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Reducing toxicity and enhancing efficacy of doxorubicin by liposomal doxorubicin and aprepitant in breast cancer

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This study investigates the efficacy and toxicity profiles of pegylated liposomal doxorubicin (Doxil) compared to conventional doxorubicin. Additionally, it evaluates the potential of combination therapy involving Doxil and doxorubicin with aprepitant, an FDA-approved agent for the management of chemotherapy-induced nausea and vomiting. Using a mouse model induced with 4T1 breast cancer cells, tumor size, and weight were assessed following treatment with either single doses or combination therapies. The study also examined oxidative and antioxidant stress markers in tumor, liver, and cardiac tissues, complemented by histopathological analysis of these tissues using hematoxylin and eosin staining. Results indicated that prepared liposomal doxorubicin significantly enhanced antitumor efficacy, as evidenced by decreased tumor size and weight. Moreover, it positively influenced oxidative stress markers, promoting apoptosis in tumor tissues. Notably, Doxil also reduced adverse effects compared to standard doxorubicin, as indicated by lower oxidative stress levels and increased antioxidant activity in both cardiac and liver tissues. The combined administration of doxorubicin and aprepitant further improved therapeutic efficacy and reduced side effects. Consequently, the formulation of doxorubicin in liposomes and aprepitant-based combination therapy represents a promising strategy for enhancing treatment effectiveness while minimizing adverse effects in breast cancer management.

Keywords Breast cancer, Doxorubicin, Aprepitant, Doxil, Cardiotoxicity, Hepatotoxicity

Breast cancer is one of the malignancies that is increasing all over the world[1–3]. According to the statistics of the World Health Organization (WHO), breast cancer was the cause of death of 670,000 people in the world in 2022. Also, the World Health Organization has announced the most common cancer in women in 157 out of 185 countries in 2022[4]. Many risk factors play a role in the occurrence of breast cancer, including genetic and hereditary predisposition, hormonal imbalance, and lifestyle[1,2,5]. Due to the high heterogeneity of breast cancer, despite scientific advances and clinical care, as well as knowledge about the molecular mechanisms involved in this malignancy, the prognosis is relatively poor, and the mortality rate is relatively high. In addition, the mechanisms related to the pathogenesis of this disease are not yet fully defined[5,6].

Doxorubicin (DOX) is an anthracycline that is often used to manage and treat a variety of malignancies, including lung, breast, and testicular cancer[7]. Also, this drug is widely considered to be the most active drug available for the treatment of breast cancer. However, DOX is known to cause cardiac side effects such as electrocardiographic changes, heart failure, and acute coronary syndromes in some patients[8,9]. Importantly, DOX frequently leads to chemoresistance in patients with breast cancer, especially in patients with triple-negative breast cancer (TNBC). Considering that DOX is very effective in the treatment of TNBC, it can have poor results due to the induction of chemical resistance[10,11]. Thus, due to the lack of valid molecular targets

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and poor outcomes in TNBC, there is an urgent need to develop new treatments for breast cancer to prevent chemoresistance.

A strategy for reducing the toxicity associated with doxorubicin and increasing its effectiveness in treating patients with TNBC is to encapsulate it in liposomes[12,13]. Doxil is a liposomal formulation of doxorubicin approved by the United States Food and Drug Administration (FDA) and is used as an anti-cancer drug in various cancers, including breast, prostate, and ovarian cancer[12–14]. Several studies have shown that Doxil is more effective than doxorubicin because of its lower toxicity, stability, and prolonged half-life in the blood[13,15–17]. O'Brien et al. conducted a study comparing the efficacy of pegylated liposomal doxorubicin (PLD) to traditional doxorubicin in patients with metastatic breast cancer. They found similar median treatment durations (149 days for PLD vs. 133 days for doxorubicin) and comparable progression-free survival rates (6.9 months for PLD vs. 7.8 months for doxorubicin), with a hazard ratio of 1.00 indicating noninferiority of PLD. Both treatments exhibited similar safety profiles, but PLD had reduced cardiotoxicity and led to lower rates of myelosuppression, vomiting, and hair loss. Overall, PLD was found to be as effective as doxorubicin while minimizing cardiac side effects[13]. Maher Alhaja and colleagues indicated in a single-center retrospective cohort study that administration of pegylated liposomal doxorubicin had limited cardiotoxicity in patients with breast cancer and sarcoma who had formerly received doxorubicin[18]. Another observational study indicated that liposomal doxorubicin decreased the side effects of doxorubicin in patients with metastatic breast cancer[19].

On the other hand, it has been shown that the neuropeptide system Substance P (SP) and its specific receptor (NK-1R), which is involved in the progression of many cancers, including breast cancer, increases the side effects associated with chemotherapy, especially cardiotoxicity[20–22]. Activation of NK1 by SP leads to activation of PI3K, NF-κB and MAPK pathways[23]. In addition, it has been observed that the SP/NK1R system is related to cardiac complications such as encephalomyocarditis, hypertrophy, and apoptosis of cardiac cells through increasing the production of reactive oxygen species (ROS)[22,24,25]. Also, blocking the NK-1R receptor by the specific antagonist, aprepitant has shown promising results in treating various cancers, including breast, gallbladder, ovarian, and prostate cancer[20,25–27].

Aprepitant has been approved by the FDA as a clinical drug for the treatment of chemotherapy-induced vomiting and nausea (CINV). Studies have shown that aprepitant via blocking the NK1 receptor acts as an antitumor agent, inhibiting the proliferation and migration of cancer cells as well as inducing cell death by apoptosis[20,27–29]. The study conducted by Muñoz et al. showed that NK-1 receptor antagonists could serve as a new antitumor drug for breast cancer[22]. Another study showed that aprepitant exerts a neuroprotective effect against DOX-mediated chemo brain by reducing inflammatory, oxidative, and apoptotic responses, partly by reducing oxidative stress in the endoplasmic reticulum, SP levels, and miR-146a[30]. In addition, various studies have reported that aprepitant, when used in combination with DOX, demonstrates synergistic effects in anti-tumor activity and induction of apoptosis on hepatoblastoma cancer cells, and also, the effects of reducing the cardiac side effects of DOX have been seen in the treatment of breast cancer cells with aprepitant[22,31]. Considering the properties of aprepitant and Doxil in reducing side effects caused by chemotherapy, in this study, we investigated the cardiotoxic effects of liposomes containing DOX and aprepitant in mice with breast cancer.

Results

Physicochemical properties of Doxil

To investigate the physicochemical properties of liposomes, particle size, PDI and surface charges were measured. The prepared liposome particles' final size, PDI and zeta potential were calculated as 103.83 ± 0.75, 0.086 ± 0.0049 and − 14.1 mV, respectively. Consistent with past evidence, the presence of mPEG2000-DSPE leads to negative zeta potential[32,33]. The concentration of doxorubicin in the prepared liposome was 2 mg/ml. The TEM analysis revealed that the prepared liposomes exhibited a uniform spherical morphology with a well-defined lipid bilayer structure, confirming their nanoscale size and structural integrity (Fig. S1).

Evaluation of antitumor effects

A comparative study was conducted in a 4T1 tumor-bearing mouse model to evaluate the antitumor efficacy of nanoliposomal doxorubicin (Doxil) alone and in combination with aprepitant, in comparison to free doxorubicin and its combination with aprepitant. Tumor size and weight were monitored as endpoints. The results demonstrated that the tumor size in mice receiving Doxil and its combination with aprepitant was significantly smaller than that in mice receiving free doxorubicin and its combination with aprepitant, respectively, suggesting the enhanced efficacy of the liposome formulation of doxorubicin (Fig. 1). Furthermore, the combination of Doxil with aprepitant was found to be effective in reducing tumor size, while the combination of Doxil and free doxorubicin with aprepitant resulted in synergistic tumor growth inhibitory effects. Consistent with these findings, the examination of tumor weight revealed that the liposome formulation was more effective in reducing tumor weight (Fig. 1).

Antioxidant activity and oxidative stress in tumor tissue

To assess the oxidative stress and antioxidant activities of the examined drugs within tumor tissue, we measured the levels of MDA, SOD, and CAT. As shown in the Fig. 2, the treatment of mice with Doxil resulted in a significant increase in oxidative stress, as evidenced by elevated MDA levels compared to the other groups. Conversely, aprepitant demonstrated notable antioxidant properties by reducing MDA levels and enhancing SOD and CAT levels, both when administered alone and in combination with Doxil and doxorubicin.

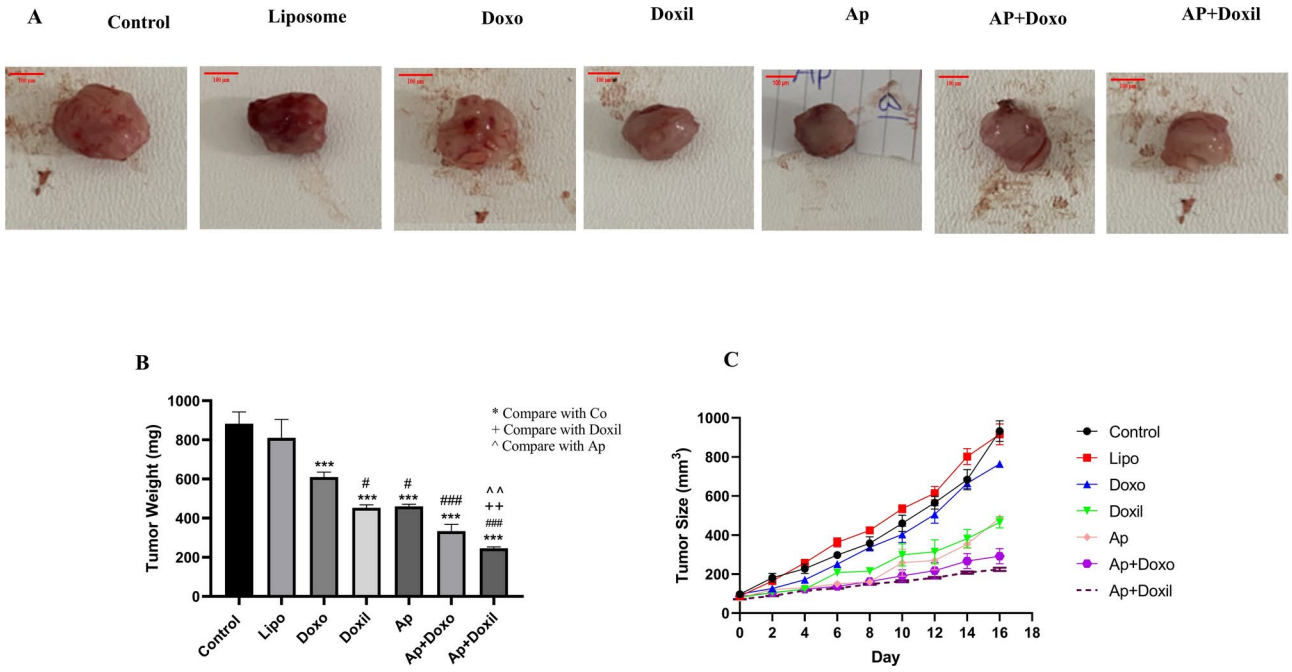

Fig. 1. Comparison of antitumor effects of doxorubicin, Doxil, and aprepitant in single dose and combination therapy. Tumor images were taken on the last day of treatment (**A**). Mean tumor weight in each group (**B**). Mean tumor size in each group (**C**) (*P < 0.05; **P < 0.01; ***P < 0.001 vs. control).

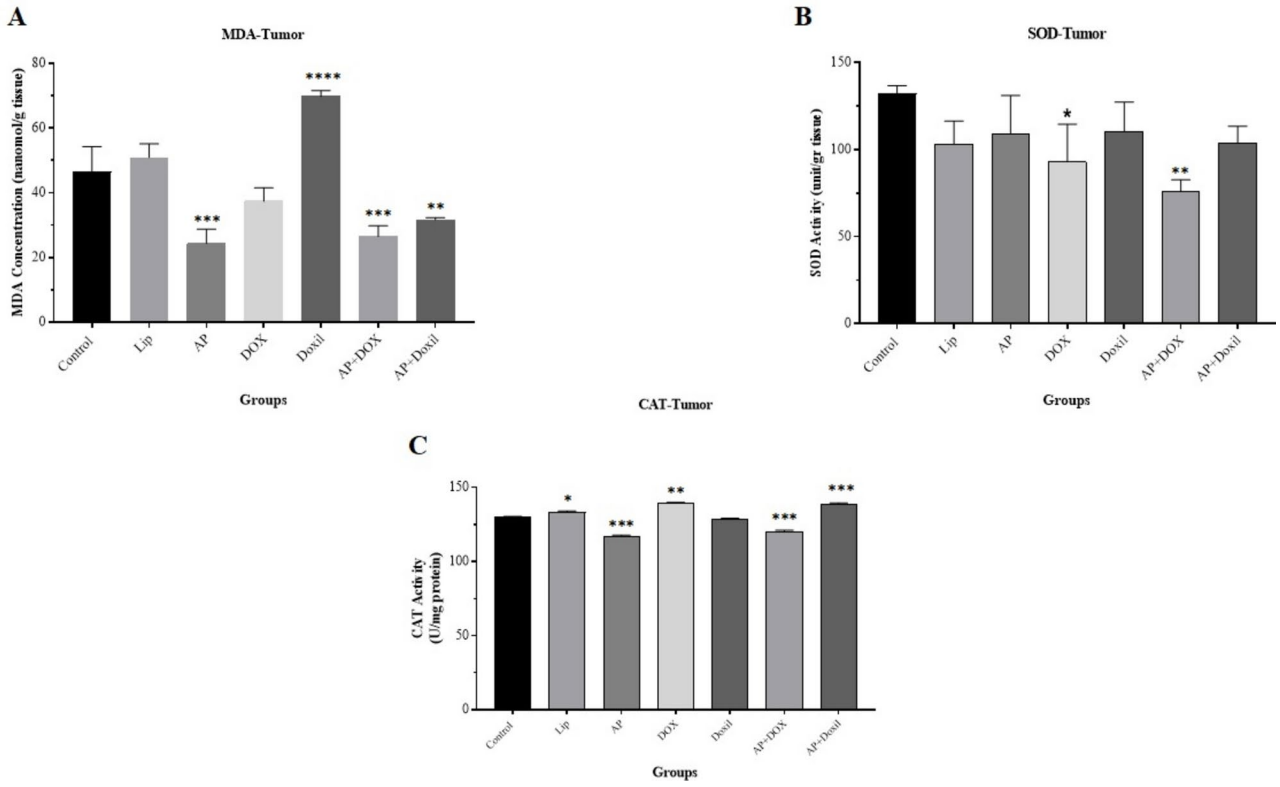

Fig. 2. Examining the effects of the studied drugs on the level of Malondialdehyde (MDA) (**A**), Superoxide dismutase (SOD) (**B**), and Catalase (CAT) (**C**) on tumor tissue (*P < 0.05; **P < 0.01; ***P < 0.001 vs. control).

Antioxidant activity and oxidative stress in heart tissue

Doxorubicin-induced cardiotoxicity has been extensively documented in the literature[34]. Consequently, we investigated the levels of oxidative and antioxidant stress markers in heart tissue following treatment with liposomal doxorubicin (Doxil) alone and in combination with aprepitant (Fig. 3). The results revealed that the MDA levels, a marker of oxidative stress, were significantly elevated in the group treated with doxorubicin. In contrast, MDA levels were reduced in the groups treated with Doxil both alone and in combination with aprepitant. Additionally, levels of SOD and CAT were found to increase in the groups treated with aprepitant and Doxil. Interestingly, SOD and CAT levels were lower in the combination treatment groups compared to those receiving single-agent treatment.

Antioxidant activity and oxidative stress in liver tissue

The liver plays a crucial role in the metabolism of various drugs, particularly those formulated as liposomal preparations[35]. Therefore, we examined the impact of the examined drugs on oxidative stress levels and antioxidant enzyme activity. Our findings indicated that MDA levels were significantly higher in the Doxil and liposomal treatment group compared to the other groups. Conversely, the combination therapies resulted in a reduction of MDA levels relative to those observed in the groups treated with single doses of doxorubicin and Doxil. Furthermore, the combination of aprepitant with Doxil and doxorubicin was associated with an increase in SOD and CAT levels. Notably, antioxidant levels in the Doxil group were also enhanced compared to those in the doxorubicin group (Fig. 4).

Histological assessment

Histopathological analysis utilizing H&E staining indicated that liposomal doxorubicin enhances therapeutic efficacy, resulting in increased necrosis within tumor tissues. Furthermore, the combination of Doxil and doxorubicin with aprepitant demonstrated an improvement in antitumor properties (Fig. 5). Additionally, histopathological assessments of both liver and heart tissues revealed that aprepitant mitigates the adverse effects associated with Doxil and doxorubicin. Notably, the histopathological images illustrated that Doxil exerts fewer side effects on cardiac and hepatic tissues compared to doxorubicin. Besides, Histopathological analysis of HE-stained sections revealed distinct tissue damage in both the heart and liver across different treatment groups. In cardiac tissue, myocardial fiber disorganization, interstitial edema, increased spaces between myofibers, and evidence of cardiomyocyte vacuolization (Black arrows) were observed, particularly in the Doxo- and Doxil-treated groups. In liver sections, hepatocyte degeneration, including vacuolization (Black arrows), sinusoidal congestion, and infiltration of inflammatory cells (Black Stars) were apparent. The inflammatory response was

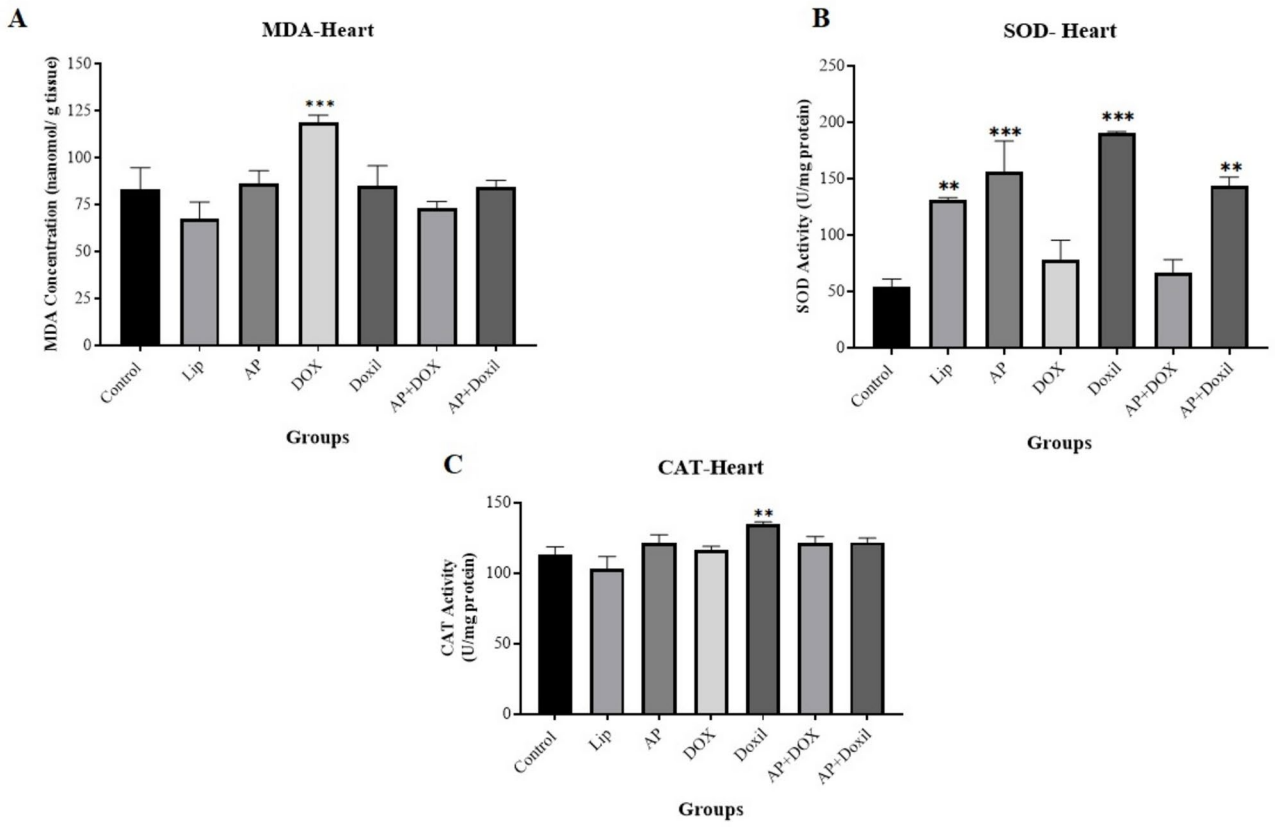

Fig. 3. Examining the effects of the studied drugs on the level of Malondialdehyde (MDA) (**A**), Superoxide dismutase (SOD) (**B**), and Catalase (CAT) (**C**) on heart tissue (*P < 0.05; **P < 0.01; ***P < 0.001 vs. control).

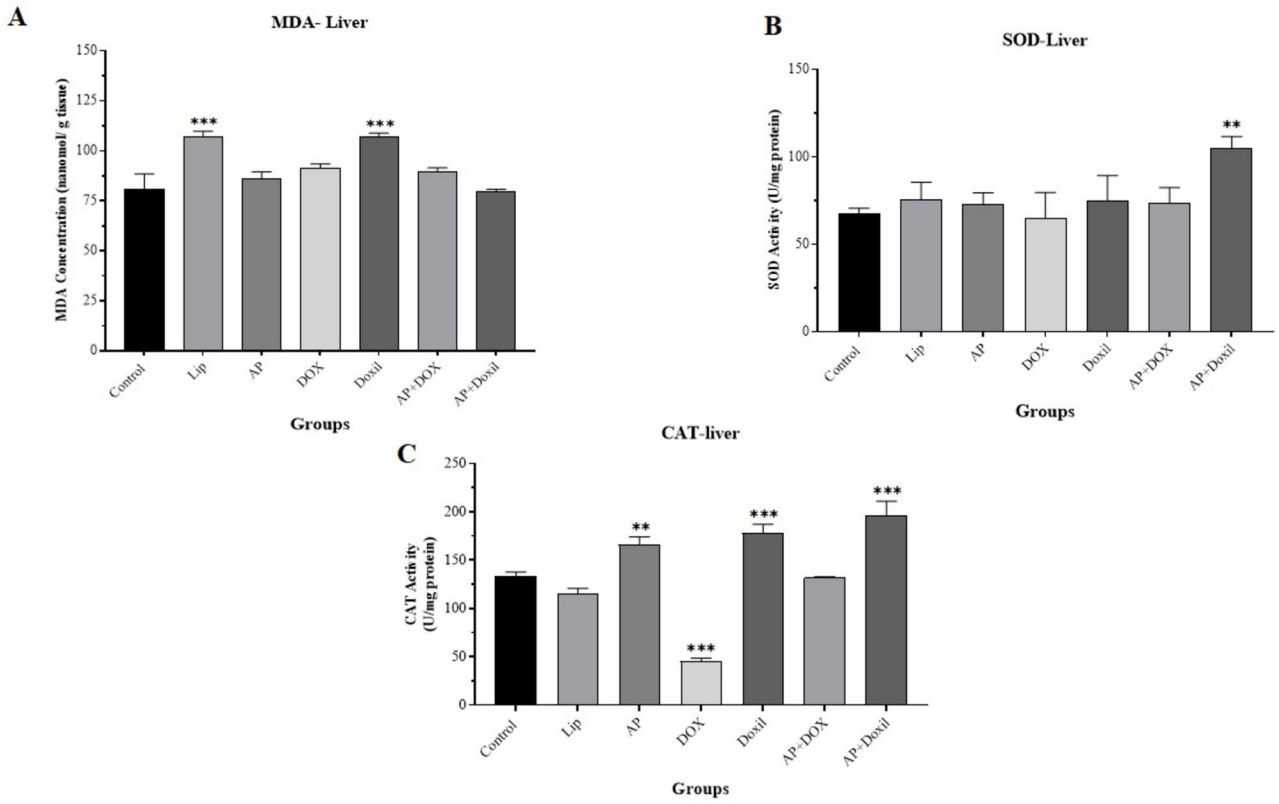

Fig. 4. Examining the effects of the studied drugs on the level of Malondialdehyde (MDA) (**A**), Superoxide dismutase (SOD) (**B**), and Catalase (CAT) (**C**) on liver tissue (*P < 0.05; **P < 0.01; ***P < 0.001 vs. control).

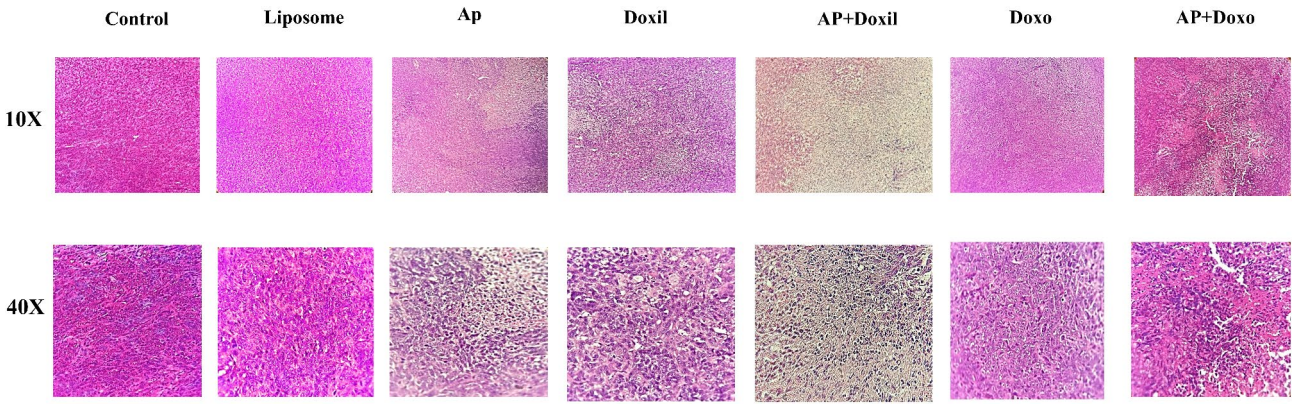

Fig. 5. Investigation of the tumor cell necrosis in the microscopic images of tumor tissue in the studied cases using H&E staining.

characterized by prominent infiltration of lymphocytes, macrophages, and neutrophils, along with increased vascular permeability and congestion, indicating immune activation. Importantly, Aprepitant treatment appeared to mitigate these adverse effects. Compared to Doxo/Doxil alone, Aprepitant co-administration resulted in reduced cardiac muscle disruption, lower inflammatory cell infiltration in liver sections, and better preservation of normal tissue architecture (Fig. 6).

Discussion

Considering the high prevalence of breast cancer and its fatality among women in the world, research is very valuable in order to obtain effective drugs with few side effects[36]. According to the evidence, resistance to chemotherapy drugs as well as side effects caused by the injection of chemotherapy drugs is an important challenge in cancer therapy research[37,38]. Toward this end, alternative treatments using new formulations have been considered. In this regard, the present study designed a pegylated liposome containing doxorubicin

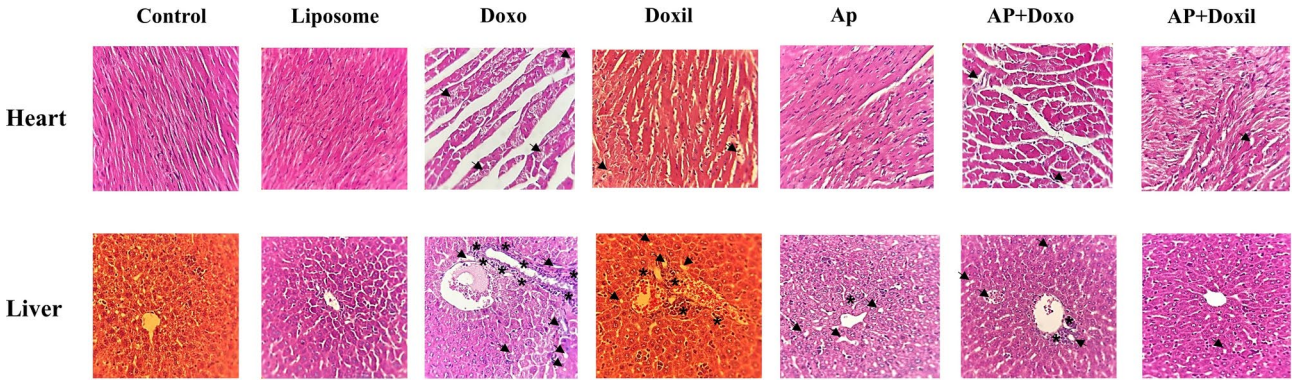

Fig. 6. Histopathological analysis revealed tissue damage in heart and liver across treatment groups. Cardiac findings: myocardial fiber disorganization, edema, myofiber spacing, and cardiomyocyte vacuolization (Black arrows). Liver: hepatocyte degeneration, vacuolization (Black arrows), sinusoidal congestion, and inflammatory cell infiltration (Black Stars). Aprepitant (AP) treatment mitigated adverse effects, showing reduced cardiac disruption, decreased liver inflammation, and improved tissue preservation compared to Doxo/Doxil treatments.

(Doxil) to reduce side effects and increase the efficiency of doxorubicin. In addition, the aprepitant drug, which is approved by the FDA as a drug to reduce diarrhea and vomiting caused by chemotherapy drugs, was studied in combination with Doxil and doxorubicin.

The liposomes designed in this study had desirable characteristics such as suitable size, favorable PDI, and anionic zeta potential. Evidence has shown that nanoliposomes with anionic surface charge are less toxic and more tolerable to cells and tissues. The preparation of nanoliposomes with favorable characteristics plays a key role in stability, efficiency, drug release, and cellular absorption. Several studies have indicated that nanoliposomes with a size of 50–200 nm are effective in studies related to cancer therapy and are absorbed by tumor cells by the enhanced permeability and retention (EPR) effect[39].

A comparison of tumor size and weight changes in mice receiving doxorubicin and Doxil showed that Doxil was more effective in reducing tumor size and weight than doxorubicin. In line with the present study, several researchers have prepared liposomes containing doxorubicin by designing several formulations, which obtained promising results[40–42]. For example, Askarizadeh et al. designed a liposome engineered with 1,2-dioleoylsn-glycero-3-phosphoethanolamine matrix metalloproteinase-2 (MMP-2) cleavable peptide-polyethylene glycol (PEG). They found that this structure has an antitumor and a strong anti-migration effect in colorectal cancer[37]. In addition, Akhtari et al. prepared a liposome containing doxorubicin conjugated with anti-HER2. They reported that the designed liposome has effective anti-tumor activity against HER2+ breast cancer cells and leads to a reduction in cytotoxicity in both HER2+ and HER2− tumors[40]. However, the combination of doxorubicin and Doxil with aprepitant led to an increased antitumor effect and helped to reduce tumor size and weight compared to other groups. Consistent with our results, several studies have mentioned the anti-tumor properties of aprepitant against various tumors[23,26,43]. Similarly, results from a recent paper have confirmed the enhanced antitumor efficacy of the combination therapy of Aprepitant and Doxil on breast cancer[44]. The findings from histopathological studies also demonstrated that doxorubicin and Doxil, as well as their combination with aprepitant treatment, have a pronounced effect on apoptosis in tumor cells.

A comparative analysis of oxidative and antioxidant stress levels in tumor tissues revealed that doxorubicin significantly elevates MDA concentrations within the tumor microenvironment. These findings suggest that doxorubicin may exert antitumor effects through the provocation of oxidative stress and the concomitant reduction of antioxidant defenses. Interestingly, epithelial cells appear to possess intrinsic antioxidant properties and exhibit distinct functionalities within the tumor context. Furthermore, existing literature indicates that doxorubicin induces oxidative stress, which can activate apoptotic factors such as poly (ADP-ribose) polymerase (PARP) in cancer cells[34]. Interestingly, the analysis of oxidative and antioxidant stress levels in cardiac tissue revealed that Doxil and the combination therapy with aprepitant can lead to an increase in antioxidant capacity while simultaneously reducing oxidative stress in the heart tissue. These findings support the notion that employing liposomal formulations of doxorubicin, along with combination therapy with aprepitant, may help mitigate the cardiotoxic effects traditionally associated with doxorubicin treatment. Similarly, promising results were observed in terms of reducing oxidative stress and increasing antioxidants in the combination therapy groups in the liver tissue. It is worth noting that Doxil increased MDA levels in liver tissue, which could be due to the importance of liver in metabolizing liposome drugs[35]. Consistent with our findings, multiple studies have reported that liposomal doxorubicin can reduce cardiotoxicity and protect myocardial tissue. This effect is attributed to the decrease in cardiac enzyme levels and the inhibition of free radical formation, highlighting the potential of liposomal formulations in mitigating the adverse cardiac effects commonly seen with conventional doxorubicin therapy[45–48]. Additionally, several studies have highlighted the antioxidant properties of aprepitant. These properties may contribute to its therapeutic benefits, particularly in mitigating oxidative stress and cellular damage associated with various conditions[49,50]. Histopathological data also showed the reduction of side effects due to Doxil and combination therapy.

While our study provides valuable insights into the therapeutic potential of our self-prepared PEGylated liposomal doxorubicin, it has certain limitations. A direct in vivo comparison with the FDA-approved DOXIL formulation was not performed in this study. Conducting such a comparison would require a separate preclinical investigation under identical experimental conditions to ensure accurate and reliable results. Additionally, only the 4T1 cell line was used in our experiments, which may limit the generalizability of our findings. Future studies should incorporate additional breast cancer cell lines, including both triple-negative and non-triple-negative subtypes, to further validate the efficacy of our formulation. Moreover, further research is needed to comprehensively assess the pharmacokinetics, drug release profiles, and long-term toxicity of our formulation compared to commercial DOXIL. Future investigations should focus on these aspects to strengthen the clinical relevance of our liposomal formulation.

In sum it up, chemotherapy agents selected as first-line treatments for various cancers, including breast cancer, often encounter numerous side effects that necessitate the administration of lower doses. Therefore, it may also affect the effectiveness of these drugs. To address this challenge, researchers have turned to nanotechnology to minimize adverse effects and enhance drug effectiveness. The present study investigates the efficacy of doxorubicin in combating tumor cells while concurrently reducing side effects, particularly cardiotoxicity, through the development of doxorubicin-encapsulated liposomes. Furthermore, the incorporation of aprepitant, known for its effectiveness in alleviating nausea and vomiting, may enhance the antitumor effects of both Doxil and doxorubicin while mitigating side effects. Given these considerations, liposomal doxorubicin presents a promising alternative to conventional doxorubicin, and the concurrent use of aprepitant may represent a favorable addition to chemotherapy regimens. Nevertheless, further investigations in both cellular and clinical settings are essential to thoroughly explore the potential complications and benefits of this combined approach.

Methods and materials

Drug and chemical

Aprepitant and doxorubicin (DOX) were purchased from Molekula (United Kingdom, Product Code: 90028467) and Sigma-Aldrich (USA, Product Code: D5220) companies. 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), and hydrochloric acid (HCl) and MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) powder was purchased from Merck company (Germany). Distearoyl phosphatidylcholine (DSPC), cholesterol, and distearoyl phosphatidylethanolamine-*N*-monomethoxy polyethylene glycol (*M*w 2,000, MPEG2000-DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL).

Preparation liposomal doxorubicin and characterization

First, lipids were mixed (DSPC, cholesterol, and MPEG2000-DSPE) in chloroform, and the solvent was evaporated under vacuum conditions to form a lipid film. This film was subsequently hydrated with citric buffer, and the resulting multi-lamellar vesicles (MLVs) were downsized by freeze–thaw cycles and extrusion to form large unilamellar vesicles (LUVs). The liposomes were then pH-adjusted to 6.5–7.0 using sodium carbonate. Finally, a preheated doxorubicin solution was added to the liposomes and incubated to form the final DOX-loaded liposomes, similar to the commercial drug Doxil[51]. To investigate the characteristics of the prepared liposome, particle size and zeta potential were evaluated using a particle size analyzer (Nano-ZS, Malvern Panalytical, Malvern, UK). The concentration of doxorubicin in the liposome (entrapment efficiency) was calculated using ultraviolet–visible spectrophotometry (UV–Vis). Transmission electron microscopy (TEM) was used to assess vision of the morphology-prepared liposomes.

Animals

The animal management regulations are approved by the Institutional Ethics Committee and Research Advisory Committee of the local laboratory (ethical code: IR.MUMS.AEC.1401.047). All methods were performed in accordance with the relevant guidelines and regulations by Institutional Ethics Committee and Research Advisory Committee of the local laboratory. Besides, the procedure followed the ARRIVE guidelines. Female BALB/c mice, aged 4–6 weeks and weighing an average of 18–22 g, were obtained from the Pasteur Institute in Tehran, Iran. The mice were kept in a housing facility that met local standard requirements.

Antitumor efficacy

We assessed the anti-tumor efficacy of aprepitant and Doxil, as well as doxorubicin, on breast cancer, both separately and in combination, using a 4T1 tumor mouse model established with xenografts derived from a cell line (CDX). Subcutaneously, 1×10^6 4T1 cells in 100 µl of PBS were injected into the right flank of the mice. After two weeks, the tumor size was measured by a digital caliper, and when their volume reached 80 to 110 mm^3, the mice were haphazardly divided into seven groups (n = 5) as follows:

1. The group receiving PBS (control group). 2. The group doxorubicin treated (was treated via the tail vein with a single intravenous dose of 10 mg/kg)[37]. 3. The group receiving aprepitant (0.3 mg/kg intraperitoneally every other day)[52]. 4. The group receiving empty liposomes (was administered via the tail vein a single intravenous dose of 10 mg/kg)[37]. 5. The group the Doxil treated (similar to the doxorubicin treated group). 6. The group combination aprepitant plus doxorubicin (aprepitant 0.3 mg/kg intraperitoneally every other day and doxorubicin 10 mg/kg intravenously as a single dose). 7. The group combined the Doxil with aprepitant (similar to the combination of aprepitant and doxorubicin). After the untreated group reached a tumor volume of 1000 mm^3, mice in different groups were first anesthetized with 10% ketamine (90 mg/kg) and 2% xylazine (9 mg/kg) intraperitoneally and then euthanized by cervical dislocation. The mice's sacrifice was confirmed by cessation of breathing and paleness of the eyes.

Histopathological analysis

Upon completion of the study, the mice were anesthetized in accordance with ethical guidelines for animal research. Subsequently, requisite tissues, including tumors, heart, and liver, were excised for pathological analysis. These tissues were then fixed in 10% formalin and subjected to hematoxylin and eosin staining. The prepared slides were ultimately examined under a light microscope (Olympus Co., China) to assess histological alterations.

Measurement of antioxidant parameters

Measurement of catalase (CAT) activity

The activity of CAT was determined using the Aebi method[53]. Catalase activity is calculated based on the reduction of hydrogen peroxide at a wavelength of 240 nm. For this purpose, 8 µl of tissue homogenate was added with 650 µl of a solution containing 30% hydrogen peroxide and sodium phosphate (PBS) and then read after 1 min with the spectrophotometer, and catalase activity was reported as units per milligram of protein (U/mg protein).

Measurement of superoxide dismutase (SOD) activity

The activity of SOD was measured with the Madesh and Balasubramanian colorimetric method at the wavelength of 570 nm by a plate reader (Biotek Epoch Microplate Spectrophotometer). The basis of this method is that the autoxidation of pyrogallol leads to the production of superoxide anion, and then the reaction with MTT leads to the production of formazan, which measures the inhibitory effect of the SOD enzyme on the MTT reaction. Finally, the enzyme activity was calculated based on international units per milligram of protein (U/mg protein).

Measurement of malondialdehyde (MDA) concentration

To determine the concentration of MDA, 1 ml of tissue homogenate was combined with two mL of solution (TBA + TCA + HCl) and placed in a boiling water bath for 45 min. After it was allowed to cool to room temperature, the mixture was centrifuged for 10 min. The absorbance at 535 nm was then measured, and MDA concentration was calculated using the formula $(C(M) = A/1.56 \times 10^5)$[54].

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 software (San Diego, CA, USA) on the data obtained from the antioxidant parameter test. Results are expressed as mean ± standard deviation (SD) ($n = 3$), and findings were deemed significant when the p-value was less than 0.05 ($p < 0.05$).

Data availability

The data generated in this study are available upon reasonable request from the corresponding author.

Received: 23 December 2024; Accepted: 12 March 2025
Published online: 21 March 2025

References

1. Zhang, Y. N., Xia, K. R., Li, C. Y., Wei, B. L. & Zhang, B. Review of breast cancer pathologigcal image processing. *Biomed. Res. Int.* **2021**, 1994764. https://doi.org/10.1155/2021/1994764 (2021).
2. Katsura, C., Ogunmwonyi, I., Kankam, H. K. N. & Saha, S. Breast cancer: presentation, investigation and management. *Br. J. Hosp. Med.* **83**, 1–7. https://doi.org/10.12968/hmed.2021.0459 (2022).
3. Akram, M., Iqbal, M., Daniyal, M. & Khan, A. U. Awareness and current knowledge of breast cancer. *Biol. Res.* **50**, 33. https://doi.org/10.1186/s40659-017-0140-9 (2017).
4. *World Health Organization. Breast cancer*. https://www.who.int/news-room/fact-sheets/detail/breast-cancer (2021).
5. Hong, R. & Xu, B. Breast cancer: an up-to-date review and future perspectives. *Cancer Commun. (Lond)* **42**, 913–936. https://doi.org/10.1002/cac2.12358 (2022).
6. Hutchinson, L. Challenges, controversies, breakthroughs. *Nat. Rev. Clin. Oncol.* **7**, 669–670. https://doi.org/10.1038/nrclinonc.2010.192 (2010).
7. Alghorabi, A., Kabel, A. & Abdelmaboud, M. Doxorubicin: Insights into dynamics, clinical uses and adverse effects. *J. Cancer Res. Treat.* **7**, 17–20. https://doi.org/10.12691/jcrt-7-1-3 (2019).
8. Franco, Y. L., Vaidya, T. R. & Ait-Oudhia, S. Anticancer and cardio-protective effects of liposomal doxorubicin in the treatment of breast cancer. *Breast Cancer (Dove Med Press)* **10**, 131–141. https://doi.org/10.2147/bctt.S170239 (2018).
9. Wu, G. S. et al. Synergistic anti-cancer activity of the combination of dihydroartemisinin and doxorubicin in breast cancer cells. *Pharmacol. Rep.* **65**, 453–459. https://doi.org/10.1016/s1734-1140(13)71021-1 (2013).
10. Liang, Y. et al. HSPB1 facilitates chemoresistance through inhibiting ferroptotic cancer cell death and regulating NF-κB signaling pathway in breast cancer. *Cell Death Dis.* **14**, 434. https://doi.org/10.1038/s41419-023-05972-0 (2023).
11. Zeng, W. et al. Advances in doxorubicin-based nano-drug delivery system in triple negative breast cancer. *Front. Bioeng. Biotechnol.* **11**, 1271420. https://doi.org/10.3389/fbioe.2023.1271420 (2023).
12. Gabizon, A., Shmeeda, H. & Barenholz, Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin. Pharmacokinet.* **42**, 419–436. https://doi.org/10.2165/00003088-200342050-00002 (2003).
13. O'Brien, M. E. et al. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann. Oncol.* **15**, 440–449. https://doi.org/10.1093/annonc/mdh097 (2004).
14. Barenholz, Y. Doxil®—The first FDA-approved nano-drug: Lessons learned. *J. Control. Release* **160**, 117–134. https://doi.org/10.1016/j.jconrel.2012.03.020 (2012).
15. Gabizon, A. A., Patil, Y. & La-Beck, N. M. New insights and evolving role of pegylated liposomal doxorubicin in cancer therapy. *Drug Resist. Updates* **29**, 90–106. https://doi.org/10.1016/j.drup.2016.10.003 (2016).
16. Füredi, A. et al. Pegylated liposomal formulation of doxorubicin overcomes drug resistance in a genetically engineered mouse model of breast cancer. *J. Control. Release* **261**, 287–296. https://doi.org/10.1016/j.jconrel.2017.07.010 (2017).

17. Hu, W. et al. Pegylated liposomal doxorubicin versus epirubicin as adjuvant therapy for stage I–III breast cancer. *Front. Genet.* **12**, 746114. https://doi.org/10.3389/fgene.2021.746114 (2021).
18. Alhaja, M., Chen, S., Chin, A. C., Schulte, B. & Legasto, C. S. Cardiac safety of pegylated liposomal doxorubicin after conventional doxorubicin exposure in patients with sarcoma and breast cancer. *Cureus* **15**, e44837. https://doi.org/10.7759/cureus.44837 (2023).
19. Huober, J. et al. A multicentric observational trial of pegylated liposomal doxorubicin for metastatic breast cancer. *BMC Cancer* **10**, 2. https://doi.org/10.1186/1471-2407-10-2 (2010).
20. Muñoz, M., Rosso, M. & Coveñas, R. Triple negative breast cancer: how neurokinin-1 receptor antagonists could be used as a new therapeutic approach. *Mini Rev. Med. Chem.* **20**, 408–417. https://doi.org/10.2174/1389557519666191112152642 (2020).
21. Davoodian, M. et al. Evaluation of serum level of substance P and tissue distribution of NK-1 receptor in breast cancer. *Mol. Biol. Rep.* **46**, 1285–1293. https://doi.org/10.1007/s11033-019-04599-9 (2019).
22. Robinson, P. et al. Substance P receptor signaling mediates doxorubicin-induced cardiomyocyte apoptosis and triple-negative breast cancer chemoresistance. *Biomed. Res. Int.* **2016**, 1959270. https://doi.org/10.1155/2016/1959270 (2016).
23. Ghahremanloo, A., Javid, H., Afshari, A. R. & Hashemy, S. I. Investigation of the role of neurokinin-1 receptor inhibition using aprepitant in the apoptotic cell death through PI3K/Akt/NF-κB signal transduction pathways in colon cancer cells. *Biomed. Res. Int.* **2021**, 1383878. https://doi.org/10.1155/2021/1383878 (2021).
24. Mozafari, M., Ebrahimi, S., Darban, R. A. & Hashemy, S. I. Potential in vitro therapeutic effects of targeting SP/NK1R system in cervical cancer. *Mol. Biol. Rep.* **49**, 1067–1076. https://doi.org/10.1007/s11033-021-06928-3 (2022).
25. Legi, A., Rodriguez, E., Eckols, T. K., Mistry, C. & Robinson, P. Substance P antagonism prevents chemotherapy-induced cardiotoxicity. *Cancers (Basel)* https://doi.org/10.3390/cancers13071732 (2021).
26. Ebrahimi, S. et al. The in vitro pro-inflammatory functions of the SP/NK1R system in prostate cancer: a focus on nuclear factor-kappa B (NF-κB) and its pro-inflammatory target genes. *Appl. Biochem. Biotechnol.* **195**, 7796–7807. https://doi.org/10.1007/s12010-023-04495-w (2023).
27. Cao, X. et al. Aprepitant inhibits the development and metastasis of gallbladder cancer via ROS and MAPK activation. *BMC Cancer* **23**, 471. https://doi.org/10.1186/s12885-023-10954-8 (2023).
28. Aapro, M. et al. Aprepitant and fosaprepitant: a 10-year review of efficacy and safety. *Oncologist* **20**, 450–458. https://doi.org/10.1634/theoncologist.2014-0229 (2015).
29. Momen Razmgah, M. et al. The effect of substance P and its specific antagonist (aprepitant) on the expression of MMP-2, MMP-9, VEGF, and VEGFR in ovarian cancer cells. *Mol. Biol. Rep.* **49**, 9307–9314. https://doi.org/10.1007/s11033-022-07771-w (2022).
30. Gomaa, A. A., Abdallah, D. M., El-Abhar, H. S. & El-Mokadem, B. M. Repurposing Aprepitant: Can it protect against doxorubicin-induced Chemobrain beyond its antiemetic role?. *Life Sci.* **359**, 123210. https://doi.org/10.1016/j.lfs.2024.123210 (2024).
31. Berger, M. et al. Hepatoblastoma cells express truncated neurokinin-1 receptor and can be growth inhibited by aprepitant in vitro and in vivo. *J. Hepatol.* **60**, 985–994. https://doi.org/10.1016/j.jhep.2013.12.024 (2014).
32. Alavizadeh, S. H. et al. Therapeutic efficacy of cisplatin thermosensitive liposomes upon mild hyperthermia in C26 tumor bearing BALB/c mice. *Mol. Pharm.* **14**, 712–721. https://doi.org/10.1021/acs.molpharmaceut.6b01006 (2017).
33. Gheybi, F. et al. Liposomal silymarin anti-oxidative and anti-apoptotic features in lung cells: An implication in cadmium toxicity. *J. Trace Elem. Med. Biol.* **80**, 127291. https://doi.org/10.1016/j.jtemb.2023.127291 (2023).
34. Kong, C. Y. et al. Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death. *Int. J. Biol. Sci.* **18**, 760–770. https://doi.org/10.7150/ijbs.65258 (2022).
35. Hilmer, S. N., Cogger, V. C., Muller, M. & Le Couteur, D. G. The hepatic pharmacokinetics of doxorubicin and liposomal doxorubicin. *Drug Metab. Dispos.* **32**, 794–799. https://doi.org/10.1124/dmd.32.8.794 (2004).
36. Ferlay, J. et al. Cancer statistics for the year 2020: An overview. *Int. J. Cancer* https://doi.org/10.1002/ijc.33588 (2021).
37. Askarizadeh, A. et al. Doxorubicin-loaded liposomes surface engineered with the matrix metalloproteinase-2 cleavable polyethylene glycol conjugate for cancer therapy. *Cancer Nanotechnol.* **14**, 18. https://doi.org/10.1186/s12645-023-00169-8 (2023).
38. Doagooyan, M. et al. Anti-tumor activity of silymarin nanoliposomes in combination with iron: In vitro and in vivo study. *Int. J. Pharm. X* **6**, 100214. https://doi.org/10.1016/j.ijpx.2023.100214 (2023).
39. Ray, S. et al. Magnetic heating stimulated cargo release with dose control using multifunctional MR and thermosensitive liposome. *Nanotheranostics* **3**, 166–178. https://doi.org/10.7150/ntno.31164 (2019).
40. Akhtari, J. et al. Targeting, bio distributive and tumor growth inhibiting characterization of anti-HER2 affibody coupling to liposomal doxorubicin using BALB/c mice bearing TUBO tumors. *Int. J. Pharm.* **505**, 89–95. https://doi.org/10.1016/j.ijpharm.2016.03.060 (2016).
41. Haftcheshmeh, S. M. et al. Liposomal doxorubicin targeting mitochondria: A novel formulation to enhance anti-tumor effects of Doxil® in vitro and in vivo. *J. Drug Deliv. Sci. Technol.* **62**, 102351. https://doi.org/10.1016/j.jddst.2021.102351 (2021).
42. Zahmatkeshan, M., Gheybi, F., Rezayat, S. M. & Jaafari, M. R. Improved drug delivery and therapeutic efficacy of PEgylated liposomal doxorubicin by targeting anti-HER2 peptide in murine breast tumor model. *Eur. J. Pharm. Sci.* **86**, 125–135. https://doi.org/10.1016/j.ejps.2016.03.009 (2016).
43. Ebrahimi, S. et al. The in vitro anti-cancer synergy of neurokinin-1 receptor antagonist, aprepitant, and 5-aminolevulinic acid in glioblastoma. *BioFactors (Oxford, England)* **49**, 900–911. https://doi.org/10.1002/biof.1953 (2023).
44. Ghahremanloo, A. et al. Synergistic anticancer effects of liposomal doxorubicin and aprepitant combination therapy in breast cancer: Preclinical insights and therapeutic potential. *J. Drug Deliv. Sci. Technol.* **105**, 106620 (2025).
45. Dorostkar, H. et al. Reduction of doxorubicin-induced cardiotoxicity by co-administration of smart liposomal doxorubicin and free quercetin: In vitro and in vivo studies. *Pharmaceutics* https://doi.org/10.3390/pharmaceutics15071920 (2023).
46. Paliwal, S. R. et al. Estrogen-anchored pH-sensitive liposomes as nanomodule designed for site-specific delivery of doxorubicin in breast cancer therapy. *Mol. Pharm.* **9**, 176–186. https://doi.org/10.1021/mp200439z (2012).
47. Xing, M., Yan, F., Yu, S. & Shen, P. Efficacy and cardiotoxicity of liposomal doxorubicin-based chemotherapy in advanced breast cancer: a meta-analysis of ten randomized controlled trials. *PLoS One* **10**, e0133569. https://doi.org/10.1371/journal.pone.0133569 (2015).
48. Stark, C., Taimen, P., Savunen, T. & Koskenvuo, J. Pegylated and liposomal doxorubicin is associated with high mortality and causes limited cardiotoxicity in mice. *BMC Res. Notes* **11**, 148. https://doi.org/10.1186/s13104-018-3260-6 (2018).
49. Zarei Shandiz, S., Assaran Darban, R., Javid, H., Ghahremanloo, A. & Hashemy, S. I. The effect of SP/NK1R on expression and activity of glutaredoxin and thioredoxin proteins in prostate cancer cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **397**, 5875–5882. https://doi.org/10.1007/s00210-024-02996-x (2024).
50. Javid, H., Ghahremanloo, A., Afshari, A. R., Salek, R. & Hashemy, S. I. The emerging role of neurokinin-1 receptor blockade using aprepitant in the redox system of esophageal squamous cell carcinoma. *Int. J. Peptide Res. Ther.* **28**, 89. https://doi.org/10.1007/s10989-022-10399-w (2022).
51. Niu, G., Cogburn, B. & Hughes, J. In *Cancer Nanotechnology: Methods and Protocols* (eds Grobmyer, S. R. & Moudgil, B. M.) 211–219 (Humana Press, 2010).
52. Ebrahimi, S., Mirzavi, F., Aghaee-Bakhtiari, S. H. & Hashemy, S. I. SP/NK1R system regulates carcinogenesis in prostate cancer: Shedding light on the antitumoral function of aprepitant. *Biochim. Biophys. Acta (BBA) Mol. Cell Res.* **1869**, 119221. https://doi.org/10.1016/j.bbamcr.2022.119221 (2022).
53. Aebi, H. Catalase in vitro. *Methods Enzymol.* **105**, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3 (1984).

54. Asgharzadeh, F. et al. Thymoquinone prevents myocardial and perivascular fibrosis induced by chronic lipopolysaccharide exposure in male rats: Thymoquinone and cardiac fibrosis. *J. Pharmacopuncture* **21**, 284–293. https://doi.org/10.3831/kpi.2018.21.032 (2018).

Author contributions

A. G: Conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. B.E,formal analysis, investigation, methodology, writing—original draft, writing—review and editing. F. A, Formal analysis, investigation, methodology. S.M, investigation, methodology. F. G, methodology, investigation, supervision. S.I. H: Conceptualization, resources, supervision, funding acquisition.

Funding

This research was funded by the Mashhad University of Medical Sciences (Grant number: 4021510).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94291-9.

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