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Combination of Silver Nanoparticles Synthesized Using Karaerik Extract and Cisplatin: Effects on Breast Cancer Cells

Tuğba ATICI[1], Deniz ALTUN ÇOLAK[2] ✉
[1]Ministry of Education, Erzincan, Turkey, [2]Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, Erzincan, Türkiye,
[1]https://orcid.org/ 0000-0002-4798-935X, [2]https://orcid.org/ 0000-0002-3576-0355
✉: daltun@erzincan.edu.tr

ABSTRACT

Nanoparticle-based therapies provide targeted drug delivery with reduced side effects, making them promising options for cancer treatment. This study examines the anticancer and oxidative effects of green-synthesized silver nanoparticles (AgNPs) derived from the Karaerik grape (*Vitis vinifera* L., Vitaceae), a variety known for its high antioxidant capacity, both alone and in combination with cisplatin (CP) on MDA-MB-231 breast cancer cells. AgNPs were synthesized using the plant leaf extract and characterized through UV-Vis, SEM-EDX, and FT-IR techniques. MDA-MB-231 cells were exposed to 10 and 20 µg mL^{-1} AgNPs, 25 µM CP, and their combinations for 24, 48, and 72 hours. Cytotoxicity was analyzed using the MTT assay, and IC_{50} values were determined. Parameters of oxidative stress, including total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI), were evaluated to assess oxidative balance disruption. The combination of AgNPs and CP led to a significantly greater reduction in cell viability compared to individual treatments (p<.05). The IC_{50} value of CP decreased in the presence of AgNPs, indicating an enhanced cytotoxic effect. Additionally, a significant increase in TOS and OSI levels, along with a decrease in TAS, suggested that the combination therapy induced oxidative stress-mediated cell death. Green-synthesized AgNPs from Karaerik grape leaves enhance CP-induced cytotoxicity by modulating oxidative stress pathways, indicating their potential role as adjuvants in breast cancer therapy. These findings underscore the importance of green nanotechnology-based strategies in oncology.

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Karaerik Ekstraktı ile Sentezlenen Gümüş Nanopartiküllerin Sisplatin ile Kombinasyonu: Meme Kanseri Hücrelerine Etkileri

ÖZET

Nanopartikül bazlı tedaviler, azaltılmış yan etkilerle hedefe yönelik ilaç dağıtımı sağlayarak kanser tedavisinde umut vaat etmektedir. Bu çalışmada, yüksek antioksidan kapasiteye sahip bir üzüm çeşidi olan Karaerik üzümünden (*Vitis vinifera* L., Vitaceae) elde edilen yeşil sentezlenmiş gümüş nanopartiküllerin (AgNPs) tek başına ve sisplatin (CP) ile kombinasyon halinde MDA-MB-231 meme kanseri hücreleri üzerindeki antikanser ve oksidatif etkileri araştırılmaktadır. AgNP'ler bitki yaprağı ekstraktı kullanılarak sentezlenmiş ve UV-Vis, SEM-EDX ve FT-IR teknikleri ile karakterize edilmiştir. MDA-MB-231 hücreleri 10 ve 20 µg mL^{-1} AgNP'lere, 25 µM CP'ye ve bunların kombinasyonlarına 24, 48 ve 72 saat boyunca maruz bırakılmıştır. Sitotoksisite MTT deneyi kullanılarak değerlendirilmiş ve IC_{50} değerleri belirlenmiştir. Oksidatif denge bozulmasını belirlemek için toplam oksidan durum (TOS), toplam antioksidan durum (TAS) ve oksidatif stres indeksi (OSI) dahil olmak üzere oksidatif stres parametreleri analiz edilmiştir. AgNPs ve CP kombinasyonu, tek tek uygulamalara kıyasla hücre canlılığında önemli ölçüde daha büyük bir azalma ile sonuçlanmıştır (p<.05). CP'nin IC_{50} değeri AgNP'lerin varlığında azalmış ve bu da sitotoksik etkinin arttığını

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göstermiştir. Ek olarak, TOS ve OSI seviyelerindeki kayda değer artış ve TAS'daki düşüş, kombinasyon tedavisinin oksidatif stres aracılı hücre ölümüne neden olduğunu göstermiştir. Karaerik üzüm yapraklarından yeşil olarak sentezlenen AgNP'ler, oksidatif stres yolaklarını modüle ederek CP kaynaklı sitotoksisiteyi güçlendirmekte ve meme kanseri tedavisinde adjuvan olarak potansiyel rollerini ortaya koymaktadır. Bu bulgular, onkolojide yeşil nanoteknoloji tabanlı stratejilerin önemini vurgulamaktadır.

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INTRODUCTION

Cancer is a complex disease caused by a combination of genetic, epigenetic, and environmental factors leading to uncontrolled cell differentiation and proliferation, disruption of key cellular processes like division and DNA repair, and the immune system's failure to eliminate mutated cells (Lodish et al., 2000; Yılmaz & Altunok, 2011). The first tumors that form due to the uncontrolled proliferation of a single mutated cell are generally referred to as benign; these tumors cannot spread to other tissues. However, tumors that gain the ability to spread (metastasize) to other tissues over time are classified as malignant (Alberts et al., 2002; Yokota and Kohno, 2004; Hoeppner and Bronsert, 2021). Malignant tumors are often described as cancer. GLOBOCAN, part of the International Agency for Research on Cancer (IARC) of the World Health Organization, provides global cancer statistics. There was a total of 50.6 million cancer cases worldwide in 2020; 19.3 millions of these cases were newly diagnosed, and 10 million people died due to cancer. Statistics predict that more than 20 million new cancer cases will occur every year by 2025. In 2022, lung cancer was the most diagnosed cancer worldwide (12.4%) and the leading cause of cancer deaths (18.7%), followed by breast, colorectal, liver, and stomach cancers. Breast cancer is most common in women, while lung cancer dominates in men for both cases and deaths. (Bray et al., 2024). Common cancer treatment methods, such as chemotherapy and radiotherapy, often come with significant side effects. Furthermore, the efficacy of many chemotherapy therapies is diminished by their limited bioavailability and water solubility (Yi et al., 2022). The primary goal of cancer research is to find and develop new approaches that significantly improve cancer treatment and prevention.

Nanotechnology-based therapies have recently gained significant attention due to their high specificity and minimal side effects. Nanoparticles are particularly useful in cancer treatment, enabling the precise delivery of chemotherapy drugs to targeted organs, cells, or organelles, enhancing drug efficacy, and reducing side effects (Zhao et al., 2019). These nanoscale carrier systems, with unique chemical, physical, and biological properties, are increasingly employed in disease prevention, diagnosis, and treatment (Cao et al., 2022).

Cisplatin (CP) is a powerful platinum-containing chemotherapeutic agent widely used in chemotherapy (Florea and Büsselberg, 2011; Becit et al., 2017). To work, CP forms a cross, or covalent connection, with purine bases on cell DNA (Kelland, 2007; Gültekin, 2013). This process interferes with DNA repair mechanisms, causing DNA damage and ultimately triggering apoptosis, that is, programmed cell death (Gürbüz et al., 2011). CP is widely used against various types of cancer, such as breast, ovarian, testicular, bladder, lung, prostate, esophageal carcinomas, and sarcomas, alone or in combination with different antineoplastic agents or radiotherapy (Boulikas & Vougiouka, 2003; Frezza et al., 2010; Gürbüz et al., 2011; Becit et al., 2017).

In recent years, nanoparticles obtained by the green synthesis method using plant extracts have attracted great attention due to their antimicrobial, antioxidant, and anticancer effects and have found use in the field of health (Nematollahi, 2015; Nikam et al., 2019; Souza et al., 2019). Given the limited treatment options for this type of cancer, the discovery of novel and effective therapeutic agents remains essential (Chavez et al., 2010). The Karaerik plant is significant in this study due to its rich phytochemical profile and potential biomedical applications. It has been chosen specifically for its high content of bioactive compounds such as polyphenols, flavonoids, and resveratrol, which are known for their strong antioxidant, anti-inflammatory, and anticancer properties (Kaur et al., 2009; García-Oliveira et al, 2021; Kupe et al., 2021; Pérez-Navarro et al., 2022). Silver nanoparticles (AgNPs), in particular, have an important place in the field of nanotechnology due to their chemical stability, good conductivity properties, antibacterial, antiviral, and antifungal effects, and also exhibit promising anticancer activities against various cancer cells (Klaus-Joerger, 2001). Silver nanoparticles with CP are designed to increase cytotoxicity, induce apoptosis, and overcome drug resistance, enhancing the anti-tumor efficacy of CP.

This molecule uses oxidative stress and gene regulation mechanisms to target cancer cells and minimize the effects of cancer. It selectively has little or no effect on healthy cells. The use of functionalized nanoparticles can further improve the transport and release of drugs. With the increasing use of these combinations, it is seen as a possible approach to cancer treatment. (Elbaz et al., 2016; Yuan & Gurun, 2017; Rank Miranda et al., 2020).

The study was designed to investigate the antioxidant and anticancer properties of CP and green-synthesized AgNPs from the Karaerik plant on the human breast cancer cell line MDA-MB-231, known for being one of the most aggressive and lethal breast cancer subtypes. The plant's extracts have demonstrated promising activity in nanoparticle synthesis through green methods, offering a sustainable and efficient approach to producing nanoparticles with enhanced therapeutic potential. Its importance lies in utilizing natural and readily available plant-based resources to develop advanced nanotechnology applications, particularly in combating diseases like cancer while minimizing environmental impact.

MATERIAL and METHOD

Cell Lines

MDA-MB-231 human breast cancer cells and MCF-10A non-malignant mammary epithelial breast cells (ATCC®, USA) were used in this study for cytotoxicity assays. MCF-10A cell lines were utilized to test the biocompatibility of the synthesized silver nanoparticles in living cells. The MCF-10A cells (passage number <8) were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (GIBCO Life Technologies, USA) medium supplemented with 10% heat-inactivated fetal bovine serum (GIBCO Life Technologies, USA), 20ng/mL epidermal growth factor (Sigma-Aldrich, USA), 100µg/mL hydrocortisone (Sigma-Aldrich, USA), insulin and 1% penicillin-streptomycin (GIBCO Life Technologies, USA) in 5% CO_2 at 37°C. MDA-MB-231 with a passage number less than 30 was propagated in Roswell Park Memorial Institute (RPMI) 1640 (GIBCO) medium supplemented with 10% heat-inactivated FBS (GIBCO Life Technologies, USA) and 1% 10 U/mL penicillin, and 100µg/mL streptomycin (GIBCO Life Technologies, USA) in 5% CO_2 at 37°C. Cells were harvested for treatment once they had reached 70-80% confluency.

Preparation of Karaerik Leaves Extract

Vitis vinifera L. cv. Karaerik (Vitaceae), the main material of this research, was obtained from the vineyards in Üzümlü, Erzincan, Turkey, in September 2018. Dr. Ali Kandemir expertly performed the taxonomic identification of the plant species using "Flora of Turkey and the East Aegean Islands" (Davis, 1985; Davis et al., 1988; Güner et al., 2000), and all plant specimens were subsequently deposited in the Herbarium of the Science and Art Faculty at Erzincan Binali Yıldırım University. Grape leaves were washed, air-dried for a week to remove moisture, and finely cut. 5 grams of these leaves were heated with 100 mL of sterile distilled water at 80 °C for an hour, and the solution turned light yellow. After cooling, the extract was filtered, centrifuged at 3500 rpm for 5 minutes to remove residues, and stored at 4 °C for further use.

Green Synthesis of Silver Nanoparticles

A 10 mL $AgNO_3$ (100 mmol L^{-1}) solution was added to 40 mL of Karaerik grape leaf extract. The mixture was heated on a hot plate at a temperature of 70 °C, stirring continuously for two hours. The color of the reaction mixture gradually changed from pale yellow to orange, red, and brown, demonstrating the reduction of silver ions to silver atoms and indicating the formation of silver nanoparticles. The colloidal suspension of the synthesized nanoparticles (AgNPs) was then centrifuged at 6000 rpm for 10 minutes, and the resulting solid product was washed several times with distilled water to remove any unbound silver and plant extract. A similar experimental procedure was followed to repeat the synthesis of AgNPs, this time with the reaction mixture exposed to sunlight.

Characterization of Green Synthesized Silver Nanoparticles

The characterization of silver nanoparticles was performed using UV-vis spectroscopy (PerkinElmer Lambda 35), SEM equipped with EDX (FEI- Quanta FEG 450), and FT-IR (Thermo Scientific Nicolet 6700) analyses. UV-vis spectra were recorded on a UV-vis Spectrophotometer (200- 700 nm, 1 nm resolution), and the optical properties of AgNPs were confirmed. The structural and morphological shape and size of AgNPs were revealed by scanning electron microscopy (120,000X, 30 kV). FTIR analysis (400- 4000 cm^{-1}) was used to identify the surface biomaterials and oxide forms.

Preparation of Stock Solutions

The CP stock solution was freshly prepared in the dark by fully dissolving 1 mg of CP (molecular weight 300.05 g

mol^{-1}) in 50 µL of dimethyl sulfoxide (DMSO). This solution was then diluted with RPMI 1640 medium to a final volume of 1 mL. The final concentration of CP in this stock solution was 1 mg mL^{-1}, and aliquots were further diluted to achieve the desired working concentrations (4, 8, 16, 32, and 64 µM) for the MTT assay. The AgNP stock solution was also freshly prepared by fully dissolving 3 mg of AgNP in 100 µL of distilled water. This solution was then added to RPMI 1640 medium to reach a final volume of 1 mL. The final concentration of AgNP in the stock solutions was 3 mg mL^{-1}, and working concentrations were prepared to get the desired final concentrations (2.5, 5, 10, 15, 20, 25, and 50 µg) for the MTT assay.

MTT Assay

The dosages for AgNP and cisplatin were selected based on preliminary dose-response experiments to identify concentrations that would provide a broad range of effects without causing immediate cytotoxicity, allowing for accurate IC_{50} calculation. The concentrations of AgNP (2.5, 5, 10, 15, 20, 25, and 50 µg mL^{-1}) and cisplatin (4, 8, 16, 32, and 64 µM) were chosen to cover a broad spectrum of potential biological activity. In this study, two different dosage selection approaches were adopted. First, a wide range of AgNP and cisplatin concentrations was selected to explore overall biological activity. Secondly, the concentrations of 10 µg mL^{-1} and 20 µg mL^{-1} AgNP were specially preferred based on previous literature, which demonstrated effective biological responses within this range without causing high toxicity levels in cells. These specific doses were applied to both cell lines (MCF-10A and MDA-MB-231) for 24, 48, and 72 hours to assess cytotoxic potential. These concentrations were further validated in preliminary trials where cell viability was evaluated using MTT assays at various time points. The IC_{50} values were determined by analyzing the MTT assay results using AAT Bioquest software. This software accurately estimated the concentration required to inhibit 50% cell viability at each time point. These values were then used to evaluate the comparative efficacy of AgNP and cisplatin in inducing cytotoxic effects in the treated cells. Therefore, during the main experimental phase, only the doses of 10 and 20 µg mL^{-1} AgNP and 25 µM cisplatin—applied individually or in combination—were selected for cytotoxicity assessment based on the preliminary screening results.

For the MTT assay (Mosmann, 1983), MCF-10A and MDA-MB-231 cells were counted, and 7.5×10^3 cells/well were seeded into 96-well plates containing 100 µL of RPMI 1640. After 24 hours of incubation to allow cell attachment, selected concentrations of AgNPs green-synthesized from the Karaerik leaves (10 and 20 µg mL^{-1}) and cisplatin (25 µM), both alone and in combination, were applied to the cells. For the untreated control group, cells were cultured in the medium without any treatment to represent maximum viability. Following treatment periods of 24, 48, and 72 hours, cell viability was assessed using the Roche MTT kit protocol. All experiments were performed in triplicate. The obtained cell viability rates were calculated with the following formula:

% Viability = [100 × (mean of compound-treated cell absorbance - blank mean) / (mean of control cell absorbance - blank mean)].

Total Oxidant Status and Total Antioxidant Status

Commercial kits (Rel Assay Diagnostic kits) were used to determine cellular antioxidant capacity and oxidant damage. Firstly, MDA-MB-231 cells were counted and seeded in 6-well plates with 50×10^3 cells in 500 µL medium in each well for the antioxidant status. After a 24-hour incubation period, cells were applied to the cultures separately or in combination with 25 µM CP and 10 and 20 µg mL-1 AgNP. A medium alone was added to the wells and was designated as an untreated control cell in the experiments. After 48 hours of incubation, the total oxidant status (TOS), total antioxidant substance capacity (TAS), and oxidative stress index (OSI) of the cells were determined. All experiments were repeated three times. Oxidative stress index (OSI) was calculated using the formula:

$$OSI\ (arbitrary\ unit) = TOS\ (\mu mol\ H2O2\ Eq/L) / TAS\ (\mu mol\ Trolox\ Eq/L) \times 100$$

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 22) software. The AAT Bioquest program was utilized to calculate the IC_{50} values. MTT cytotoxicity test results and oxidative stress parameter values were evaluated using one-way analysis of variance (ANOVA) based on the data's conformity to normal distribution. A significance threshold of $p<.05$ was applied in all statistical assessments.

RESULTS

Characterization of Synthesized Silver Nanoparticles

AgNPs green-synthesized by Karaerik plant characterization was performed using UV-vis (PerkinElmer Lambda

35), SEM equipped with EDX (FEI- Quanta FEG 450) and FT-IR (Thermo Scientific Nicolet 6700) techniques. In the study, the highest peak for AgNP was observed at 335 nm (Figure 1a). SEM analysis was carried out to examine the morphology and particle size distribution of AgNPs. The SEM image of silver nanoparticles is displayed in Figure 1b. The morphology of the obtained nanoparticles is mostly spherical; this result matches the shape of the surface plasmon resonance peak in the UV-vis spectrum. The average particle size of silver nanoparticles is about 30 nm. The huge particles could be related to the crystallized biomolecules. The FTIR analysis of AgNPs derived from plant extract is shown in Figure 1c. The IR spectrum of the biosynthesized silver nanoparticles (Figure 1c) shows broad peaks. Therefore, it is evident from the FTIR spectrum that the biomolecules in the plant extract, specifically the polyphenolic moieties, stabilize the silver nanoparticles.

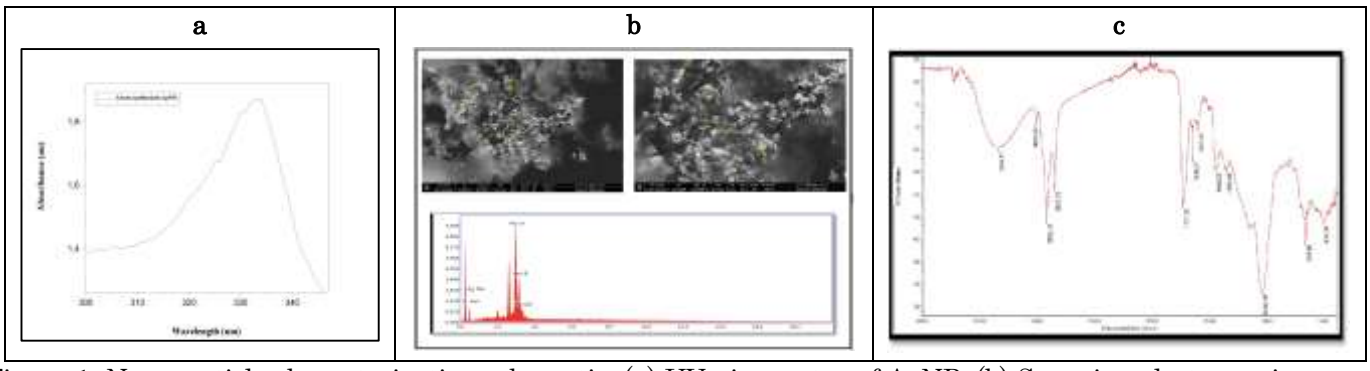

Figure 1. Nanoparticle characterization schematic. (a) UV-vis spectra of AgNP. (b) Scanning electron microscopy (SEM) images of AgNP. The scale bar is 1 µm, and the magnification during imaging are 60.000 and 120.000×. (c) The FT-IR spectrum of AgNP.

Şekil 1. Nanopartikül karakterizasyon şeması. (a) AgNP'nin UV-vis spektrumları. (b) AgNP'nin taramalı elektron mikroskobu (SEM) görüntüleri. Ölçek çubuğu 1 µm'dir ve görüntüleme sırasındaki büyütme 60.000 ve 120.000× idi. (c) AgNP'nin FT-IR spektrumu.

MTT Assay

In the MDA-MB-231 breast cancer cell line, it was observed that AgNP doses below 10 µg mL^{-1} were not lethal, and cell growth was comparable to the control group. Based on preliminary screening, doses of 10 and 20 µg mL^{-1} were selected as working doses for AgNP, since doses above 20 µg mL^{-1} resulted in more than 50% cell death. For CP, no lethal effects were observed at doses of 8 µM and below, whereas significant reductions in cell viability occurred at doses above 16 µM. Therefore, 25 µM was selected as the working dose for CP, as it exceeded the IC_{50} value observed at 72 hours, indicating an enhanced cytotoxicity over time. The IC_{50} values at 24, 48, and 72 hours were calculated based on dose-response data using a range of AgNP doses (2.5- 50 µg mL^{-1}) and CP doses (4- 64 µM), as shown in Table 1.

Table 1. Dose-dependent cell viability values of AgNP and Cisplatin in MDA-MB-231 cells at 24, 48, and 72 hours
Tablo 1. 24, 48 ve 72 saatlerde MDA-MB-231 hücrelerinde AgNP ve Sisplatin'in doza bağlı hücre canlılığı değerleri

Treatments		Time		
		24h	48h	72h
AgNP (µg mL^{-1})	0	100	100	100
	2.5	95.15±0.96	81.21±0.25	71.45±0.55
	5	95.36±0.02	73.12±0.15	58.15±0.37
	10	93.45±0.85	62.45±0.05	47.24±0.90
	15	89.35±0.36	54.55±0.01	42.70±0.52
	20	89.85±0.15	48.75±0.30	38.85±0.15
	25	88.45±0.75	40.12±0.45	26.11±0.10
	50	88.10±0.05	22.28±0.63	17.52±0.01
CP (µM)	0	100	100	100
	4	94.01±0.16	76.18±0.08	64.74±0.55
	8	94.58±0.20	69.35±0.18	51.17±0.09
	16	91.75±0.34	52.45±0.33	43.35±0.14
	32	88.96±0.31	41.85±0.30	31.18±0.23
	64	87.20±0.04	34.75±0.65	25.85±0.36

Based on the literature review, suggesting the potential effectiveness of moderate doses of AgNP and CP, 10 and 20 µg mL^{-1} AgNP and 25 µM CP were selected as test doses for further evaluation. Cells were treated with these doses and incubated for 24, 48, and 72 hours. A progressive, dose-dependent inhibition of cell proliferation was observed, with the most pronounced cytotoxic effects recorded at 72 hours. At this point, IC_{50} values were calculated as 8.05 µg mL^{-1} for AgNP and 9.26 µM for CP, indicating a strong time- and dose-dependent cytotoxic response. The detailed IC_{50} values across all time points are summarized in Table 2.

Table 2. IC_{50} values for 24, 48, and 72 hours after AgNP and CP treatment of the MDA-MB-231 cell line
Tablo 2. MDA-MB-231 hücre hattının AgNP ve CP tedavisinden 24, 48 ve 72 saat sonra IC_{50} değerleri

Cytotoxicity activity (IC_{50})			
Time	Cell	Treatments	
		CP	AgNP
24h	MDA-MB-231	> 64 µM	>50 µg mL^{-1}
48h	MDA-MB-231	21.77±0.8 µM	18.11±0.12 µg mL^{-1}
72h	MDA-MB-231	9.26±0.15 µM	8.05±0.45 µg mL^{-1}

MTT assay results showed that treatment with 10 µg mL^{-1} AgNP for 24, 48, and 72 hours resulted in cell viability of 100.15±0.14%, 96.31±0.14%, and 95.25±0.45%, respectively, in MCF-10A cells. Similarly, treatment with 20 µg mL^{-1} AgNPs resulted in cell viability of 95.05±0.55%, 93.45±0.11%, and 93.02±0.32% at 24, 48, and 72 hours. All AgNP treatments-maintained cell viability above 92%, suggesting good biocompatibility with normal cells and indicating that the AgNP formulations have minimal cytotoxicity at the tested concentrations. Statistical analysis showed a significant difference compared to the control group (F=10.997, p=.009), but cell viability remained high, indicating minimal cytotoxicity to healthy cell lines at these concentrations.

In the MDA-MB-231 cell line, treatment with 25 µM CP alone for 24 hours resulted in cell viability of 65.40±0.32%, which was significantly lower than that of the control group (p<.05). Treatment with green-synthesized AgNPs at doses of 10 and 20 µg mL^{-1} resulted in cell viability of 82.12±0.20% and 70.15±0.12%, respectively, both significantly reduced compared to the control group (p<.05). When combined with 25 µM CP, the treatment with 10 µg mL^{-1} AgNP reduced cell viability to 63.85±0.45%, and with 20 µg mL^{-1} AgNP, it further decreased to 59.75±0.26% (Figure 2). Both combination treatments showed a statistically significant reduction in cell viability compared to the control group (p<.05). There was a negative correlation between dose increase and cell viability (r=-.93, p=.007).

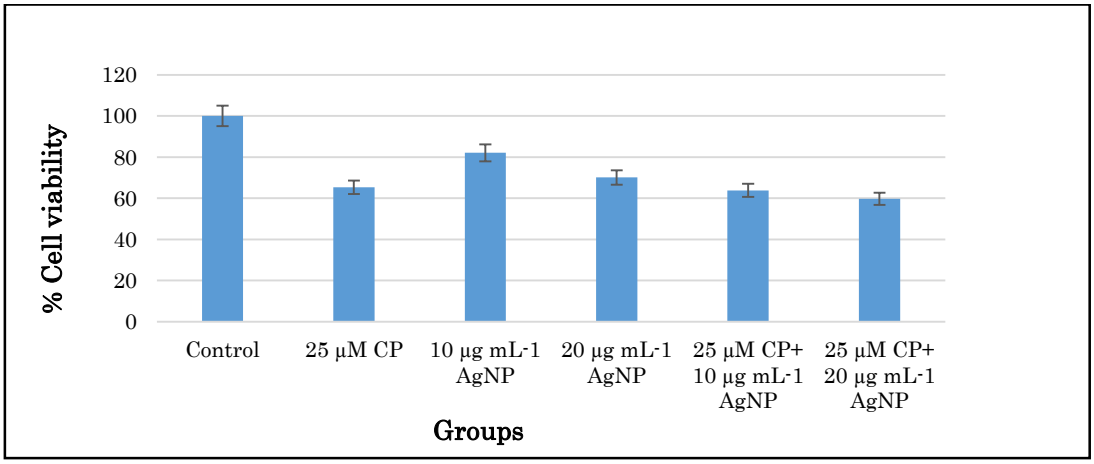

Figure 2. Cell viability rates in 24 hours on MDA-MB-231 cells
Şekil 2. MDA-MB-231 hücrelerinde 24 saat içinde hücre canlılık oranları

After 48 hours of treatment with 25 µM CP alone, the MDA-MB-231 cell line displayed cell viability of 46.31±0.25%, significantly lower than the control (p<.05). Treatment with 10 and 20 µg mL^{-1} green-synthesized AgNPs resulted in cell viabilities of 75.53±0.65% and 48.75±0.21%, respectively, both showing notable reductions compared to the control group (p<.05). In the groups receiving 25 µM CP and 10 or 20 µg mL^{-1} AgNP, cell viability rates were 43.28±0.82% and 38.80±0.79%, respectively. A statistically significant decrease in cell viability was observed at both doses of AgNP when compared to the control group (p<.05) (Figure 3). There was a negative correlation between dose increase and cell viability (r=-.84, p=.008).

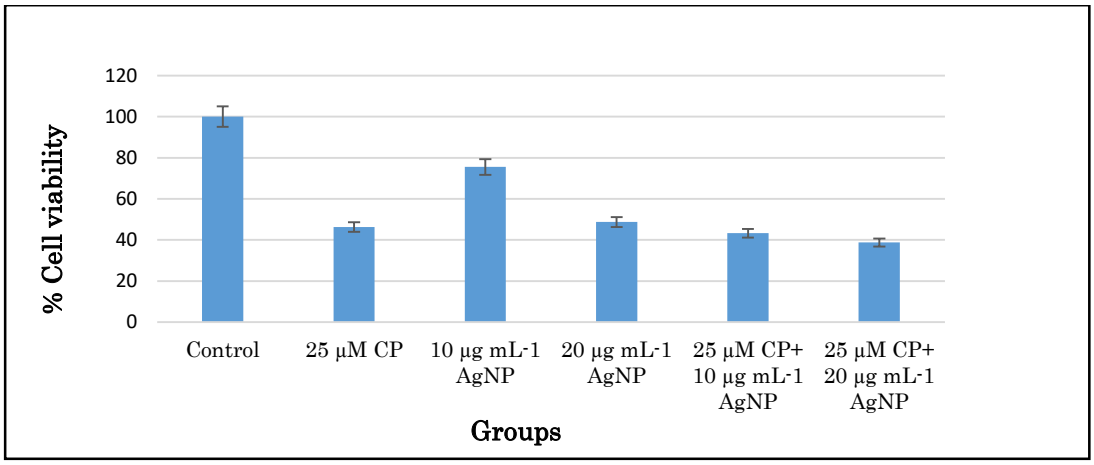

Figure 3. Cell viability rates in 48 hours on MDA-MB-231 cells
Şekil 3. MDA-MB-231 hücrelerinde 48 saat içinde hücre canlılık oranları

After 72-hour treatment with 25 µM CP alone, the MDA-MB-231 cell line showed a cell viability of 36.20±0.42%, which was significantly lower than the control (p<.05). Treatment with 10 and 20 µg mL^{-1} green-synthesized AgNPs resulted in cell viabilities of 47.13±0.75% and 39.47±0.11%, respectively, both significantly lower than the control (p<.05). The combination of 25 µM CP with 10 µg mL^{-1} AgNP reduced cell viability to 33.85±0.28%, and the combination with 20 µg mL^{-1} AgNP further decreased viability to 29.65±0.52%, both combinations showing significant reductions compared to the control group (p<.05) (Figure 4). There was a negative correlation between dose increase and cell viability (r=-.81, p=.004).

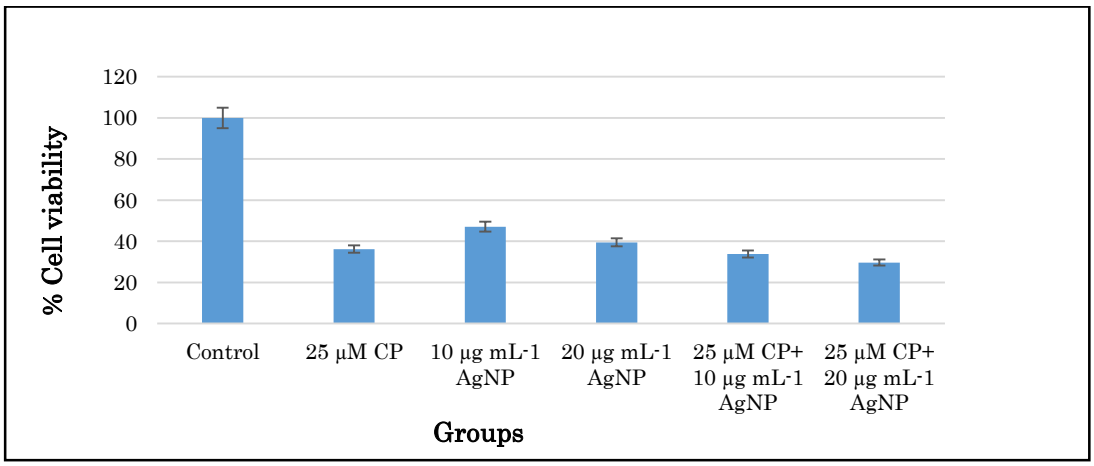

Figure 4. Cell viability rates in 72 hours on MDA-MB-231 cells
Şekil 4. MDA-MB-231 hücrelerinde 72 saat içinde hücre canlılık oranları

Oxidative Parameter Results

In this study, parameters of oxidative stress, including oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI), were measured, with results presented in Table 3. In the control group, oxidative stress significantly increased in all treated groups except for the 10-µg mL^{-1} AgNP group (F= 152.670, p=.001).

In terms of TOS levels, a significant increase was observed in the groups treated with 25 µM CP, 20 µg mL^{-1} AgNP, and their combination compared to the control group (p<.05). However, in the 10-µg mL^{-1} AgNP group, no significant difference was observed compared to the control (p=.412). Combinations of CP and AgNP caused significantly higher TOS levels than the groups in which CP or AgNP was administered alone (p<.05), indicating that oxidative stress increased more in combination. Regarding TAS levels, a significant decrease was observed in all experimental groups compared to the control group (p<.05). No statistically significant difference was found between the CP group and the CP and AgNP combination groups (p>.05), indicating that CP already significantly decreased antioxidant capacity and its combination with AgNP did not contribute to this effect. In terms of OSI levels, a significant increase was observed in all experimental groups compared to the control group (p<.05). The significant difference observed between the CP group and the CP + 20 µg mL^{-1} AgNP combination (p=.043) suggests that higher AgNP concentrations elevate oxidative stress. Likewise, a significant difference was detected between

the 10-µg mL^{-1} and 20 µg mL^{-1} AgNP groups (p=.011), confirming that an increased AgNP dosage enhances the OSI levels. Correlation analyses revealed that TOS levels were negatively correlated with TAS (r=-.84) but positively correlated with OSI (r=.97). CP and AgNP combinations increased oxidative stress while decreasing antioxidant capacity, as indicated by a strong negative correlation with TAS. These findings suggested that the CP and AgNP combination elevates oxidative stress and suppresses antioxidant defense mechanisms. Notably, the combination of 25 µM CP and 20 µg mL^{-1} AgNP resulted in the highest oxidative stress levels (p<.05).

Table 3. Oxidative stress parameters of the CP and AgNP combination in the MDA-MB-231 cell line
Tablo 3. MDA-MB-231 hücre hattındaki CP ve AgNP kombinasyonunun oksidatif stres parametreleri

Groups	TOS (µmol H_2O_2 Eqv./L)	TAS (mmol Trolox Eqv./L)	OSI (AU)
Control	5.59±0.89^{a}	1.29±0.36^{a}	4.3±0.39^{a}
25 µM CP	12.34±0.15^{b}	0.10±0.78^{b}	123±0.96^{b}
10 µg mL^{-1} AgNP	6.27±0.80^{a,c}	0.36±0.11^{c}	17±0.57^{c}
20 µg mL^{-1} AgNP	8.98±0.45^{d}	0.24±0.52^{c}	37±0.11^{d}
25 µM CP+10 µg mL^{-1} AgNP	14.95±0.80^{e}	0.12±0.78^{b,c}	124±0.83^{b}
25 µM CP+20 µg mL^{-1} AgNP	16.20±0.45^{f}	0.11±0.01^{b,c}	147±0.13^{c}

Different letters (a-f) in the same column indicate statistically significant differences between the corresponding groups (p<.05).

Consequently, the antioxidant capacity was significantly diminished. Notably, a marked increase in the total oxidative status (TOS) value was observed in the groups treated with CP and AgNP, with the highest recorded values. These findings provide strong evidence that the combined treatment further elevates oxidative stress. Furthermore, the combination of cisplatin and green-synthesized silver nanoparticles disrupts the antioxidant defense system, leading to increased oxidative stress and ultimately contributing to cell death.

DISCUSSION

Cancer, a leading cause of death worldwide, particularly in developed countries, continues to show an upward trend. This rise in cancer cases has underscored the need for innovative diagnostic and treatment methods, prompting the exploration of alternative therapies, such as herbal medicine and acupuncture. It is essential to evaluate the compatibility of these methods with conventional techniques, assess their side effects, and minimize potential risks. Extensive research has investigated the therapeutic potential of herbal treatments and their active compounds (Pecere et al., 2000; Kuo et al., 2002; Pecere et al., 2003). Our study focused on the anticancer potential of green-synthesized silver nanoparticles derived from Karaerik grape leaves and the chemotherapeutic agent cisplatin.

In the literature, several studies have examined the anticancer effects of AgNPs derived from different plant sources. In a previous study, the MTT test was used to assess the anticancer activity of AgNPs synthesized from the unripe fruits of the *Solanum trilobatum* plant in the MCF-7 cell line, demonstrating anticancer effects (Munusamy et al., 2015). Similarly, Suman et al. (2013) reported that AgNPs green-synthesized from *Morinda citrifolia* root extract exhibited significant cytotoxicity against the HeLa cell line. Green-synthesized AgNPs play a vital role in delivering antitumor drugs to cancer cells through the apoptosis pathway, offering controlled drug delivery advantages (Sadat et al., 2017). The size of AgNPs, ranging from 20- 52 nm, is critical for maximizing their anticancer efficacy, as demonstrated by several studies (Al-Sheddi et al., 2018; Jadhav et al., 2018; Padinjarathil et al., 2018; Kumkoon et al., 2023).

In our study, the highest peak for AgNPs at 335 nm is likely attributed to aromatic compounds in the Karaerik grape leaf extract. The *V. vinifera* extracts primarily produced spherical AgNPs, with a polydisperse distribution ranging in size from 28.79 nm to 34.24 nm. Larger particles may result from the crystallization of biomolecules. The FTIR analysis of AgNPs derived from plant extract revealed characteristic bands, indicating the presence of polysaccharides, phenolic compounds, and proteins, emphasizing flavonoids. Key peaks in the FTIR spectrum, such as those at 3343 cm^{-1} (O-H stretching of alcoholic and phenolic groups), 3009 cm^{-1} (aromatic C-H stretching), and 1737 cm^{-1} (C=O stretching of carboxylic groups), highlight the structural features of these biomolecules (Divya et al., 2018; Acay et al., 2019; Said & Othman, 2019).

Previous research has demonstrated the cytotoxic effects of AgNPs derived from different plants on various cancer cell lines. Behboodi et al. (2019), AshaRani et al. (2009), and Franco-Molina et al. (2010) have also demonstrated the cytotoxicity of green-synthesized AgNPs against various cancer cell lines, including MCF-7, glioblastoma, and lung cancer cells. Silver nanoparticles, including those synthesized from *Malvaviscus arboreus* and *Plumeria alba,* have shown promising anticancer and antimicrobial effects (Rudrappa et al., 2022; Mohammed et al., 2023).

Furthermore, silver nanoparticles from *Garcinia mangostana* fruit peel extract loaded with protocatechuic acid (PCA) have been shown to have synergistic anticancer activity, disrupting mitochondrial membrane potential and increasing reactive oxygen species (ROS) levels in colon cancer cells (Lee et al. 2019). Our study aligns with these findings, supporting the idea that AgNPs could serve as effective anticancer agents, especially when used in combination with other treatments.

In terms of biocompatibility, our study demonstrated that all AgNP treatments exhibited over 90% cell viability in MCF-10A healthy epithelial breast cells, suggesting that these nanoparticles are non-toxic to normal cells. These results are consistent with previous studies indicating the biocompatibility of green-synthesized silver nanoparticles in L929 healthy fibroblast cells, such as those derived from *Salacia chinensis* and *Astragalus membranaceus* (Jadhav et al., 2018; Öztolüt, 2023). Additionally, the size of AgNPs (approximately 30 nm) is ideal for enhancing cancer cytotoxicity while maintaining good biocompatibility (Yu et al., 2012; Qian et al., 2019; Della Vechia et al., 2020). These findings suggest that AgNPs derived from Karaerik grape leaves, in combination with CP, could be promising tools for targeted therapy in cancer treatment.

In the MDA-MB-231 breast cancer cell line, our study found significant cytotoxicity of AgNPs at concentrations of 10 and 20 µg mL^{-1}. When AgNPs were combined with CP, cell viability was further reduced, indicating a beneficial interaction effect. This enhanced effectiveness of CP by AgNPs aligns with previous findings that AgNPs increase ROS production, disrupt cell membranes, and damage DNA and proteins in cancer cells (Patil & Kim, 2017; Hammamchi, 2019). Our study also observed increased oxidative stress, with significant increases in total oxidative status (TOS) levels and decreased antioxidant status (TAS) levels in the combination treatments. This suggests that CP and AgNPs work together to enhance oxidative stress, leading to cell death.

Several studies have explored the role of oxidative stress in AgNP-induced cytotoxicity. AgNPs are known to promote the formation of ROS, leading to the degradation of cancer cells, inflammation, and mitochondrial damage (Pugazhendhi, 2018). Additionally, AgNPs can induce apoptosis by increasing ROS levels and disrupting mitochondrial membrane potential, triggering a cascade of events that ultimately result in cell death (Gurunathan et al., 2013; Jing et al., 2011; Li et al., 2016; Dasgupta et al., 2018). In our study, the AgNPs and CP combination significantly increased oxidative stress, as evidenced by elevated TOS levels and decreased TAS levels. This suggests that AgNPs potentiate the oxidative damage caused by CP, leading to enhanced anticancer effects.

Our study investigates the effects of green-synthesized AgNPs derived from Karaerik plant leaves with CP on the MDA-MB-231 breast cancer cells. This is the study to explore this combination, and our results show that the AgNPs from Karaerik grapes enhance the cytotoxic effects of CP. This suggests that Karaerik-derived AgNPs could serve as potential anticancer agents for breast cancer treatment, particularly when used in conjunction with traditional chemotherapeutic agents like CP. These findings provide valuable insights into alternative and complementary approaches for cancer therapy. However, further *in vivo* studies are necessary to confirm these results and to better understand the underlying mechanisms of action.

CONCLUSION

Our findings highlight the promising potential of silver nanoparticles synthesized using Karaerik leaf extract, particularly in combination with cisplatin, against breast cancer cells. The results indicate that these nanoparticles may enhance the effectiveness of conventional chemotherapy. However, several limitations must be addressed before clinical translation. First, while the in vitro results are encouraging, further *in vivo* studies are essential to evaluate the therapeutic efficacy and safety of this combination in a complex biological environment. Second, the precise molecular mechanisms underlying the interaction between the synthesized nanoparticles and cisplatin require further elucidation. Lastly, the scalability and reproducibility of the green synthesis method using Karaerik leaf extract should be thoroughly investigated to ensure consistent nanoparticle characteristics for potential biomedical applications. Future research focusing on these aspects will be crucial for advancing the clinical potential of this promising nanotherapeutic approach.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that no conflicts of interest exist among them.

Ethics Committee Permission

An ethics committee permission is not required for the article.

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