Cancer Nanotechnology

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Synthesis and characterization of silver nanoparticles loaded with carboplatin as a potential antimicrobial and cancer therapy

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Abstract

In recent studies with silver nanoparticles, it has been reported that the use of nanoparticles in carrier drug systems increases tumor suppression and reduces drug-related side effects. At the same time, the combination of traditional medicine with nanotechnology provides the opportunity to develop new antimicrobial agents. The aim of this study was to determine the anticancer, antimicrobial activities and pro-apoptotic effects of silver nanoparticles (AgNPs), and carboplatin-loaded silver nanoparticles (AgNPs-Car). Characterization studies of the synthesized nanoparticles were carried out by DLS, EDX-STEM, and FTIR analysis. The antiproliferative and pro-apoptotic effects of these molecules were evaluated using XTT and Annexin V, respectively. MIC (Minimum Inhibitory Concentration) test was used to determine the antimicrobial activity. The anticancer activity of the AgNPs-Car was high in MCF-7 (human breast adenocarcinoma), A549 (human lung carcinoma), and C6 (brain glioma) cells. The cell group with the most effective selective cytotoxic activity was C6 cells. It was also shown that AgNPs-Car and AgNPs induced DNA fragmentation eventually increasing apoptosis of cells. The antimicrobial activity of AgNPs and AgNPs-Car was evaluated on Gram-positive and Gram-negative pathogenic microorganisms and yeast fungi. Among the nanomaterials that reached effective MIC values according to reference sources, AgNPs-Car achieved better results. As a result, AgNPs-Car was found to be very successful in targeting C6 glioma cells by facilitating cell entry of the drug. In addition, their anticancer activity on MCF-7 and A549 cells was high and their toxicity was low. Silver nanoparticles are preferred for creating a better drug carrier system because of their qualitative properties and effects. Therefore, it is an interesting field for research on targeting cancer cells and pathogenic microorganisms.

Keywords: AgNPs, Drug carrier, Carboplatin, Antimicrobial, Apoptosis

Introduction

Cancer has pathological features such as abnormal growth, invasion, and metastasis in healthy cells because it is a complex disease. Uncontrolled cell proliferation causes a surge in aberrant cells, which can permeate the circulation, harm healthy cells, and lead to secondary tumors. (Khan et al. 2017). The majority of chemotherapeutic drugs used to treat cancer

cause systemic toxicity because they accumulate non-specifically in normal cells (Lee et al. 2013). Therefore, the synergy and safety of chemotherapeutic agents used in monotherapy should be improved to treat cancer. (Hekmat et al. 2012; Karuppaiah et al. 2020).

Nanotechnology research is increasingly focused on the development of novel, potent, and targeted anticancer drugs. The detection and treatment of cancer, the identification of biomarkers, the comprehension of cancer progression, and the development of novel imaging and diagnostic agents are all areas of intense nanotechnology study (Mignani et al. 2015, Sehgal et al., 2022). In this field, silver nanoparticles are among the most investigated metal nanoparticles (Xu et al. 2020, Cruz et al., 2021).

Silver nanoparticles have been extensively investigated in pharmaceutical and medical fields due to their antimicrobial activity and cytotoxicity against various cancer cell lines. (Paiva et al. 2018; Huy et al. 2017). Cancer therapy using nanoparticles such as AgNPs plays an important role in increasing therapeutic efficacy against cancer by combining nanoparticles and chemotherapeutic agents. This therapy improves the pharmacokinetics and targeted delivery of loaded drugs, achieving better control of the tumor, and reducing unwanted side effects (He et al. 2015; Davis et al. 2008).

Carboplatin is widely used to treat various cancers such as breast, lung, brain, head, and neck (Shahzad et al. 2009; Aparicio-Blanco et al. 2020). Using a novel therapeutic approach to increase this drug's therapeutic efficacy will provide new therapy options for a variety of malignancies (Mofidian et al. 2019, Ebrahimi et al., 2014). It is thought that the targeted agent may help nanoparticles cross the blood–brain barrier more effectively and deliver higher amounts of carboplatin to tumor tissue (Hassanzadeganroudsari et al. 2020).

An important activity of these metal nanoparticles is their use as antimicrobial agents. Overdoses of essential metals and non-essential metals have microbicidal lethal effects even in minimal doses, proving that using metals as antimicrobial agents has powerful effects (Lemire et al. 2013). For this reason, research on antimicrobial substances involves the production of objects at the nanoscale using metals including titanium, copper, zinc, gold, magnesium, and silver. (Morones-Ramirez et al., 2013; Roy et al. 2019). Among metal nanoparticles, AgNPs are stated to have strong antimicrobial activity. These nanoparticles inhibit microorganisms' growth by various mechanisms that affect the cell wall and metabolic processes (Liao et al. 2019, Sezari et al., 2021).

In this study, considering that carboplatin-added silver nanoparticles are an accurate carrier for targeting cancer cells, the antiproliferative effect of these AgNPs-Car on various cancer cells was examined. It was also aimed to determine the antimicrobial activity of AgNPs on various pathogens. For this purpose, AgNPs with low cost, easy synthesis, biocatalytic and photocatalytic advantages, MCF-7 (human breast adenocarcinoma cell line), A549 (human lung carcinoma cell line), C6 (rat brain glioma cell line), and healthy WI-38 (human lung fibroblast normal cell line) cells and ten different human pathogenic microorganisms were used.

Materials and methods

Materials and chemicals

Cell lines used in the study were MCF-7 (human breast adenocarcinoma cell line; ATCC [@]HTB-22), A549 (human lung carcinoma cell line; ATCC[@]CCL-185), C6 (rat brain glioma cell line; ATCC[@]CRL-2199) cells, non-cancerous WI-38 (human fibroblast normal

cell line; ATCC@CCL-75), and microorganisms used in the experiment were ATCC (American Type Culture Collection). $Ag(NO_3)$ (Sigma cat. no. 209139), $NaBH_4$ (Sigma Cat No:452882), and trisodium citrate chemicals of analytical grade from Sigma–Aldrich (St.Louis, USA), Dimethylsulfoxide (DMSO; Sigma Cat No:472301), XTT cell proliferation kit from Sigma (Sigma Cat No:4636 St.Louis, USA), Dulbecco's Modified Eagle's Medium (DMEM), penicillin–streptomycin, trypsin–EDTA, Fetal bovine serum (FBS) from Gibco (Gibco; Thermo Fisher Scientific, Inc.), 20 × Phosphate buffered saline (PBS) from Thermo Fisher (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 2,3,5-Triphenyltetrazolium chloride (TTC) from Merck (Merck Millipore, Darmstadt, Germany), Annexin V/PI assay kit from BD (Cat. No. 556547, USA), Carboplatin from Sandoz (Sandoz, Princeton, New Jersey, USA) was supplied.

Synthesis of AgNPs and AgNPs-Car

Stock solutions of 2×10^{-3} M $Ag(NO_3)$, 8.6×10^{-3} M trisodium citrate, and 4×10^{-3} M sodium boron hydride were prepared. 0.0169 g $Ag(NO_3)$ powder is weighed with analytical scales and dissolved at 50 mL pure water, as $Ag(NO_3)$ stock solution. 0.1109 g trisodium citrate powder is weighed with analytical scales and dissolved at 50 mL pure water, as trisodium citrate stock solution. 0.0075 g sodium boron hydride powder is weighed with analytical scales and dissolved at 50 mL pure water, as sodium boron hydride stock solution. Then, 12 mL trisodium citrate, 12 mL sodium boron hydride, and 24 mL pure water were mixed for 30 min at 60 °C. The temperature was raised to 90 °C, and 48 mL silver nitrate was added to the stock solution. The reaction was stopped after 5 min. AgNPs stock solution was kept in a cool dark environment. 10 mg / 10 mL Car stock solution was prepared. The mixture of 1 mL Car stock solution and 1 mL AgNPs stock solutions was sonicated with an ultrasonication instrument for 10 s. And then, the mixing is placed in the dark for at least 30 min (Danışman-Kalındemirtaş et al. 2021).

Characterization of AgNPs and AgNPs-Car

Zetasizer, FTIR, and SEM analysis were used to control Carboplatin binding to AgNPs.

DLS measurements

Zetasizer measurements were made with DLS (dynamic light scattering) using the Zetasizer Nano ZS, employing a 4 mW He–Ne laser operating at 633 nm and 173°detection angle at room temperature. The reference liquid was distilled water. Dimensional and surface load analyses were performed.

EDX–STEM analysis

The samples were dropped onto amorphous glass substrates, which had been washed with detergent and rinsed with distilled water. They were left to dry for one night in a clean room at room temperature and under normal atmospheric conditions. Then they were used in STEM–EDX analyses. Surface properties were examined using a Gemini 500 computer-controlled digital transmission electron microscopy (STEM). An EDX spectrometer attached to STEM was used to perform quantitative elemental analysis (Khashan et al. 2018; Mohammed et al. 2020).

FTIR analysis

FTIR (Fourier Transform Infrared spectroscopy) analysis was performed with the Bruker Alpha instrument at 4 cm × 1 resolution in diffuse reflection mode. Each sample was measured and recorded after ten scans (Jihad et al. 2021; Bahjat et al. 2021).

Biological assays

Preparation of microorganism cultures

Microorganisms to be tested in the experiment *Escherichia coli (*ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Acinetobacter baumanii (*ATCC 17978), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Methicillin-resistant Staphylococcus aureus* (MRSA)(ATCC 43300), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 11778), *Candida albicans* (ATCC 10231), and *Candida tropicalis* (ATCC 4563) were obtained from the American Type Culture Collection (Rockville, MD, United States). Bacteria were cultured on Mueller Hinton Broth (MHB–HI Media Cat No: H05-M173-500G) and fungi Sabouraud Dextrose Broth (SDB—HI Media Cat. No.:GM033-500G) media at 37 °C for 24 h (Loo et al. 2018).

Antimicrobial assay

AgNPs and AgNPs-Car molecules' antimicrobial activity was determined using the MIC (Minimum Inhibition Concentration) method. U-bottomed 96-well plates were used for the experiment. 10 µl of the sample (100 µg/ml) was put into the first row of the plates, and twofold serial dilutions were made in a total of ten concentrations. Microorganisms adjusted to McFarland turbidity of 0.5 were diluted to 5×10^5 CFU/mL for bacteria and $0.5–2.5 \times 10^3$ CFU/mL for yeasts and 50 µl was applied to the wells (CLSI (M07-A9), 2012, CLSI (M27-A2) 2012). The plates were incubated at 37 °C for 24 h for bacteria and 48 h for yeasts. At the end of incubation, 50 µl of 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) 2 mg/ml solution was added to each well to see growth better. The plates were incubated at 37 °C for 2 h. A decrease in Formazan color was observed in the first well depending on the presence of viable microorganisms and was considered as MIC. According to reference literature, MIC results were taken as Effective (MIC < 100 µg/mL), Moderate (100 < MIC ≤ 625 µg/mL), and Weak (MIC > 625 µg/mL) (Kuete 2010; Awouafack et al. 2013).

Cell culture

For experiments, cancerous MCF-7 (human breast adenocarcinoma cell line), A549 (human lung carcinoma cell line), C6 (rat brain glioma cell line) cells, and healthy WI-38 (human fibroblast cell line) from ATCC (American Type Culture Collection) were provided and used. Frozen cells for cell culture experiments were thawed and passaged for 2 weeks to reach the appropriate density. 20 µL of the cells examined under the microscope, which were observed to adhere and multiply, were separated for counting under the microscope, and the rest of the cells were seeded into the flask.

Cells were placed in an incubator kept at 37 °C and 5% CO_2, and the same procedure was repeated every 2 days (Ibrahim et al. 2021, Machana et al. 2010).

Cell proliferation assay

The XTT test was used to investigate cell viability. Cells counted immediately after passage were divided into 10,000 cells per well in a 96-well plate. 100 µL of cells were seeded per well. The seeded cells were incubated for 24 h, discarding the medium after incubation. Then, samples of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml concentrations were added to the cells, and they were incubated for 24 h. After the incubation, 10 µL of XTT was added to the cells, and measurements were taken with a spectrophotometer device at 1-hour intervals (Morais et al. 2020, Koca et al., 2018).

Cell viability (%) = [(As−Ab)/(Ac−Ab)] × 100.

As: Absorbance Sample **Ab:** Absorbance Blank **Ac:** Absorbance Viable cell (control).

IC_{50} values were calculated using XTT results. Based on experiments performed on A549, C6, MCF-7, and WI-38 cells, the maximum selectivity of nanoparticle and carboplatin dose was determined, and AgNPs-Car interaction was revealed (Hepokur et al. 2019).

Apoptosis analysis (Annexin/PI flow cytometry analysis)

Commercial Annexin V/PI kits were used in flow cytometric analysis. IC_{50} values of AgNPs, AgNPs-Car, and Carboplatin were processed in C6 cells for 24 h. At the end of this period, the plate contents were removed; cells were washed with PBS and treated with trypsin–EDTA, then centrifuged for 8 min at 800 rpm. The resulting cells were counted with trypan blue, and 5 µL of Annexin V and 5 µL of PI were added. They were kept in the dark for 15 min at room temperature. After the incubation, 400 µL of binding buffer was placed on ice, and flow cytometry measurements were carried out (emission: 530 nm, excitation: 488 nm) (Hepokur et al. 2019).

Statistical analysis

Two-sample t tests were used for two groups or one-way ANOVA for multiple groups. The significance was $p \leq 0.05$ (Jabir et al. 2022).

Results and discussion

AgNPs' unique chemical, biological, and physical properties increase the research focusing on them. AgNPs have many antibacterial and anticancer properties. For example, the uptake of AgNPs by living cells or bacteria leads to severe cellular damage and death due to the deregulation of critical cellular mechanisms after silver ions are released, and radical species are formed (Talapko et al. 2020; Krishnan et al. 2020). AgNPs can be used to transfer anticancer drugs to the tumor; AgNPs will release the drug "in situ," and they will be taken up by cells and act against cancer cells, a promising treatment approach against cancer (Gomes et al. 2021).

Synthesis and characterization of AgNPs and AgNPs-Car

DLS results are given in Table 1 as a whole. While the average size of AgNPs produced by a standard method, as in the literature, was 2.32 nm, the particle size distribution was also determined at 6.5 nm in the nanoparticle size distribution. When the carboplatin drug was attached to AgNPs, the average particle size increased to 28.85 nm, and the largest particle was detected at 43.82 nm. While the PDI value of AgNPs was 0.200, this value increased to 0.625 when the drug was bound. This means that although the drug is attached to AgNPs, the nanoparticles are stable according to the warnings of the device.

STEM images are given in Fig. 1. Considering the 100 nm scale, it is seen that AgNPs are 10 nm and below, while drug-bound AgNPs-Car particles have particle sizes around 30–40 nm. Of course, after the lamella is dripped on the glass and dried, agglomeration is also observed in the nanoparticles with the evaporation of the solvent. However, STEM images are sufficient to confirm the DLS results.

In Fig. 2, EDX analyses of AgNPs and AgNPs-drug particles are also given. Of course, the elements that do not affect the work are neglected here. According to the analysis results, 1.01% Ag was detected in AgNPs, while this rate decreased to 0.48% with the

Table 1 DLS results of the AgNPs and AgNPs-Car

Nano	Mean particle size (nm)	Particle size distribution (nm)	PDI
AgNPs	2.32	1.5–6.5	0.200
AgNPs-Car	28.85	21.04–43.82	0.625

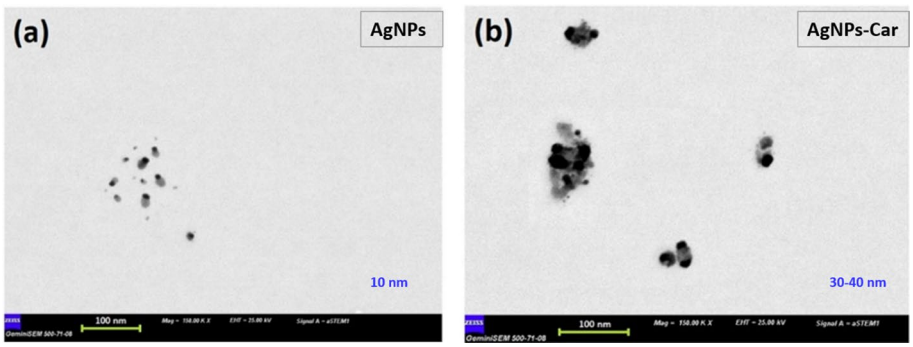

Fig. 1 STEM image of AgNPs (**a**), STEM image of AgNPs-Car (**b**)

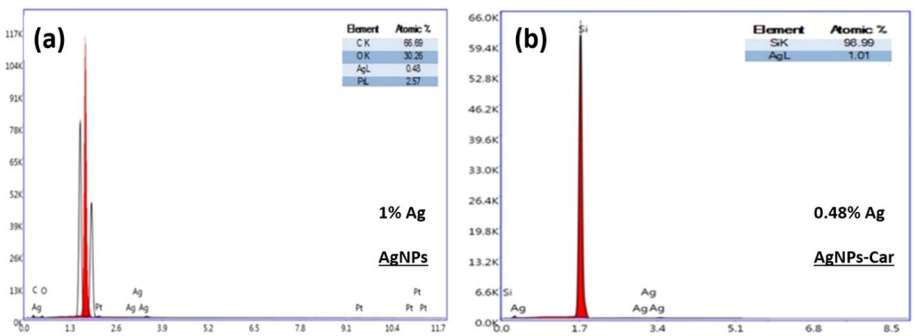

Fig. 2 EDX analysis of AgNPs (**a**), EDX analysis of AgNPs-Car (**b**)

effect of drug-bound AgNPs in the drug. In addition, drug-bound AgNPs were determined as 2.57% of Pt, one of the drug's specific elements. At the same time, it is a surprising result that it is detected at very high rates in C and O. However, it cannot be seen because the nitrogen amount is meager.

Figure 3 shows AgNPs' and AgNPs-Car's FTIR spectra. Vibration peaks of –OH and –NH groups are observed at 3252 cm-1.–C = O stretching vibrations peaks are observed at 1631 cm-1; In general, a specific characteristic peak of nanoparticles was observed at 631 cm. (Shaik et al. 2018). On the other hand, symmetric vibration peaks belonging to aliphatic groups were also detected at 2853–2956 cm-1. At 1715–1463 cm-1, –NH and -NH2 bending vibration contributions were detected (Iliescu et al. 2011). It means that when AgNPs are assembled with drugs, they bind to the –NH groups in AgNPs-Car, and the drug surrounds the AgNPs.

Antimicrobial activity

Although the antimicrobial properties of silver nanoparticles have been known for a long time, the development of nanotechnology may help to utilize this property much more effectively. The use of drug-loaded AgNPs also has great potential to help solve the growing microbial resistance crisis (Fernando et al. 2018, Vishwanath et al., 2021). In our study, the antimicrobial effect of AgNPs and drug-loaded AgNPs-Car was evaluated on ten different pathogenic microorganisms including Gram-positive, Gram-negative bacteria, and yeast fungi using the minimum inhibition concentration (MIC) method. Among the Gram-positive bacteria, the antimicrobial effect of AgNPs and AgNPs-Car on *S. aureus*, MRSA and *E. faecalis* was found below the MIC < 100 µg/mL. Among Gram-negative bacteria, especially *P. aeruginosa* (25 and 12.5 µg/mL) demonstrated a high antimicrobial effect due to low MIC value. Effective antimicrobial activity below MIC < 100 µg/mL was observed in all Gram-negative bacteria. AgNPs and AgNPs-Car demonstrated moderate antimicrobial activity in *B. cereus, C. albicans, and C. tropicalis* with values in the range of 100 < MIC ≤ 625 µg/mL (Table 2). According to these results, AgNPs and AgNPs-Car were demonstrated to be highly effective compared to reference sources (Awouafack et al. 2013).

Bharadwaj et al. reported that green-synthesized silver nanoparticles using fruit extract had an antimicrobial effect on *S. aureus* and *E. coli* (Bharadwaj et al. 2021). In the research of Yaseen et al., the best antibacterial potential of GBP-AgNPs was observed

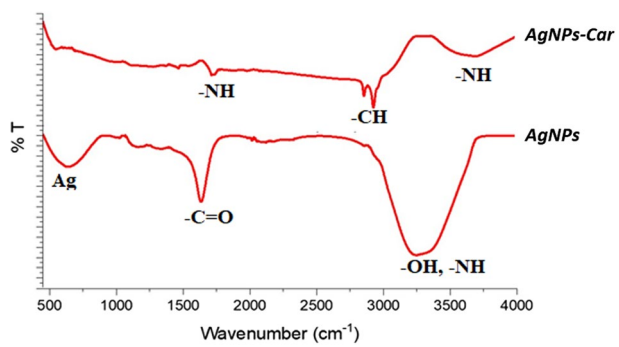

Fig. 3 FTIR spectrum of AgNPs-Car and AgNPs

Table 2 Antimicrobial activity results of the AgNPs and AgNPs-Car

Microorganisms (bacteria and yeasts)	MIC (µg/mL) AgNPs	MIC (µg/mL) AgNPs-Car
Escherichia coli	50	25
Klebsiella pneumoniae	50	50
Acinetobacter baumannii	100	50
Pseudomonas aeruginosa	25	12.5
Staphylococcus aureus	50	50
Methicillin-resistant Staphylococcus aureus (MRSA)	25	25
Enterococcus faecalis	50	25
Bacillus cereus	> 100	> 100
Candida albicans	> 100	> 100
Candida tropicalis	> 100	> 100

The data obtained were studied as n = 3

Effective (MIC < 100 µg/mL), Moderate (100 < MIC ≤ 625 µg/mL), Weak (MIC > 625 µg/mL)

Table 3 IC_{50} results of the AgNPs-Car, carboplatin, and AgNPs

IC_{50}(µg/mL)	MCF-7	A549	C6	WI-38
AgNPs-Car	25,08 ± 0,74[a]	42,91 ± 0,24[a]	16,18 ± 0,45[a]	153,63 ± 3,56
Carboplatin	97,26 ± 4,12[b]	130,16 ± 2,14 [b]	54,66 ± 1,34[b]	121,35 ± 4,05
AgNPs	Not effective	Not effective	Not effective	Not effective

Standard deviation values are given as ± Std, and all values are calculated as n = 3

[a] AgNPs-Car was found to be significant according to $p \leq 0.05$ by one-way test (Dunnett's Method) against WI-38

[b] One-way test (Dunnett's method) was performed against WI-38, with a significance level of $p \leq 0.05$

for two different Gram-negative bacterial species, *A. baumannii* and *P. aeruginosa,* and the MIC value was found to be 40 µg/mL (Yaseen et al. 2022). Mohsen et al. tested the antibacterial activity of the synthesized CIP-AgNPs composite on *S. aureus* and *E. coli*. They found that the antibacterial effect was higher against Gram-positive bacteria than Gram-negative bacteria (Mohsen et al. 2020). Kaur et al. demonstrated in their research that Vancomycin-citrate-AgNPs exhibited enhanced remarkable activity against both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) (Kaur et al. 2019,). Porous silicon nanoparticles synthesized by Jabir et al. were shown to have antibacterial activity against Gram-positive and Gram-negative bacteria. This is mediated by their ability to penetrate the bacterial wall and bind with the ROS produced (Jabir et al. 2018). Our results are compatible with other research in the literature.

Anticancer activities

Nanocarrier systems (NCs) transfer clinically approved anticancer drugs; they are designed to cope with drug solubility problems, improve circulation times, and enable controlled drug release. Moreover, passive (EPR effect) or active targeting promotes drug accumulation at the tumor site (Miranda et al. 2022). Regarding Table 3, AgNPs did not significantly cause cytotoxicity in any cell group. This result shows the harmlessness of synthesized nanoparticles regarding size and physical properties and their usability in cancer treatment.

On the other hand, AgNPs-Car showed higher activity than carboplatin alone in all cancer cells, thus lower IC_{50} values and cell viability (Fig. 4 and Fig. 5). When the SI index was calculated, it was determined that the AgNPs-Car was more effective in C6 cell lines than in other cancer cells (Table 4). The low toxicity of the AgNPs-Car in WI-38 healthy cell lines is also a great advantage.

In a study synthesizing gemcitabine-loaded AgNPs developed for use in breast cancer treatment, GEM–AgNPs conjugates were found to increase cytotoxicity significantly more than GEM alone (Karuppaiah et al. 2020). Carboplatin, one of the cytotoxic

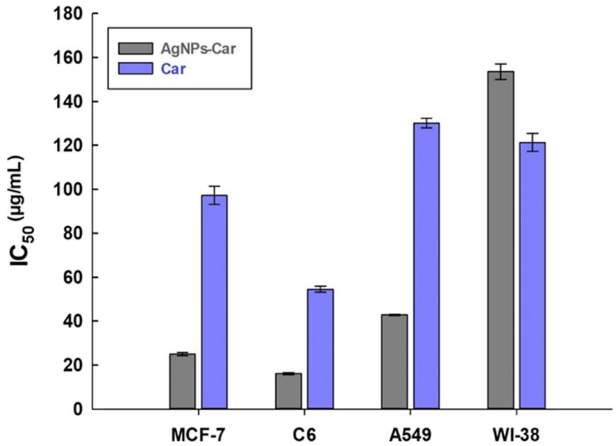

Fig. 4 IC_{50} results of the AgNPs-Car and carboplatin

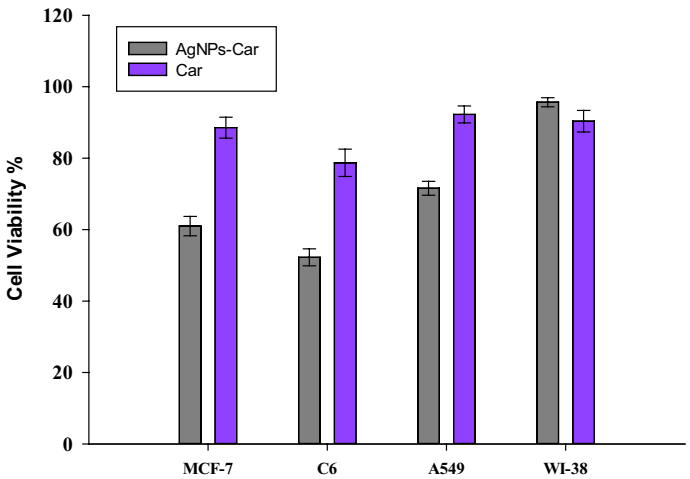

Fig. 5 Cell viability results of the AgNPs-Car and carboplatin

Table 4 Index of SI

IC_{50}(µg/mL)	WI-38 / MCF-7	WI-38 / A549	WI-38 / C6
AgNPs-Car[a]	6.13	3.58	9.49
Carboplatin	1.25	0.93	2.22

Standard deviation values are given as ± Std, and all values are calculated as $n = 3$

[a] AgNPs: silver nanoparticles, AgNPs-Car: carboplatin-loaded silver nanoparticles

anticancer drugs, was loaded into chitosan nanoparticles to reduce dose-limiting side effects and improve their therapeutic response. Its anticancer effects were investigated, and its antiproliferative effect on the MCF-7 cell line was strong (Khan et al. 2017). Hepokur et al. tested AgNPs loaded with capecitabine in MCF-7 breast cancer cells. AgNPs–Capecitabine's antitumor activity was higher than the drug alone (Hepokur et al. 2019). In the study of Khedr et al., green-synthesized AgNPs showed strong cytotoxicity by reaching lower IC_{50} values on A549 and PC-3 cell lines (Khedr et al. 2022). Mahmood et al. demonstrated that CuONPs have potent antiproliferative and pro-apoptotic effects on AMJ-13 and MCF-7 cell lines (Mahmood et al. 2022).

In our study, the AgNPs-Car was synthesized but was not found in other artıcles. AgNPs-Car's anticancer activity was high on MCF-7 and A549 cells, as in the drug-loaded nanoparticles mentioned in other studies. Glioblastoma, one of the most malignant tumors affecting the brain, accounts for over 60% of all brain tumors in adults (Rock et al. 2012). Carboplatin is an anticancer drug that reduces tumors in glioblastoma treatment (Aparicio-Blanco et al. 2020). In a glioblastoma treatment development study using carboplatin-loaded pegylated nanoparticles, drug-loaded nanoparticles were much more effective than a single drug on C6 cells (Hassanzadeganroudsari et al. 2020). In brain tumor treatment through intravenous drug administration, plasma half-life being short, enzymatic degradation, innate or other immune response development, insufficient drug concentration in or around tumors, and problems in targeting tumors and achieving controlled drug release pose severe difficulties. Apart from causing systemic side effects, these problems create difficulties in preparing appropriate doses to treat brain tumors effectively. Thus, nanoparticles are accepted as a suitable approach to increase drug release efficiency, improve therapeutic effects, and reduce drug side effects (Giakoumettis et al. 2018; Ghaferi et al. 2022). The AgNPs-Car was found to have a highly effective selective cytotoxic activity on glioma cells compared to other cell lines studied. Our results support the literature, and the synthesized nanomaterial has the capacity to provide a substantial benefit in the treatment of glioblastoma.

The comparison of the Carboplatin, which is known to have serious side effects despite its clinical use, and the AgNPs-Car we synthesized showed that the drug dose could be reduced up to four times. This finding is crucial regarding reducing the drug's side effects. In addition, AgNPs-Car had no toxic effects on healthy cells, indicating that the AgNPs-Car is specific to cancer cells.

Apoptosis analysis (Annexin V/PI flow cytometry analysis)

Non-cytotoxic concentrations of AgNPs-Car 10 nm have an antiproliferative effect on C6 cells. This antiproliferative effect is due to the induction of apoptosis as shown by the Annexin-V-flow cytometric approach. Apoptosis data are shown in both Table 5 and Fig. 6.

Our results indicate that we also compared carboplatin with carboplatin-loaded silver nanoparticles. It is recognized that in cancer therapies, the number of necrosis cells is expected to be as low as possible, while the number of early and late apoptosis cells is expected to be higher (Hassan et al. 2014). When carboplatin was used alone for therapy, the number of necrosis cells was maximized and was, therefore, found to be the most toxic. A comparison of silver nanoparticles and drug-loaded nanoparticle samples

Table 5 Percentage ranges of C6 cells after Annexin V/PI

Cells	Molecules	Normal	Early Apop	Late Apop	Dead
C6	AgNPs-Car	66.50 ± 1.09	9.90 ± 0.80	17.85 ± 0.92	5.75 ± 0.65
	Carboplatin	69.20 ± 1.12	6.40 ± 0.76	7.90 ± 0.78	16.50 ± 0.91
WI-38	AgNPs-Car	97.90 ± 0.14	0.80 ± 0.01	1.00 ± 0.07	0.30 ± 0.02
	Carboplatin	96.10 ± 1.24	1.40 ± 0.20	1.70 ± 0.06	0.80 ± 0.03

Standard deviation values are given as ± Std, and all values are calculated as $n = 3$

AgNPs: silver nanoparticles, AgNPs-Car: carboplatin-loaded silver nanoparticles

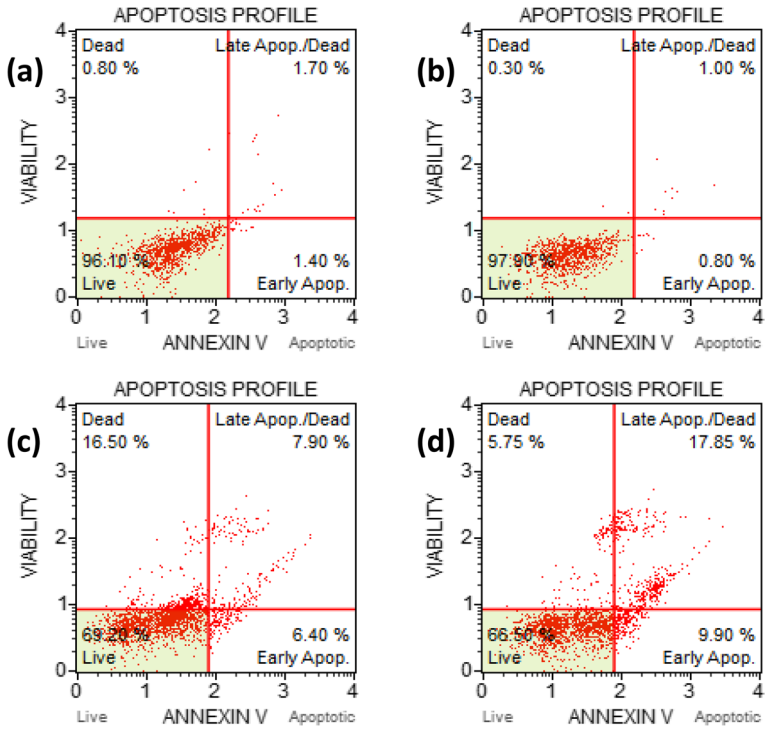

Fig. 6 Annexin V. Carboplatin on WI-38 cells (**a**), AgNPs-Car WI-38 cells (**b**), carboplatin on C6 cells (**c**), AgNPs-Car on C6 cells (**d**)

showed that the nanoparticle-bound drug significantly reduced the amount of C6 cells. AgNPs-Car was observed to significantly increase the number of early and late apoptotic cells, while it did not alter necrosis. This indicates that AgNPs-Car and AgNPs induce apoptosis in C6 glioma cells. We found that AgNPs-Car and AgNPs induced DNA fragmentation and eventually increased apoptosis of cells.

It has been reported in different studies that AgNPs induce cell death through apoptosis in cancer cells in a dose–dependent response. In one of these studies, Al-Nuairi et al. showed that silver nanoparticles with *Cyperus conglomeratus* root extract drove cells to apoptosis by inhibiting or activating proteins in different pathways involved in the apoptosis mechanism in cancer cell lines (Al-Nuairi et al. 2020). The exact mechanism of destroying cancer cell lines through biologically synthesized nanoparticles is not fully understood. However, different studies have implicated the mechanism of action of silver nanoparticles on cancer cells (Plackal et al. 2018). Changes in the viable cell

population indicate that the cell becomes apoptotic due to AgNP-induced antitumor activities (Ullah et al. 2020). Sriram et al. examined the anticancer effects of AgNPs in a tumor model and observed a reduction in tumor volume (Sriram et al. 2010). Our findings are similar to previous results on cancer cells in the literature.

Conclusion

In this study, our results indicate that synthesized AgNPs and AgNPs-Car hold promise as a potential candidate for the treatment of different cancers, especially brain cancer. AgNPs and AgNPs-Car with antitumoral activity on cancer cell lines affecting death mechanism demonstrated. Also, AgNPs and AgNPs-Car showed effective antimicrobial activity on Gram-positive and Gram-negative microorganisms. When silver nanoparticles with known antimicrobial effects are conjugated with drugs, they can be more specific and enable proper drug delivery to the site of infection. In addition, the use of drug-loaded AgNPs has great potential to help solve the growing crisis of microbial resistance. AgNPs and AgNPs-Car has a huge range of application such as antimicrobial, and anticancer agent. Future research is necessary to conclude the safety aspects of AgNPs and AgNPs-Car. Our results need to be supported by further studies, and their in vivo efficacy should be evaluated.

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Author contributions
TT planned the project, performed the experiments, and prepared and arranged the manuscript. TT analyzed the data, interpreted the data, and designed and revised the manuscript. TT provided the chemicals and reagents. The author read and approved the final manuscript.

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Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author wishes to confirm that there are no known competing interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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