies show that silver nanoparticles biosynthesized from herbal extract have a potent cytotoxic effect against tumor cells. (46-48). In the literature, cytotoxic and anticancer activities of *Salvia officinalis* extract and nanoparticles synthesized from *Salvia officinalis* against various cancer cells have been reported (27,49,50). The viability and proliferation of cancer cells treated with bio-AgNPs were reduced at low doses (51-53). In the study, in vitro cytotoxic effect of So-AgNPs prepared by biosynthesis was examined with the MTT assay. MTT analysis exhibited the presence of cytotoxic effects in MCF-7 and CRL-4010 cells at 24 h owing to the increasing dose of biosynthesized So-AgNPs. The inhibitory concentration of So-AgNps was found to be 8.49 and 9.69 µg/mL, respectively. These results are in good numbness with data in the literature reporting concentration-dependent toxicity of silver nanoparticles on MCF-7 cells, especially at low concentration levels (54, 55, 56). The synthesized AgNPs via Tamarindus indica have cytotoxicity against MCF-7 cell lines and the inhibitory concentration (IC50) was found to be 20 µg/mL by Gomathi et.al. (57). Cytotoxic activity of the green synthesized AgNPs by using C. gilliesii against normal skin fibroblast (BJ-1) and human breast cancer cell (MCF-7) were found to 80.1 and 36.5 g/mL at 48 hours incubation, respectively by Emam et al. (58). In the literature reporting, green synthesized silver nanoparticles using *Salvia officinalis* extract have cytotoxic and inhibitory effect on the cell viability of the MCF-7 cells in a dose-dependent manner have been reported (26, 59, 60).

CONCLUSION

In the present work, AgNPs were successfully synthesized and characterized by using *Salvia officinalis* extract. Green synthesis of AgNPs using *Salvia officinalis* extract exhibited a significant cytotoxic effect on MCF-7 cell lines compared to control. It can be a potential alternative antitumour agent in breast cancer treatment. However, to promote the findings, further studies are needed to clarify the mechanism of action at the molecular level and to test it in experimental breast cancer models in animals.

Disclosure statement

The authors declare that they have no conflicts of interest.

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