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Cytotoxic Effects of Cisplatin and Carboplatin Loaded Albumin Nanoparticles on Breast Cancer Cells

Sisplatin ve Karboplatin Yüklü Albumin Nanopartiküllerin Meme Kanseri Hücreleri Üzerindeki Sitotoksik Etkileri

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ABSTRACT

Objective: This study aims to investigate and compare the anticancer effects of carboplatin and cisplatin, frequently used in cancer treatment, by loading them on albumin nanocarrier.

Materials and Methods: Carboplatin (CP) and Cisplatin (Cis) loaded albumin nanoparticles were synthesized using ultrasonication as CP-NPs and Cis-NPs, respectively. Nanoparticle size and distribution were evaluated by Dynamic light scattering (DLS). Cytotoxicities of NPs were evaluated in MDA-MB-231 and MCF-7 breast cancer cells and HUVEC using MTT test and their morphological images were compared.

Results: While the average size of CP-NPs was around 2-3 nm, Cis-NPs was 7-8 nm. It was observed that both NPs groups were homogeneously dispersed. According to the cytotoxicity results, both CP-NPs and Cis-NPs were more cytotoxic on MCF-7 breast cancer cells. In addition, CP-NPs and Cis-NPs showed significant cytotoxicity on MCF -7, MDA-MB-231 breast cancer cells, while low cytotoxicity was detected in normal HUVEC cells. The NPs treated MCF-7 was compared with the untreated MCF-7 and statistical significance was calculated as $P<0.01$ for CP-NPs and Cis-NPs.

Conclusions: Abumin-based CP-NPs and Cis-NPs showed high cytotoxicity in breast cancer cells, they have low cytotoxicity in healthy cells, making them promising for breast cancer treatment.

Keywords: Cytotoxicity, breast cancer, MCF-7, MDA-MB-231, nano drug

ÖZ

Amaç: Bu çalışmanın amacı kanser tedavisinde sıklıkla kullanılan karboplatin ve sisplatin ilaçlarının albümin nanotaşıyıcıya yüklenerek antikanser etkilerinin araştırılması ve karşılaştırılmasıdır.

Materyal ve Metot: Karboplatin (CP) ve Sisplatin (Cis) yüklenmiş albümin nanopartiküller, sırasıyla CP-NPs ve Cis-NPs olarak ultrasonikasyon kullanarak sentezlendi. Nanopartikül boyutu ve dağılımın homojenitesi Dinamik ışık saçılımı (DLS) ile değerlendirildi. Nanopartiküllerin sitotoksik aktiviteleri MDA-MB-231 ve MCF-7 meme kanseri hücrelerinde ve HUVEC hücrelerinde, MTT testi kullanılarak değerlendirildi ve morfolojik görüntüleri karşılaştırıldı.

Bulgular: CP-NPs'lerin boyutu ortalama 2-3 nm civarında iken, Cis-NPs'lerin 7-8 nm idi. Her iki nanopartikül grubunun da homojen bir şekilde dağıldığı görüldü. Sitotoksisite sonuçlarına göre CP-NPs ve Cis-NPs, MCF-7 meme kanseri hücrelerinde daha sitotoksikti. Ayrıca CP-NPs ve Cis-NPs'ler MDA-MB-231 meme kanseri hücrelerinde önemli sitotoksisite gösterirken, normal HUVEC hücrelerinde düşük sitotoksisite tespit edildi. CP-NPs ve Cis-NPs ile tedavi edilen MCF-7, tedavi edilmeyen MCF-7 ile karşılaştırıldı ve NPs'ler için istatistiksel anlamlılık $P<0,01$ olarak hesaplandı.

Sonuç: Meme kanseri hücrelerinde yüksek sitotoksisite gözlenirken, sağlıklı hücrelerde belirgin sitotoksisite gözlenmemiş olup albümine bağlı CP-NPs ve Cis-NPs kanser tedavisinde umut vadedici bir tedavi seçeneği olabilir.

Anahtar Kelimeler: MCF-7, MDA-MB-231, meme kanseri, nano ilaç, sitotoksisite

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INTRODUCTION

Breast cancer is the most frequent malignant tumor in women and one of the top causes of cancer death in many nations.[1] In particular, chemotherapy is widely used in treating breast cancer, but the frequency of side effects and drug reactions limits the efficiency of treatment.[2] Consequently, new and selective anticancer drugs with fewer side effects and ways to reduce side effects are needed.[3]

There has been a surge of interest in using nanotechnology in cancer treatment in recent years.[4,5] Nanodrug delivery systems offer considerable advantages in terms of enhanced permeability and retention (EPR), particularly when targeting tumors, and can lessen the negative effects of anticancer medications.[6] Nanoparticles smaller than 100 nm, in particular, are more effective in passively targeting tumors and are ideal carriers for inducing the drug's therapeutic potential while minimizing its side effects. Furthermore, nanocarriers may increase medication half-life in therapy, enhance pharmacokinetic characteristics, and improve patient compliance.[7]

In particular, protein-based nanocarriers are preferred over other nanomaterials as they have many favourable properties and are safe to use in biological environments.[8]Albumin is a multifunctional protein used as a medicine carrier because it is nontoxic, non-immunogenic, biocompatible, and biodegradable. As a result, it is an excellent material for use as a drug delivery platform.[9]

The principal effect of albumin-based drug delivery systems is dependent on tumor targeting and nanoparticle accumulation within the tumor. Higher drug accumulation within the tumor is caused by increased absorption, which is passively mediated by the increased permeability and retention effect.[9] Albumin can bind to certain receptors overexpressed in cancer cells, increasing nanoparticle binding and absorption. The 60 kDa glycoprotein (gp60) receptor[10] is overexpressed in a variety of malignancies, as is the acidic and cysteine-rich SPARC protein.[11] Albumin can bind selectively to gp60 and SPARC, hence actively increasing nanoparticle absorption. This mechanism prevents albumin-based nanoparticles from ejecting the medication from tumor cells.[12,13]

Although many studies on nanocarriers have been conducted in recent years, there is still a need for further inquiry regarding their effects in the field of cancer. In this study, cisplatin (Cis) and carboplatin (CP) nanoparticles were loaded independently into albumin and their anticancer effects on breast cancer cells were investigated. The cytotoxic effects of these medications, which we manufactured on the nanotechnological platform, were evaluated and compared on MCF-7, MDA-MB-231 breast cancer cells, and normal HUVEC cells.

MATERIALS AND METHODS

Ethics Committee Approval: An ethical approval for the study is not required. In this study, secondary cell culture was used.

Materials: MCF-7, MDA-MB-231, and HUVEC cell lines were purchased from The American Type Culture Collection (ATCC). Analytical purity bovine serum albumin (BSA) was purchased from Sigma (USA) while pharmaceuticals CP and Cis from Koçak Farma (Turkey). Chemicals used in cell culture are from Dulbecco's Modified Eagle's Medium (DMEM), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit, Dimethylsulfoxide (DMSO), Sigma (USA) and Fetal Bovine Serum (FBS), Penicillin, streptomycin, and trypsin were obtained from Gibco (UK).

Preparation of Nanoparticles: First, stock solutions were prepared at a concentration of 10 mg/mL of albumin and 4.5 mg/mL of CP and Cis separately in distilled water (CP and Cis were previously dissolved at 10 mg/mL separately in DMSO).[14] The albumin and stock solutions were separated into Eppendorf tubes and sonicated for 30 seconds in the ultrasonication apparatus. Following that, the produced solutions were stored separately in a sterile condition for 1 hour under a 30 W (Philips brand) UV-C lamp (200-280 nm). Compounds loaded on albumin were encoded as Cis-Nps and CP-NPs.

Distribution of Nanoparticles / Size Measurements: Using a 4 mW He-Ne laser operating at ambient temperature, a wavelength of 633 nm, and a detection angle of 173°, dynamic light scattering (DLS) measurements using Zetasizer Nano ZS were used to assess the size distribution of the compounds. As the reference liquid, distilled water was utilized.

Cell Culture and Cell Lines: MDA-MB-231, MCF-7 breast cancer, and normal HUVEC cells were employed in cell culture research. They were grown in DMEM with fetal calf serum at 37°C in a humidified oven environment with 95% O_2, and 5% CO_2. Cells were checked daily and subcultured every day or every 2-3 days, depending on their condition.

Evaluation of Cell Viability by Trypan Blue Method: The trypan blue staining technique was used to measure cell viability. For this, the cells were stained with trypan blue dye, and cell viability was measured using a Neubauer slide under a light microscope. Dead cells stained with trypan blue showed blue, whereas healthy cells did not. It was observed that cells that had not been colored were alive.

Determination of Cytotoxic Activity on Cells by MTT Measurement Based on Mitochondrial Activity: The MTT test is a quantitative colorimetric

method used to assess cytotoxicity in vitro based on metabolic viability.[15] Albumin-bound Cisplatin-loaded nanoparticles (Cis-NPs) and Carboplatin-loaded nanoparticles (CP-NPs) were produced at various doses (50, 25, 12.5, 6.25, 3.12 g/mL) and incubated for 72 hours with MDA-MB-231, MCF-7, and HUVEC cells. After 72 hours, the cells were exposed to the MTT test, and the optical densities of the cells in the plate were measured with an ELISA equipment at 540 nm. All experiments were done three times, and the average was calculated.

Statistical analysis: Using the IBM SPSS 15.0 (SPSS Inc., Chicago, IL, USA) package application, the data was examined. In order to compare the variations between the control groups and CP-NPs and Cis-NPs, the Student's t-test was performed. P values less than 0.05 were considered significant. The 72-hour MTT test was used to determine cytotoxicity. Each test was conducted three times, and the average was calculated. The data are presented as mean SD.

RESULTS

CP and Cis anticancer medicines attach to albumin nanocarriers at the nanoscale, as shown in Figure 1. The nanosizes of the cisplatin-loaded albumin nanoparticles (Cis-NPs) are in the range of 5-11 nm, the nanoparticles are homogeneously disseminated, and the average Cis-NPs size is around 6-7 nm, as shown in Figure 1a. Furthermore, Figure 1b shows that the CP-loaded albumin-bound nanoparticles (CP-NPs) are homogeneously distributed in the 2-5 nm range, with the average CP-NPs size being around 2-3 nm. Furthermore, although Cis NPs had a PDI value of 0.218, CP-NPs had a PDI value of 0.481.

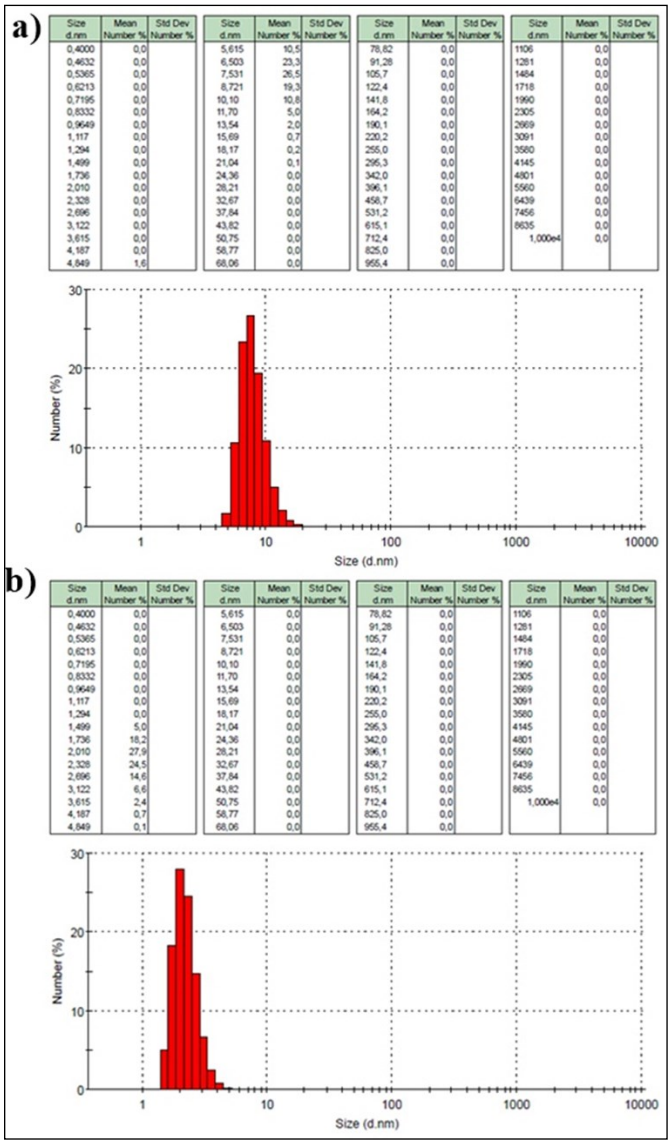

Figure 1. DLS Analysis of Albumin-based Carboplatin NPs (a) and Cisplatin NPs (b).

Figure 2 shows the cytotoxic effects of CP-NPs and Cis-NPs on MDA-MB-231, MCF-7 breast cancer cells, and normal HUVEC cells. According to our findings, both CP-NPs and Cis-NPs appear to be more cytotoxic in MCF-7 breast cancer cells. MDA-MB-231 triple-negative breast cancer cells seem to be more resistant to treatment than MCF-7. It is also noteworthy that CP-NPs and Cis-NPs samples are less cytotoxic in HUVEC cells, which are the normal cell group. Table 2 shows the IC_{50} values of Cis-NPs and CP-NPs on MCF-7, MDA-MB-231, and HUVEC cells. The IC_{50} values of Cis-NPs are 29 µg/mL in MCF-7 cells, 46 µg/mL in MDA-MB-231 cells, while the IC_{50} values of CP-NPs are 22 µg/mL in MCF-7 cells, MDA-MB-231 for 41 µg/mL (Figure 2, Table 1).

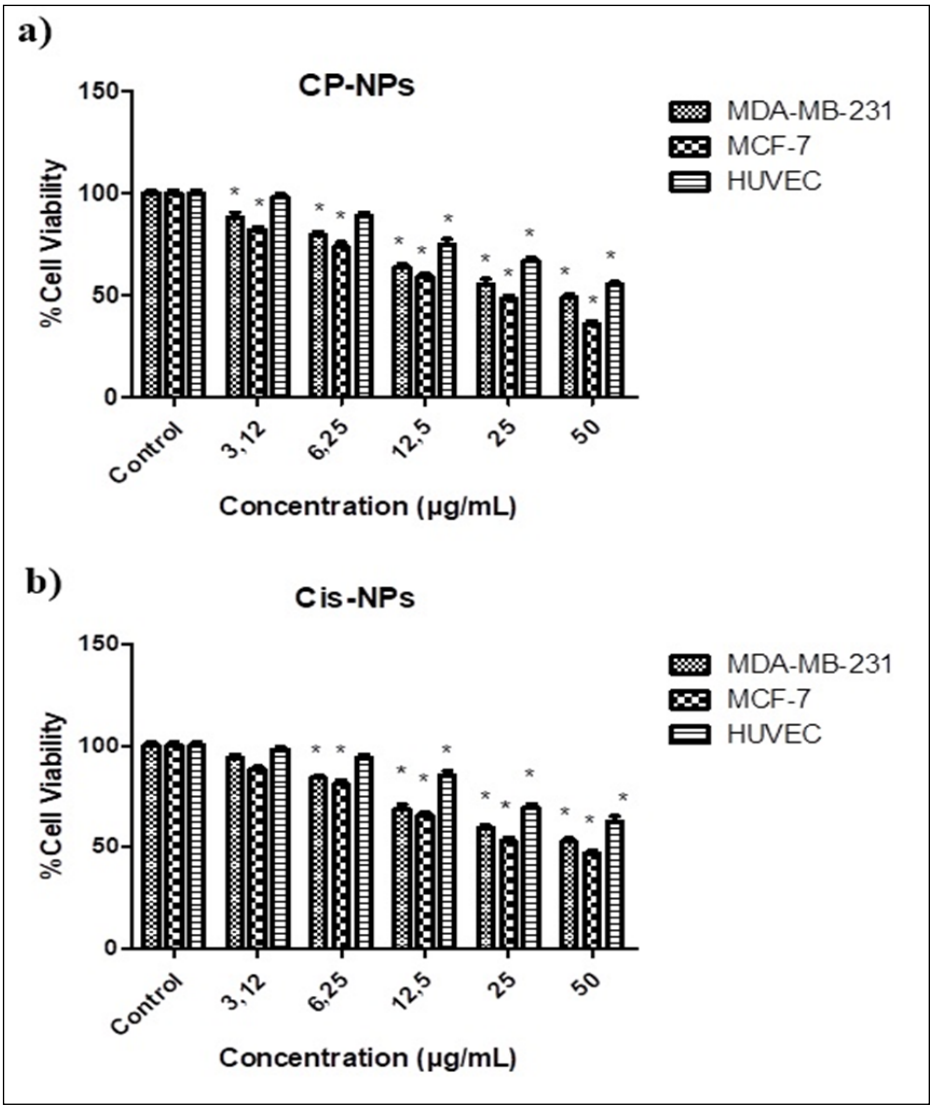

Figure 2. Cytotoxic effects of CP-NPs (a) and Cis-NPs (b) at different concentrations on MDA-MB-231, MCF -7 cancer cells and HUVEC normal cells (Concentrations are calculated based on the amount of Cis or CP loaded into the nanoparticles). Significant comparisons have a "p" value of less than 0.05. In each group, each concentration was compared with the control and the mean *: $p<0.01$ was calculated.
CP: Carboplatin, Cis: Cisplatin.

Table 1. IC_{50} concentrations of Cis-NPs and CP-NPs on MCF-7, MDA-MB-231 and HUVEC cells. Cytotoxicity was evaluated by MTT test after 72 hours.

	***IC_{50} Concentration (µg/mL)**	
Cell Line	Cis-NPs	CP-NPs
MCF-7	29	22
MDA-MB-231	46	41
HUVEC	>50	>50

IC_{50}: The concentration at which cell growth is inhibited by 50%.

Figure 3 compares the morphological changes in MDA-MB -231, MCF -7, and HUVEC cells that were not treated and in MDA-MB -231, MCF -7, and HUVEC cells treated with CP -NPs and Cis-NPs. The untreated control cells were compact, spindle-shaped, cross-linked and adherent to the surface in all cell groups. In contrast, the number of MDA-MB-231 and MCF-7 cells treated with CP-NPs, and Cis-NPs appeared to be drastically reduced. Some cells treated with the nano-drug were shrunken and round, and there was a significant decrease in cell quantity and intercellular connections. Figure 3 shows images of cells treated with CP -NPs, and Cis -NPs at IC_{50} values. According to the findings of this study, the morphological changes are consistent with cytotoxicity.

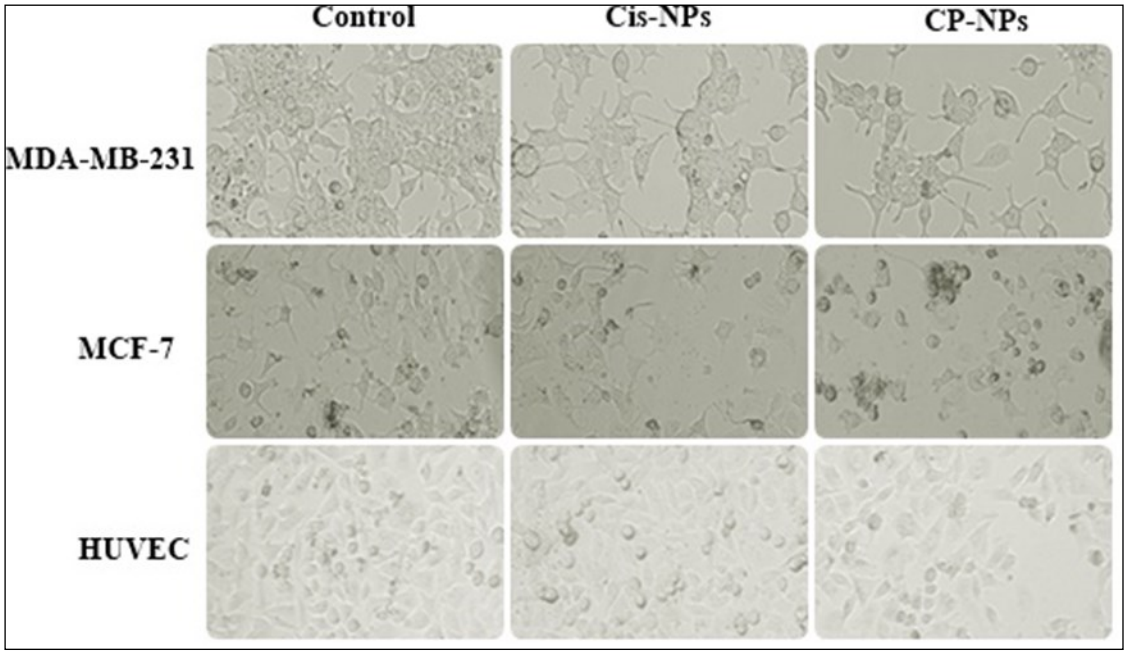

Figure 3. The morphological changes in MDA-MB-231, MCF-7, and HUVEC cells under light microscopy. Magnification 20X.

DISCUSSION AND CONCLUSION

In this study, the platinum group anticancer medicines CP and Cis were loaded on albumin nanocarrier to create two distinct nano-drugs, Cis-NPs and CP-NPs, and their cytotoxic effects on MCF-7, MDA-MB-231 and HUVEC cells were examined.

A growing body of evidence suggests that nanoparticle-based drug delivery show promise for treating cancer. Nanocarriers can effectively deliver drugs to diseased areas in a controlled and targeted manner compared to conventional drug therapy.[16] Nanocarriers may also improve pharmacokinetic characteristics, lengthen medication half-life in therapy, and improve patient compliance. Many nanoparticles have been studied for this purpose, including different polymeric nanoparticles, albumin-bound nanoparticles, lipid-based nanoparticles, inorganic nanoparticles, carbon-based nanotubes, polymer-based conjugates, and nanocrystals.[7,13] Among them, we chose albumin nanocarriers for the medications Cis and CP because it is possible to overcome the challenges posed by disease-treating drugs, such as limited solubility, surface adsorption, and high systemic toxicity, in part because of albumin nanocarriers.[8]

The literature includes synthesized nano-drug delivery technologies of various sizes.[17–19] However, passively targeting tumors with nanocarrier systems that use particles smaller than 40 nm is more effective at inducing therapeutic potential and minimizing adverse pharmacological effects.[7,13] In this study, NPs below 40 nm were obtained and their cytotoxic effects on both cancer cells and normal cells were evaluated. Albumin-bound CP-NPs and Cis-NPs with sizes of 7-8 nm and 2-3 nm, respectively (Fig.1), were created and proved to be very effective against breast cancer cells. Different sizes of gold nanoparticles (3,5,6,8,10,17 nm) were created in a study by Viyajakumar et al., and the effects of their cytotoxicity were examined. It was shown that the 3 nm nanoparticles were more cytotoxic than the others.[20]

In our study, Cis-NPs and CP-NPs were found to be highly cytotoxic in both MCF-7 and MDA-MB-231 breast cancer cell groups, but they were more effective on MCF-7 cells and had the least cytotoxicity on HUVEC normal cells (Fig. 2, Fig. 3, Table 1).

According to our results, it is expected that MDA-MB-231 cells are more resistant to treatment than MCF-7. Because MCF-7 and MDA-MB-231 breast cancer cells have different phenotypic and genotypic characteristics even though they are both invasive ductal breast cancer cells. MCF-7 cells are estrogen receptor (ER) positive and progesterone receptor (PR) positive and have low metastatic potential, while MDA-MB-231 cells known as triple-negative breast cancer (TNBC) lack ER, PR and human epidermal growth factor receptors. It is known for its lack of expression of the 2 (HER2) gene and is considered to be the most aggressive breast cancer cells that can lead to early metastasis.[21,22] For this reason, TNBC breast cancer with MDA-MB-231 cancer cells is a type of breast cancer that is difficult to treat, and it is very important that the nano-drugs we synthesize are also effective in this cell group.

Many of the drawbacks of conventional drug delivery systems can be solved by nanocarrier-dependent drug delivery applications. For instance, chemotherapeutic agents used in the treatment of cancer are traditionally non-specifically distributed systemically, causing damage to both healthy cells and cancer cells, often with low efficacy and high toxicity.[23] Nano-drug delivery systems can increase drug concentration in cancer cells by directing chemotherapeutics to the tumor site and prevent toxicity in normal cells.[24–26]

In conclusion, CP and Cis loaded on albumin nanocarriers exhibited high cytotoxicity in breast cancer cells but low cytotoxicity in healthy cells, which suggested that they are promising for the treatment of breast cancer.

Ethics Committee Approval: An ethical approval for the study is not required. In this study, secondary cell culture was used.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – FDK, AK; Materials – FDK, AK; Data Collection and/or Processing – FDK, AK; Analysis and/or Interpretation – FDK; Writing –FDK, AK. Supervision – FDK, AK.

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REFERENCES

1. Howell A, Anderson AS, Clarke RB, et al. Risk determination and prevention of breast cancer. Breast Cancer Research 2014;16(5):1-19. doi:10.1186/S13058-014-0446-2
2. Reang J, Sharma PC, Kumar Thakur V, et al. Understanding the therapeutic potential of ascorbic acid in the battle to overcome cancer. Biomolecules 2021;11(8):1130. doi:10.3390/BIOM11081130
3. Schirrmacher V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment. Int J Oncol. 2019;54(2):407-419. https://doi.org/10.3892/ijo.2018.4661
4. Zhao CY, Cheng R, Yang Z, Tian ZM. Nanotechnology for cancer therapy based on chemotherapy. Molecules. 2018;23(4). doi:10.3390/molecules23040826
5. Parvanian S, Mostafavi SM, Aghashiri M. Multifunctional nanoparticle developments in cancer diagnosis and treatment. Sens Biosensing Res. 2017;13:81-87. doi:10.1016/J.SBSR.2016.08.002
6. Wu J. The enhanced permeability and retention (epr) effect:The significance of the concept and methods to enhance its application. Journal of Personalized Medicine 2021;11(8):771. doi:10.3390/JPM11080771
7. Ma P, Mumper RJ. Paclitaxel nano-delivery systems: a comprehensive review. J Nanomed Nanotechnol. 2013;4(2):1000164. doi:10.4172/2157-7439.1000164
8. Karami E, Behdani M, Kazemi-Lomedasht F. Albumin nanoparticles as nanocarriers for drug delivery: Focusing on antibody and nanobody delivery and albumin-based drugs. J Drug Deliv Sci Technol. 2020;55:101471. doi:10.1016/J.JDDST.2019.101471
9. Elzoghby AO, Samy WM, Elgindy NA. Albumin-based nanoparticles as potential controlled release drug delivery systems. Journal of Controlled Release. 2012;157(2):168-182. doi:10.1016/J.JCONREL.2011.07.031
10. Lee ES, Youn YS. Albumin-based potential drugs: focus on half-life extension and nanoparticle preparation. Journal of Pharmaceutical Investigation 2016;46(4):305-315. doi:10.1007/S40005-016-0250-3
11. Vaz J, Ansari D, Sasor A, Andersson R. SPARC: A Potential Prognostic and Therapeutic Target in Pancreatic Cancer. Pancreas. 2015;44(7):1024. doi:10.1097/MPA.0000000000000409
12. Kratz F. A clinical update of using albumin as a drug vehicle-A commentary. Journal of Controlled Release. 2014;190:331-336. doi:10.1016/J.JCONREL.2014.03.013
13. Hassanin I, Elzoghby A. Albumin-based nanoparticles: a promising strategy to overcome cancer drug resistance. Cancer Drug Resistance. 2020;3(4):930-946. doi:10.20517/CDR.2020.68
14. Kalındemirtaş FD, Kariper İA, Sert E, Okşak N, Kuruca SE. The evaluation of anticancer activity by synthesizing 5FU loaded albumin nanoparticles by exposure to UV light. Toxicology in Vitro 2022;84:105435. doi.org/10.1016/j.tiv.2022.105435
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to prolifera-

tion and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. doi:10.1016/0022-1759(83)90303-4

16. Dang Y, Guan J. Nanoparticle-based drug delivery systems for cancer therapy. Smart Mater Med. 2020;1:10-19. doi:10.1016/J.SMAIM.2020.04.001
17. Liu Y, Guo Q, Sun H, Guo N, Zhang J. Improved therapeutic efficiency of photothermal treatment and nursing care in prostate cancer by DOX loaded PEG coated Cu@Se nano-hybrid vesicle. Process Biochemistry. 2020;92:78-84. doi:10.1016/J.PROCBIO.2020.02.022
18. Ye BL, Zheng R, Ruan XJ, Zheng ZH, Cai HJ. Chitosan-coated doxorubicin nano-particles drug delivery system inhibits cell growth of liver cancer via p53/PRC1 pathway. Biochem Biophys Res Commun. 2018;495(1):414-420. doi:10.1016/J.BBRC.2017.10.156
19. Haggag YA, Osman MA, El-Gizawy SA, et al. Polymeric nano-encapsulation of 5-fluorouracil enhances anti-cancer activity and ameliorates side effects in solid Ehrlich Carcinoma-bearing mice. Biomedicine & Pharmacotherapy. 2018;105:215-224. doi:10.1016/J.BIOPHA.2018.05.124
20. Vijayakumar S, Ganesan S. Size-dependent in vitro cytotoxicity assay of gold nanoparticles. Toxicological & Environmental Chemistry 2013;95(2):277-287. doi:10.1080/02772248.2013.770858
21. Kocak C, Kocak FE, Ozturk B, Tekin G, Vatansev H. Cytotoxic, anti-proliferative and apoptotic effects of noscapine on human estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell lines. Bratisl Lek Listy. 2020;121(1):43-50. doi:10.4149/BLL_2020_007
22. Noh JI, Mun SK, Lim EH, et al. Induction of apoptosis in MDA-MB-231 cells treated with the methanol extract of lichen physconia hokkaidensis. Journal of Fungi 2021;7(3):188. doi:10.3390/JOF7030188
23. Yetisgin AA, Cetinel S, Zuvin M, Kosar A, Kutlu O. Therapeutic nanoparticles and their targeted delivery applications. Molecules. 2020;25(9). doi:10.3390/MOLECULES25092193
24. Zinger A, Koren L, Adir O, et al. Collagenase nanoparticles enhance the penetration of drugs into pancreatic tumors. ACS Nano. 2019;13(10):11008-11021 doi: 10.1021/acsnano.9b02395
25. Yan L, Shen J, Wang J, Yang X, Dong S, Lu S. Nanoparticle-based drug delivery system: a patient-friendly chemotherapy for oncology. Dose-Response. 2020;18(3). doi:10.1177/1559325820936161
26. Edis Z, Wang J, Waqas MK, Ijaz M, Ijaz M. Nanocarriers-mediated drug delivery systems for anticancer agents: an overview and Perspectives. Int J Nanomedicine. 2021;16:1313-1330. doi:10.2147/IJN.S289443