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Dichloroacetate nanoparticles ameliorate doxorubicin's cardiotoxicity and inflammatory effect in Ehrlich ascites carcinoma

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ABSTRACT

Objective: The objective of our study is to mitigate doxorubicin-induced toxicity by combining it with dichloroacetate nanoparticles (DCA-PNPs) in Ehrlich ascites carcinoma (EAC) mice model. ***Material & Methods:*** Seventy female CD1 mice were split into ten groups (n=7). (Gp1) negative control. Groups 2 to 4 were administered intraperitoneal (*i.p.*) with DCA (50 mg/kg), DCA-PNPs (50 mg/kg), and Dox (0.2 mg/kg) for 2 weeks. From Gp5 to Gp10, mice were injected *i.p.* with EAC cells at a concentration of 0.5×10^6 cells/mouse. GP6 to GP10 were treated with Dox, DCA, DCA-PNPs, Dox/DCA, and Dox/DCA-PNPs. On day 14, biochemical parameters were evaluated. ***Results:*** The results demonstrated that the combination of Dox/DCA-PNPs exhibited reduction in cardiotoxicity and restoration of mammary gland inflammation compared to Dox alone treated group. That evidenced by improved cardiac functions tests as troponin I, T levels reduction (-91.81, -92.15 %) and decreased inflammation as CRP concentration reduced by (-69.2 %). Furthermore, decreased apoptosis of normal cardiac and mammary gland cells into (3.5 and 0.5 %), respectively. ***Conclusions***: These findings suggest that combination of Dox/DCA-PNPs is a promising strategy for improving the therapeutic efficacy and mitigating the cardiotoxic and inflammatory side effects of Dox in BC treatment.

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Introduction:

Breast cancer (BC) represents a major health issue affecting individuals and healthcare systems worldwide [1]. Ehrlich ascites carcinoma (EAC) serves as a breast cancer model sharing similarities with human breast cancer [2]. Research advances have improved treatments that enhance patient outcomes [3]. Chemotherapy remains crucial in BC management, with doxorubicin (Dox) widely used [4]. Dox works by intercalating with DNA, inhibiting topoisomerase II, and producing free radicals to kill cancer cells [5]. However, its clinical use is limited by dose-dependent cardiotoxicity, causing heart failure [6]. Additionally, Dox-induced inflammation impact cancer progression and treatment [7]. BC research must devise strategies for reducing cardiotoxicity while retaining Dox's anti-cancer effects [4]. One approach combines Dox with agents that lessen toxicity while maintaining efficacy [8].

Dichloroacetate (DCA) has emerged as a promising compound targeting cancer cell metabolism by inhibiting pyruvate dehydrogenase kinase [9]. DCA acts as a metabolic regulator, boosting treatment effectiveness and reducing Dox cardiotoxicity and inflammation [10]. Nanoparticle-based delivery systems enhance drug solubility and retention in tumor tissues, improving outcomes while minimizing systemic toxicity [11]. These nanoparticles increase drug concentration in tumors while protecting healthy organs [12]. The hypothesis of the current study was to explore anti-cancer impact of the Dox/DCA-PNPs combinatorial treatment against EAC cells, with minimizing tissue toxicity, inflammation in heart and mammary gland tissues.

Materials and Methods:

Chemicals and drugs

Sodium dichloroacetate (DCA) (≥98%; Cat. no. 347795), and Doxorubicin hydrochloride (Dox) (98.0-102.0% HPLC, Mw 579.98, Cat. no. D1515) were purchased from Sigma-Aldrich, (Germany). Troponin I, T, and C-reactive protein ELISA Kits (cTnI, cTnT, and CRP) (Cat. no. MBS2708301, MBS726068, and MBS564066), were purchased from MyBioSource, (San Diego, CA, USA). L-malondialdehyde (MDA) (Cat. no. MD 25 29), Nitric oxide (NO) (Cat. no. 25 33), catalase (CAT) (Cat. no. CA 25 17), and glutathione (GSH) (Cat. no. R 2511) were purchased from Bio Diagnostic company, (Egypt). All other chemicals used were high grades.

In vivo studies

Experimental animals

Seventy female CD1 mice (21 ± 0.5 g) were purchased from Alexandria University's animal facility, Egypt. The mice were acclimated for a week at 23-25ºC, 53 ± 4% humidity, and a 12-hour light/dark cycle, with free access to water and standard feed. The research was conducted by Tanta University's Faculty of Science, following protocol no. IACUC-SCI-TU-0210.

Implantation for tumor cells

EAC cells were given from the National Institute of Cancer at Cairo University, Egypt, and then suspended in sterile PBS [13]. The viability of the cells was assessed using the trypan blue exclusion method [14]. The cell concentration was 0.5×10^6 cells/mouse for intraperitoneal (*i.p.*) injection [15].

Experimental design

Mice were (10 groups, 7 mice/each). (Gp1) acted as normal control. (Gp2-Gp4) control groups received *i.p.* DCA (50 mg/kg/day), DCA-PNPs (50 mg/kg/day) [16], and Dox (20 mg/kg) thrice weekly [17]. Groups 5-10 were inoculated with EAC cells (0.5×10^6/mouse). Groups 6-10 received *i.p.* treatment of Dox, DCA, DCA-PNPs, Dox/DCA, and Dox/DCA-PNPs. Treatment lasted 14 days.

Sampling

Upon the experiment end, mice were slaughtered per each group with sodium barbiturate (300 mg/kg) according to [18], and serum samples were collected for biochemical analysis, then stored at −20 °C. Heart and mammary gland tissue sections were also frozen at −20 °C to evaluate

antioxidant, oxidative stress parameters, and apoptotic profile.

Homogenate preparation

Heart and mammary gland samples were cut, weighed, and finely chopped. Using a glass homogenizer, 10 percent homogenates were prepared by mixing the tissue with nine times its volume of chilled 0.05 mM potassium phosphate buffer (pH 7.4). The homogenates were then centrifuged at 6000 r.p.m. for 15 minutes at 4°C [19].

Biochemical analyses

Serum Troponin I/T levels were determined using an ELISA kit (cTnI and cTnT), with standards (0–1000 pg/mL) used to create a standard curve, as shown by [20]. CRP was quantified using a sandwich ELISA kit following manufacturer's instructions, as shown by [21]. Cardiac and mammary gland levels of MDA, NO, and GSH were measured using methods described by Li and [22], [23], and [24]. CAT activity was evaluated according to [25].

Apoptotic profile

By propidium iodide (PI) and annexin V, percentage of apoptosis were determined. This involves fixing heart and mammary glands cells with ice-cold ethanol, washing with PBS, and staining with PI in darkness. Stained cells were examined with a flow cytometer, utilising forward and side scatter measurements to identify individual cells [26].

Statistical analysis

The results were expressed as mean ± SE. All statistics were conducted using GraphPad Prism version 6. Two-way analyses of variance (ANOVA) were utilized to determine statistical significance. Group comparisons were made to highlight significant effects of the treatment conditions, where p-value of ≤ 0.05 was deemed statistically significant.

Result :

DCA-PNPs restore heart oxidative stress and increased antioxidants

Compared to normal control (Gp1), there was no significant change in MDA and NO levels in Ctrl/DCA (Gp2) and Ctrl/DCA-PNPs (Gp3). However, Ctrl/Dox (Gp4) showed a significant increase in MDA and NO levels by +17.5, and +30 %, respectively (p<0.0001). Untreated EAC (Gp5) showed a significant increase in MDA and NO levels by +136%, and +148 %, (p<0.0001). Compared to untreated EAC (Gp5), there was significant reduction in MDA and NO levels in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10), that EAC/Dox/DCA-PNPs (Gp10) was the most effected group, decreasing by -65 % for MDA, and -66 % for NO (p<0.0001).

Compared to normal control (Gp1), there was a significant reduction in Ctrl/DCA (Gp2), Ctrl/DCA-PNPs (Gp3), and Ctrl/Dox (Gp4) in GSH level by -12, -11, and -20 %, (p<0.0001). There was a significant reduction in Gp2, Gp3, and Gp4 in CAT activity by -9, -9, and -41 %, (p<0.0001). Untreated EAC (Gp5) showed a significant drop-in CAT activity and GSH level by -66 and -65%, (p<0.0001). Compared to untreated EAC (Gp5), there was a significant increase in CAT activity and GSH level in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10) (p<0.0001), where EAC/Dox/DCA-PNPs (Gp10) was the most remarkable group, increasing by +185% for CAT and +179% for GSH (Table 1).

DCA-PNPs restore mammary gland oxidative stress and increase antioxidants

Compared to normal control (Gp1), there was no significant change in MDA and NO levels in Ctrl/DCA (Gp2) and Ctrl/DCA-PNPs (Gp3). However, Ctrl/Dox (Gp4) showed a significant increase in MDA and NO levels by +242, and +41 %, respectively (p<0.0001). Untreated EAC (Gp5) showed a significant increase in MDA and NO levels by +314%, and +126 %, (p<0.0001). Compared to untreated EAC (Gp5), there was significant reduction in MDA and NO levels in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10), that EAC/Dox/DCA-PNPs (Gp10) was the most effected group, decreasing by -76 % for MDA, and -53 % for NO (p<0.0001).

Compared to normal control (Gp1), there was a significant reduction in Ctrl/DCA (Gp2) and Ctrl/DCA-PNPs (Gp3) in GSH level and CAT activity. However, Ctrl/Dox (Gp4) showed a significant reduction in their levels by -59 and -61 %, respectively for GSH and CAT, (p<0.0001). Untreated EAC (Gp5) showed a significant drop-in CAT activity and GSH level by -65 and -63%, (p<0.0001). Compared to untreated EAC (Gp5), there was a significant increase in CAT activity and GSH level in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10) (p<0.0001), where EAC/Dox/DCA-PNPs (Gp10) showed more significance, increasing by +190% for CAT and +170% for GSH (Table 2).

DCA-PNPs restore cardiac troponins activities

Compared to normal control (Gp1), there was no significant change in both troponin I and T levels in Ctrl/DCA (Gp2) and Ctrl/DCA-PNPs (Gp3). However, Ctrl/Dox (Gp4) showed a significant increase in their levels by 300 and 350 %, respectively for troponin I and T (p<0.0001), which indicated Dox induced cardiotoxicity. Untreated EAC (Gp5) showed significant increase in troponin I and T levels represented as 110 ± 0.154 and 102 ± 0236 pg/mL (p<0.0001) in compared to normal control (Gp1). Compared to untreated EAC (Gp5), significant reductions in troponin I level were observed in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10) by -68.18, -81.81, -86.36, -90.9, -91.81 %, (p<0.0001). That the most effected group was EAC/Dox/DCA-PNPs (Gp10), represented as 9.2 ± 0.762 pg/mL. Furthermore, there was significant reductions in troponin T level were observed in EAC/Dox (Gp6) to EAC/Dox/DCA-PNPs (Gp10) by -60.78, -72.54, -82.35, -85.29, -92.15 %, respectively (p<0.0001). That the most effected group was EAC/Dox/DCA-PNPs (Gp10), represented as 8.42 ± 0.485 pg/mL, which indicated normal heart function (Fig. 1).

DCA-PNPs minimize CRP level

Compared to normal control (Gp1), there was no significant change in CRP level in Ctrl/DCA (Gp2) and Ctrl/DCA-PNPs (Gp3). Ctrl/Dox (Gp4) showed a significant increase in its level by 80 %, (p<0.0001), which indicated Dox induced high inflammation rate. Untreated EAC (Gp5) showed significant increase in CRP level represented as 150 ± 0.154 pg/mL (p<0.0001) in compared to normal control (Gp1). Compared to untreated EAC (Gp5), significant rise in CRP level was observed in EAC/Dox (Gp6) by 16 %, (p<0.0001), which indicate inflammation occurred. While there was a significant reduction in CRP level were observed in EAC/DCA (Gp7) to EAC/Dox/DCA-PNPs (Gp10) by -53.3, -57.3, -60, -62 %, (p<0.0001). That the most effected group was EAC/Dox/DCA-PNPs (Gp10), represented as 57 ± 0.762 pg/mL, which indicated restoration into normal functions without inflammations (Fig. 2).

DCA-PNPs restrict apoptotic profile in cardiac and mammary gland tissues

As compared to Untreated EAC (Gp5), chart revealed that EAC/Dox (Gp6) had significantly decreased (p < 0.0001) in heart viable cells as 25.9 % and significantly increased (p < 0.0001) in number of cells undergoing apoptosis by annexin V stain and PI as 73.6 %, which indicated Dox cardiotoxicity. While from EAC/DCA (Gp7) to EAC/Dox/DCA-PNPs (Gp10) had significantly increased (p < 0.0001) in the percentage of viable cells as 66.2, 72.5, 85.6, 95.3 %, and significantly decreased in apoptotic cells as 31.2, 20.3, 13.4, and 3.5 %, as compared to untreated EAC (Gp5). According to that, EAC/Dox/DCA-PNPs (Gp10) was the most effected group, demonstrating a synergistic effect in reduced cell death by DCA-PNPs combination (Fig. 3A; 4A).

As compared to untreated EAC (Gp5), the chart revealed that EAC/Dox (Gp6) had significantly decreased (p < 0.0001) in mammary gland viable cells as 23.5 % and significantly increased (p < 0.0001) in number of cells undergoing apoptosis by annexin V stain and PI as 75.4 %, which indicated high Dox toxicity for mammary

gland tissue. While from EAC/DCA (Gp7) to EAC/Dox/DCA-PNPs (Gp10) had significantly increased ($p < 0.0001$) in the percentage of viable cells as 70.9, 97.4, 95.5, 99.2 %, and significantly decreased in apoptotic cells as 27.5, 1.9, 2.6, and 0.5 %, as compared to untreated EAC (Gp5). According to that, EAC/Dox/DCA-PNPs (Gp10) was the most improved group by Dox/DCA-PNPs combination (Fig. 3B; 4B).

Discussion:

Breast cancer is a major global health issue, with research advances improving treatments and their outcomes [1]. Dox remains crucial in BC management but is limited by dose-dependent cardiotoxicity and inflammation [4]. Combining Dox with agents like metabolic modulator may enhance its effectiveness and reduce its toxicity [8]. The current study investigated the synergetic effect of DCA-PNPs with Dox on oxidative stress and antioxidant markers in heart and mammary tissues, focusing on how DCA-PNPs mitigate Dox-cardiotoxicity and inflammation risk.

Dox/DCA-PNPs treatment reduced oxidative stress markers and increased antioxidants in EAC-bearing mice compared to conventional chemotherapy. The study suggests DCA-PNPs may enhance Dox efficiency in reducing tumor development while targeting cancer cells, preserving normal cells, enhancing absorption, and improving outcomes. Which corresponds with [27] and [28].

That in present study, Dox increased oxidative stress in heart and mammary tissues, showing high MDA and NO levels and ROS production. EAC-bearing exhibited increased MDA and NO levels, indicating oxidative stress from tumor-induced dysregulation, that align with [29]. Dox administration reduced MDA and NO levels, indicating tumor inhibition, while producing ROS in controls. DCA and DCA-PNPs reduce MDA/NO by modulating cancer cell metabolism from glycolysis to oxidative phosphorylation, as DCA increases mitochondrial ROS causing apoptosis. This aligned with previous studies [30] and [31]. DCA-PNPs provide enhanced bioavailability and targeting, increasing drug concentrations while preserving normal tissues [32]. The combined treatment reduces oxidative stress as: Dox intercalates genetic information [33], while DCA affects cancer metabolism [30]. The nanoparticles scavenge free radicals and enhance antioxidant defense through improved mitochondrial function [34].

Antioxidant parameters preserve redox equilibrium and safeguard against oxidative damage [35]. In this study the normal group administered Dox resulted in substantial decreases in antioxidant levels in both heart and mammary gland, especially CAT and GSH levels, indicating potential damage to normal cells which aligns with [36]. DCA-PNPs exhibited no alterations in antioxidant levels within the control group of mammary glands and a tiny decrease in heart tissue. In EAC-bearing mice, levels of CAT and GSH were markedly diminished, indicating that cancer diminishes oxidative stress resistance [37]. In our study the EAC groups administered Dox, DCA, and DCA-PNPs exhibited small, moderate, and significant enhancements in both heart and mammary gland antioxidant levels, respectively. These correspond with research demonstrating DCA's capacity to augment antioxidant enzyme activity [38]. The elevated antioxidant defences in the both tissues of treated mice, particularly with EAC/Dox/DCA-PNPs, suggest that the combination therapy augmenting endogenous antioxidant capacity.

Troponin I and T serve as highly specific markers for myocardial damage [39]. EAC cells have been shown to cause significant cardiotoxicity, as noted by [40]. Dox is known to induce cardiotoxicity, this adverse effect can manifest as acute or chronic cardiac dysfunction, potentially leading to heart failure and also able to generate ROS and interfere with mitochondrial function in cardiomyocytes [36]. Study results indicated a significant increase in troponins levels in normal group with Dox, which ensure its

cardiotoxicity in accordance with [41]. Moreover, a significant decrease in troponins level in all treated groups by comparing them to EAC-bearing mice troponins levels, especially group treated with Dox/DCA-PNPs combination. Which suggests that DCA-PNPs had effectively reduced Dox and EAC toxic effects that cause cardiac damage, that consistent with previous study [42].

CRP is an acute-phase protein produced by tissues into blood in response to inflammation or infection in the body [43]. Elevated CRP levels in the blood indicate the presence of systemic inflammation, which can be associated with various conditions such as cardiovascular disease [44]. Our results showed that control and EAC-bearing mice groups administrated Dox had significant increase in CRP level, which indicates high Dox-inflammatory response in normal, and cancer cells, demonstrates its side effects which supported by recent studies [7] and [45]. While DCA and DCA-PNPs in control groups had normal CRP levels, indicates how safer than Dox. In all treated EAC groups showed restoration of CRP normal levels, especially in Dox/DCA-PNPs groups indicated that DCA-PNPs decreased systemic inflammation.

Apoptosis, or programmed cell death, is crucial for cellular function and homeostasis [46]. Cancer treatments, particularly Dox, induce apoptosis in cancerous and normal cells [47]. Dox-induced cardiotoxicity affects cardiomyocytes through ROS production, impaired metabolism, cytochrome c release, and p53-dependent pathways, activating caspase-3 and causing cell death [48]. In breast cancer treatment, Dox-induced apoptosis targets cells as DNA breaks activate ATM/ATR signalling, upregulate pro-apoptotic genes (e.g., Bax, PUMA), and activate caspases, leading to apoptotic death [49]. In our study EAC-bearing mice treated with DCA, DCA-PNPs, Dox/DCA, and Dox/DCA-PNPs showed increased live cells and decreased apoptotic cells in cardiac and mammary tissues, indicating effective cancer targeting. The synergistic effect aligns with research showing DCA's ability to sensitize cancer cells to chemotherapy [8]. Treatment with EAC/Dox/DCA-PNPs demonstrated potent apoptosis induction while minimizing normal cell death compared to Dox alone, which increased normal cell death in both tissues. This suggests DCA-PNPs may mitigate Dox-induced normal cell death and target only cancerous cells.

Conclusion:

The combination of Dox with DCA-PNPs shows potential amelioration of cardiotoxicity and inflammation while enhancing anti-cancer efficacy in breast cancer treatment. This study provides a foundation for optimizing combinatorial therapies to improve patient outcomes, moving toward more effective and safer therapeutic strategies.

Limitations and future prospective:

The EAC model may not fully replicate breast cancer complexity. DCA-PNPs and Dox require long-term investigation and dosage optimization for clinical use. Future research may translate findings to trials, evaluating Dox/DCA-PNPs' safety and efficacy in breast cancer patients for treatment protocols.

Abbreviations list:

BC: Breast Cancer; CAT: Catalase; CTRL: Control; CRP: C-reactive protein; DCA: Dichloroacetate; NPs: Nanoparticles; Dox: Doxorubicin; EAC: Ehrlich Ascites Carcinoma; GSH: Glutathione; i.p: Intraperitoneal; MDA: Malondialdehyde; NO: Nitric Oxide; NPs: Nanoparticles; PBS: Phosphate Buffer Saline; and PI: Propidium iodide.

Declarations:

Ethics authorisation and approval for participation: The experiments were conducted following national ethical guidelines for laboratory animal care and the Animal Ethical Committee, Faculty of

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Data and material availability: The data are contained inside the paper.

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Contributions of the authors:

Methodology, resources, writing & editing – original draft **[Amira T. Khattab]**

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Conceptualization, supervision, review & editing **[Mai M. El-Keey]**

Conceptualization, Methodology, supervision, review & editing **[Maha M. Salem]**

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REFRENCE:

[1] Barrios CH. Global challenges in breast cancer detection and treatment. The Breast. 2022 Mar 1;62:S3-6.

[2] Barrios CH. Global challenges in breast cancer detection and treatment. The Breast. 2022 Mar 1;62:S3-6.

[3] Gowda NG, Shiragannavar VD, Prabhuswamimath SC, Tuladhar S, Chidambaram SB, Santhekadur PK. Ehrlich Ascites carcinoma mice model for studying liver inflammation and fibrosis. Advances in Cancer Biology-Metastasis. 2022 Jul 1;4:100029.

[4] Noser, A.A., El-Barbary, A.A., Salem, M.M., El Salam, H.A.A. and Shahien, M. Synthesis and molecular docking simulations of novel azepines based on quinazolinone moiety as prospective antimicrobial and antitumor hedgehog signaling inhibitors. Scientific Reports. 2024; Feb; 14(1):3530.

[5] Bisht A, Avinash D, Sahu KK, Patel P, Das Gupta G, Kurmi BD. A comprehensive review on doxorubicin: mechanisms, toxicity, clinical trials, combination therapies and nanoformulations in breast cancer. Drug Delivery and Translational Research. 2025 Jan;15(1):102-33.

[6] Kciuk M, Gielecińska A, Mujwar S, Kołat D, Kałuzińska-Kołat Ż, Celik I, Kontek R. Doxorubicin—an agent with multiple mechanisms of anticancer activity. Cells. 2023 Feb 19;12(4):659.

[7] Goje ID, Goje GI, Ordodi VL, Ciobotaru VG, Ivan VS, Buzaș R, Tunea O, Bojin F, Lighezan DF. Doxorubicin-induced cardiotoxicity and the emerging role of SGLT2 inhibitors: from glycemic control to cardio-oncology. Pharmaceuticals. 2025 May 3;18(5):681.

[8] Shi S, Chen Y, Luo Z, Nie G, Dai Y. Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. Cell communication and signaling. 2023 Mar 14;21(1):61.

[9] Shi S, Chen Y, Luo Z, Nie G, Dai Y. Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. Cell communication and signaling. 2023 Mar 14;21(1):61.

[10] Koltai T, Fliegel L. Dichloroacetate for cancer treatment: some facts and many doubts. Pharmaceuticals. 2024 Jun 6;17(6):744.

[11] Wang X, Wang L, Hao Q, Cai M, Wang X, An W. Harnessing glucose metabolism with nanomedicine for cancer treatment. Theranostics. 2024 Oct 17;14(17):6831.

[12] Yusuf A, Almotairy AR, Henidi H, Alshehri OY, Aldughaim MS. Nanoparticles as drug delivery systems: a review of the implication of nanoparticles' physicochemical properties on responses in biological systems. Polymers. 2023 Mar 23;15(7):1596.

[13] Ghazal H, Waqar A, Yaseen F, Shahid M, Sultana M, Tariq M, Bashir MK, Tahseen H, Raza T, Ahmad F. Role of nanoparticles in enhancing chemotherapy efficacy for cancer treatment. Next materials. 2024 Feb 28:100128.

[14] Aldayel TS, Gad El Hak HN, Nafie MS, Saad R, Abdelrazek HM, Kilany OE. Evaluation of antioxidant, anti-inflammatory, anticancer activities and molecular docking of Moringa oleifera seed oil extract against experimental model of

Ehrlich ascites carcinoma in Swiss female albino mice. BMC Complementary Medicine and Therapies. 2023 Dec 14;23(1):457.

[15] Bhat MA, Khan FA, Kataria HC. In-vivo Anticancer Activity of Root and Leaf extract of Jurinea dolomiaea Boiss (Asteraceae) against Ehrlichs Ascites Carcinoma (EAC) Cell Line. Journal of Advanced Chemical Sciences. 2022 Jul 23:770-3.

[16] Tayel F, Mahfouz ME, Salama AF, Mansour MA. Ethoxyquin inhibits the progression of Murine Ehrlich Ascites Carcinoma through the inhibition of autophagy and LDH. Biomedicines. 2021 Oct 23;9(11):1526.

[17] Gonzalez-Leon A, Schultz IR, Xu G, Bull RJ. Pharmacokinetics and metabolism of dichloroacetate in the F344 rat after prior administration in drinking water. Toxicology and applied pharmacology. 1997 Oct 1;146(2):189-95.

[18] Ren S, Li C, Dai Y, Li N, Wang X, Tian F, Zhou S, Qiu Z, Lu Y, Zhao D, Chen X. Comparison of pharmacokinetics, tissue distribution and pharmacodynamics of liposomal and free doxorubicin in tumour-bearing mice following intratumoral injection. Journal of Pharmacy and Pharmacology. 2014 Sep;66(9):1231-9.

[19] Kramer KJ, Ganzberg SI. Pharmacology of Outpatient Anesthesia Medications. InPeterson's Principles of Oral and Maxillofacial Surgery 2022 Aug 9 (pp. 53-80). Cham: Springer International Publishing.

[20] Jakhar DK, Vishwakarma VK, Singh R, Jadhav K, Shah S, Arora T, Verma RK, Yadav HN. Fat fighting liraglutide based nano-formulation to reverse obesity: design, development and animal trials. International Journal of Pharmaceutics. 2023 Mar 5;634:122585.

[21] Wang L, Lin R, Guo L, Hong M. Rosuvastatin relieves myocardial ischemia/reperfusion injury by upregulating PPAR-γ and UCP2. Molecular medicine reports. 2018 Jul;18(1):789-98.

[22] Bishayi B, Adhikary R, Nandi A, Sultana S. Beneficial effects of exogenous melatonin in acute Staphylococcus aureus and Escherichia coli infection-induced inflammation and associated behavioral response in mice after exposure to short photoperiod. Inflammation. 2016 Dec;39(6):2072-93.

[23] Li XY, Chow CK. An improved method for the measurement of malondialdehyde in biological samples. Lipids. 1994 Jan;29(1):73-5.

[24] Liu L, Fan H, Qi P, Mei Y, Zhou L, Cai L, Lin X, Lin J. Synthesis and hepatoprotective properties of Acanthus ilicifolius alkaloid A and its derivatives. Experimental and Therapeutic Medicine. 2013 Sep 1;6(3):796-802.

[25] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine. 1967 Jul 1;70(1):158-69.

[26] Aebi H. [13] Catalase in vitro. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 121-126). Academic press.

[27] Pozarowski P, Darzynkiewicz Z. Analysis of cell cycle by flow cytometry. Checkpoint Controls and Cancer: Volume 2: Activation and Regulation Protocols. 2004:301-11.

[28] Dongsar TS, Dongsar TT, Molugulu N, Annadurai S, Wahab S, Gupta N, Kesharwani P. Targeted therapy of breast tumor by PLGA-based nanostructures: The versatile function in doxorubicin delivery. Environmental Research. 2023 Sep 15;233:116455.

[29] Chekhoeva A, Nakanishi S, Sugimura Y, Toshmatova M, Assmann AK, Lichtenberg A, Akhyari P, Assmann A. Dichloroacetate inhibits the degeneration of decellularized cardiovascular implants. European Journal of Cardio-Thoracic Surgery. 2022 Jan 1;61(1):19-26.

[30] Sahoo BM, Banik BK, Borah P, Jain A. Reactive oxygen species (ROS): key components in cancer therapies. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents). 2022 Jan 1;22(2):215-22.

[31] Qin H, Zheng G, Li Q, Shen L. Metabolic reprogramming induced by DCA enhances cisplatin sensitivity through increasing mitochondrial oxidative stress in cholangiocarcinoma. Frontiers in Pharmacology. 2023 Sep 25;14:1128312.

[32] Hamada, W.M., El-Nahass, M.N., Noser, A.A., Fayed, T.A., El-Kemary, M., Salem, M.M. and Bakr, E.A. Simple

dihydropyridine-based colorimetric chemosensors for heavy metal ion detection, biological evaluation, molecular docking, and ADMET profiling. Scientific Reports. 2023.Sept. 13(1):15420.

[33] Shinde VR, Revi N, Murugappan S, Singh SP, Rengan AK. Enhanced permeability and retention effect: A key facilitator for solid tumor targeting by nanoparticles. Photodiagnosis and Photodynamic Therapy. 2022 Sep 1;39:102915.

[34] Tempel M, Green K, Prajapati D, Duaqui A, Norouzi M, Sattarifard H, Ashraf A, Wu E, Zovoilis A, Lakowski TM, Davie JR. Doxorubicin, a DNA intercalator, inhibits transcription elongation. Biochemistry and Cell Biology. 2025 Feb 14(ja).

[35] Ashok A, Andrabi SS, Mansoor S, Kuang Y, Kwon BK, Labhasetwar V. Antioxidant therapy in oxidative stress-induced neurodegenerative diseases: Role of nanoparticle-based drug delivery systems in clinical translation. Antioxidants. 2022 Feb 17;11(2):408.

[36] Valgimigli L. Lipid peroxidation and antioxidant protection. Biomolecules. 2023 Aug 24;13(9):1291.

[37] Sheibani M, Azizi Y, Shayan M, Nezamoleslami S, Eslami F, Farjoo MH, Dehpour AR. Doxorubicin-induced cardiotoxicity: an overview on pre-clinical therapeutic approaches. Cardiovascular Toxicology. 2022 Apr;22(4):292-310.

[38] Mukherjee S, Gupta P, Ghosh S, Choudhury S, Das A, Ahir M, Adhikary A, Chattopadhyay S. Targeted tumor killing by pomegranate polyphenols: Pro-oxidant role of a classical antioxidant. The Journal of Nutritional Biochemistry. 2023 May 1;115:109283.

[39] Zhou JW, Ji PC, Wang CY, Yang YJ, Zhao XY, Tang HZ. Anti-virulence activity of dihydrocuminyl aldehyde and nisin against spoilage bacterium Pseudomonas aeruginosa XZ01. Lwt. 2023 Mar 1;177:114573.

[40] Lazar-Poloczek E, Romuk E, Jacheć W, Wróbel-Nowicka K, Świętek A, Wojciechowska C. Association of NT-proBNP and sST2 with Left Ventricular Ejection Fraction and Oxidative Stress in Patients with Stable Dilated Cardiomyopathy. Biomedicines. 2024 Mar 22;12(4):707.

[41] Patil PP, Kumar P, Khanal P, Patil VS, Darasaguppe HR, Bhandare VV, Bhatkande A, Shukla S, Joshi RK, Patil BM, Roy S. Computational and experimental pharmacology to decode the efficacy of Theobroma cacao L. against doxorubicin-induced organ toxicity in EAC-mediated solid tumor-induced mice. Frontiers in pharmacology. 2023 May 31;14:1174867.

[42] Al-Kuraishy HM, Al-Gareeb AI, Alkhuriji AF, Al-Megrin WA, Elekhnawy E, Negm WA, De Waard M, Batiha GE. Investigation of the impact of rosuvastatin and telmisartan in doxorubicin-induced acute cardiotoxicity. Biomedicine & Pharmacotherapy. 2022 Oct 1;154:113673.

[43] Singh R, Prasad A, Kumar B, Kumari S, Sahu RK, Hedau ST. Potential of dual drug delivery systems: MOF as hybrid nanocarrier for dual drug delivery in cancer treatment. ChemistrySelect. 2022 Sep 27;7(36):e202201288.

[44] Bhattacharya S, Munshi C. Biological significance of C-reactive protein, the ancient acute phase functionary. Frontiers in Immunology. 2023 Oct 4;14:1238411.

[45] Cooper J, Pastorello Y, Slevin M. A meta-analysis investigating the relationship between inflammation in autoimmune disease, elevated CRP, and the risk of dementia. Frontiers in Immunology. 2023 Jan 27;14:1087571.

[46] Köroğlu Z, Kizir D, Karaman M, Demir Y, Türkeş C, Beydemir Ş. Protective effects of esculetin against doxorubicin-induced toxicity correlated with oxidative stress in rat liver: in vivo and in silico studies. Journal of Biochemical and Molecular Toxicology. 2024 Apr;38(4):e23702.

[47] Hekal, H.A., Hammad, O.M., El-Brollosy, N.R., Salem, M.M. and Allayeh, A.K.,. Design, synthesis, docking, and antiviral evaluation of some novel pyrimidinone-based α-aminophosphonates as potent H1N1 and HCoV-229E inhibitors. Bioorganic Chemistry. 2024 June;147, :107353.

[48] Nicoletto RE, Ofner III CM. Cytotoxic mechanisms of doxorubicin at clinically relevant concentrations in breast cancer cells. Cancer Chemotherapy and Pharmacology. 2022 Mar;89(3):285-311.

[49] Kong CY, Guo Z, Song P, Zhang X, Yuan YP, Teng T, Yan L, Tang QZ. Underlying

the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death. International journal of biological sciences. 2022 Jan 1;18(2):760.

[50] Zander DY, Burkart SS, Wüst S, Magalhães VG, Binder M. Cooperative effects of RIG-I-like receptor signaling and IRF1 on DNA damage-induced cell death. Cell Death & Disease. 2022 Apr 18;13(4):364.

Table 1: Cardiac oxidative stress and antioxidants parameters in different experimental groups.

Groups	MDA (nmol/g T)	NO (µM)	CAT (U/mg P)	GSH (mol/g T)
Ctrl	6.85±0.05	7.25±0.15	33.1±0.2	23.8±0.3
Ctrl/DCA	7.10 ±0.07	7.86 ±0.05	31.7 ±0.6*	20.8 ±0.4*
% Change	3.6 %	8.4 %	-9 %	-12 %
Ctrl/DCA-PNPs	6.75± 0.05	7.85 ±0.05	31.7 ±0.7*	21.1 ±0.7*
% Change	-1.5 %	8.3%	-9 %	-11 %
Ctrl/Dox	8.1 ±0.05*	9.4 ± 0.4*	20.65 ±0.3*	19.1±0.05*
% Change	17.5 %	30 %	-41 %	-20 %
EAC	16.2±0.1*	18±0.1*	11.2±0.65*	8.2±0.7*
% Change	136	148	-66	-65
EAC/Dox	6.3±0.1^{+}	7.5 ± 0.05^{+}	24.3±0.55^{+}	20.1±0.06^{+}
% Change	-61	-58	116	145
EAC/DCA	7.75±0.25^{+}	8.5±0.1^{+}	21.47±0.32^{+}	19.4±0.6^{+}
% Change	-52	-53	90	136
EAC/DCA-NPs	5.9±0.005^{+}	7.3±0.11^{+}	28.1±0.6^{+}	21.8±0.5^{+}
% Change	-63	-59	150	165
EAC/Dox/DCA	6.2±0.1^{+}	6.8± 0.18^{+}	23.8±0.2^{+}	20.2±0.1^{+}
% Change	-63	-62	112	146
EAC/Dox/DCA-PNPs	5.6±0.11^{+}	6.21±0.11^{+}	32.1±0.2^{+}	22.9±0.1^{+}
% Change	-65	-66	185	179

Data were presented as mean ± SE n=4, (*$p<0.0001$) *value*: versus normal control group, ($^{+}p<0.0001$) *value*: versus EAC bearing group. P: protein, and T: tissue. MDA: monoaldehyde, NO: nitric oxide, CAT: catalase, and GSH: glutathione reductase.

Table 2: Mammary gland oxidative stress and antioxidants parameters in different experimental groups.

Groups	MDA (nmol/g T)	NO (µM)	CAT (U/mg P)	GSH (mol/g T)
Ctrl	4.4±0.1	14±1	21.35±0.25	26.15 ±0.35
Ctrl/DCA **% Change**	4.65 ±0.05 6 %	13.8 ±0.2 -1.4%	20.8±0.3 -3 %	25.25 ±0.25 -3 %
Ctrl/DCA-PNPs **% Change**	5.25± 0.05 19 %	14.4 ±0.6 3 %	22.15±0.15 4 %	25.2 ±0.1 -4 %
Ctrl/Dox **% Change**	15.05 ±0.05* 242%	19.7 ± 0.3 41 %	8.3±0.3* -61 %	10.7 ± 0.3* -59 %
EAC **% Change**	18.2± 0.1* 314%	31.65±1.65* 126 %	7.45±0.05* -65 %	9.65±0.15* -63 %
EAC/Dox **% Change**	4.75 ± 0.25^{+} -74%	17.5 ± 2.5^{+} -45 %	19.1±0.1^{+} 156 %	17.65 ± 0.15^{+} 83 %
EAC/DCA **% Change**	5.15 ± 0.45^{+} -72%	15.3 ± 0.1^{+} -52 %	20.85±0.15^{+} 180 %	22.3 ± 0.7^{+} 131 %
EAC/DCA-NPs **% Change**	6.7 ± 0.9^{+} -63%	17.3 ± 1.3^{+} -45 %	17.8±0.8^{+} 139 %	19.25 ± 0.15^{+} < 0.0001 99 %
EAC/Dox/DCA **% Change**	5.1 ± 0.2^{+} -72%	15.6 ± 0.8^{+} -51 %	20.25±0.15^{+} 172 %	21.85 ± 0.15^{+} 126 %
EAC/Dox/DCA-PNPs **% Change**	4.45 ± 0.15^{+} -76%	15 ± 1^{+} -53 %	21.6±0.3^{+} 190 %	26.05 ± 0.65^{+} 170 %

Data were presented as mean ± SE n=4, (*$p<0.0001$) *value*: versus normal control group, ($^{+}p<0.0001$) *value*: versus EAC bearing group. P: protein, and T: tissue. MDA: monoaldehyde, NO: nitric oxide, CAT: catalase, and GSH: glutathione reductase.

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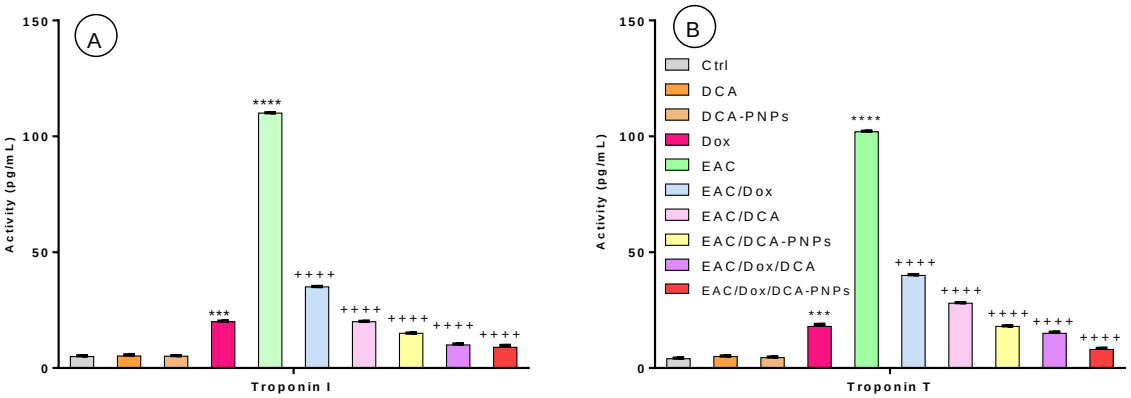

Figure 1: Serum troponin I level **(A)**, and serum troponin T level **(B)** in all groups, data are presented as mean ± SE n=4, (**p<0.0001*) value: vs. control group, ($^{+}$*p<0.0001)* value: vs. EAC-bearing group.

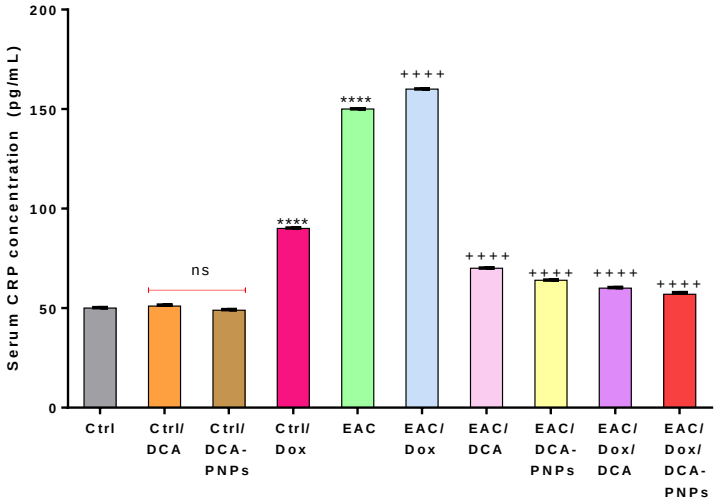

Figure 2: Serum CRP concentration in all groups, data are presented as mean ± SE n=4, (**p<0.0001*) value: vs. control group, ($^{+}$*p<0.0001)* value: vs. EAC-bearing group.

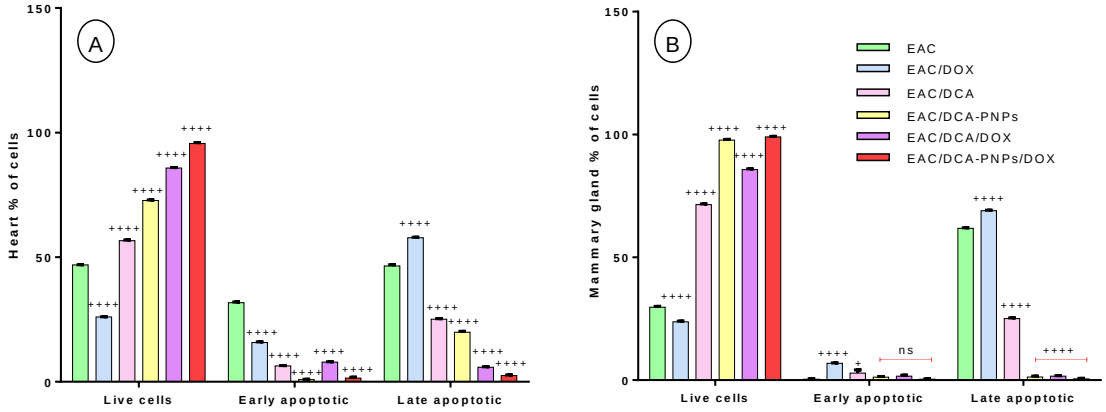

Figure 3: Cardiac annexin V of EAC-bearing cells and all treated groups **(A),** Mammary gland annexin V of EAC-bearing cells and all treated groups **(B).** Results were expressed as mean ± SE n=4. (*p* value <0.0001) is significantly expressed, where $^{+}$ significant in all treated groups vs. EAC-bearing mice.

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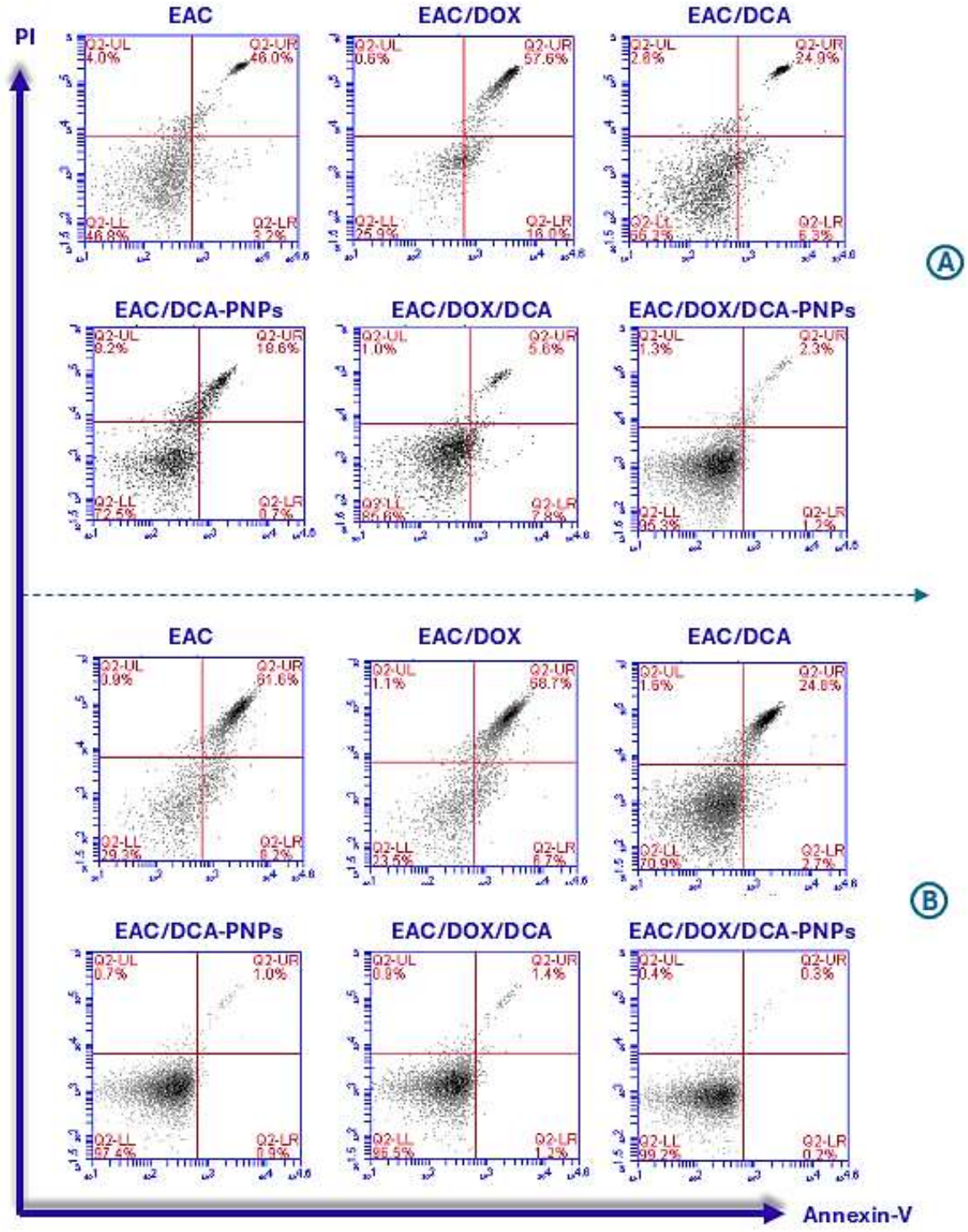

Figure 4: Heart tissue annexin V % of all experimental groups and EAC-bearing mice group **(A).** Mammary gland tissue annexin V % **o**f all experimental groups and EAC-bearing mice group **(B)**.