Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2024; Vol 23: Issue 1, https://carcinogenesis.com/

The Mitigating Role of Transresveratrol Against Doxorubicin-Induced Cardiotoxicity in Breast Cancer Patients via Modulating Mitochondrial Gene Expression: A Randomized Controlled Trial

Bendary M. Rana [1], Omar Y. El-Azzazy[1], Nabila Hefzi[3], Hanan M. Elnahas[2]

1 Department of Pharmacy Practice, Faculty of Pharmacy, Zagazig University, Egypt

2 Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Zagazig University, Egypt

3 Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University, Egypt

✉ **Corresponding author:**

Bendary M. Rana, Faculty of Pharmacy, Zagazig University, Egypt

Email ID : rbendary@pharmacy.zu.edu.eg / ranabendary00@gmail.com

Addresses: El Qawmia square, Zagazig city, Egypt

Tel: 00201014939803

Fax: 0552303266

ABSTRACT

Background: Increased awareness of the adverse cardiac effects of chemotherapeutic agents makes the paradigm of care has shifted to a double hit goal of increasing cancer survival with cardiac protection. Breast cancer (BC) patients treated with Doxorubicin (DOX, Adriamycin®) usually develop cardiotoxicity. Therefore, our trial aimed to investigate the cardioprotective role of the polyphenolic transresveratrol (Transresv) supplement with Adriamycin-Cytoxan (AC) chemotherapy protocol to mitigate DOX-induced cardiotoxicity (DIC).

Methods: This study is a randomized controlled clinical trial (RCT) recruiting 54 BC patients who were randomly allocated into two equal groups. A control group (AC group) received four cycles of AC chemotherapy, each of which was three weeks apart in a dose of 60 mg/m2 Adriamycin with 600 mg/m2 Cytoxan. An intervention group (AC/Transresv group) received the same AC protocol with 150 mg/day Transresv orally. Baseline characteristics were analyzed. Serum cTnI, CK-MB, LVEF, mitochondrial gene expression of NADH dehydrogenase, cytochrome c oxidase I, II, III subunits, CRP, MDA, and t-SOD were assessed pre and post intervention.

Results: There was no significant difference between AC and AC/Transresv groups concerning baseline characteristics. AC group revealed a significant increase in cTnI, CRP, and MDA with a decrease in LVEF, t-SOD, beside down regulation of mitochondrial genes with a non-significant change in CK-MB after 4th AC cycle compared to baseline values. AC/Transresv group exhibited a significant improvement in almost all parameters compared to AC group.

Conclusion: Transresv supplementation with AC protocol alleviates DIC in the BC patients via modulating diverse signaling pathways and targeting mitochondrial biogenesis..

Keywords: Breast cancer, Cardiotoxicity, Doxorubicin, Mitochondrial enzymes, Oxidative stress, Resveratrol.

How to Cite: Bendary M. Rana , Omar Y. El-Azzazy, Nabila Hefzi, Hanan M. Elnahas, (2024) The Mitigating Role of Transresveratrol Against Doxorubicin-Induced Cardiotoxicity in Breast Cancer Patients via Modulating Mitochondrial Gene Expression: A Randomized Controlled Trial.. Journal of Carcinogenesis, Vol.23, No.1, 751-770

1. INTRODUCTION

Breast cancer (BC) is a life-threatening devastating malignancy. An estimated 316,950 women with invasive type and 59,080 of non-invasive (in situ) type of BC will be diagnosed in the United States (U.S) by the end of 2025 [1]. Fortunately, the five year survival rate of the BC is 91% with 4 million BC survivors, this high survival rate reflects a great advancement in the chemotherapeutic strategies for BC [1]. However, these strategies are usually associated with chemotherapy-related cardiotoxicity that are not to be ignored [2]. One of such strategies is Adriamycin-Cytoxan (AC) chemotherapy protocol in which Adriamycin (Doxorubicin; DOX) is a potent cytotoxic agent that belongs to anthracyclines antibiotic family. It is widely implicated as a cornerstone for treating BC and other myriads of solid and hematogenous malignancies [3, 4].

One of DOX major drawbacks is a dose-dependent, short- and long-term cardiotoxicity that encountered in BC patients throughout the treatment and even many years after its cessation in the BC survivors. According to American Cancer Society (ACS), the BC survivors have a 42% higher risk of developing DOX-induced cardiotoxicity (DIC) compared to the age-matched individuals without cancer **[5]**. It is noteworthy that DIC is the second leading cause of heart failure among cancer survivors where its rate was 5%, 16%, 26%, and 48% following a DOX dose of 400, 500, 550, and 700 mg/m², respectively **[6, 7]**. That is why it is commonly perpetuated that "today's BC survivors are tomorrow cardiac sufferers" **[8]**.

Despite several decades of investigation, DIC continues to be a subject of great interest and remains a central focus in the field of cardio-oncology because its precise mechanism is still a conundrum that has not been solved yet.

Several hypothetical mechanisms were unveiled to be the potential contributing factors of DIC, including imbalance of calcium hemostasis, topoisomerase IIβ (Top IIβ) inhibition, DNA damage, and oxidative stress with overproduction of reactive oxygen species (ROS).These multifarious mechanisms collectively orchestrate the deterioration of cardiac architecture and function **[9, 10]**. A major mechanism of DIC is DOX-provoked mitochondrial dysfunction via an alteration in the expression of oxidative phosphorylation (OXPHOS) genes with a consequent decrease of cardiac ATP production **[11, 12]**. Additionally, DOX indirectly triggers the release of mitochondrial proapoptotic factors which activate the caspase cascades that initiate the intrinsic apoptotic pathways in the cardiomyocytes with subsequent cardiac death **[13]**.

The aforementioned deleterious effects of DOX continue to be a major challenge that impedes DOX wide clinical applicability **[8]**. In an endeavor to counteract DOX ramifications, the research realm has been directed toward discovering complementary cardioprotective adjuvants that enhance DOX tolerability without interfering with its anti-neoplastic efficacy. Hence, this study was conducted to spot a light on Transresv against DIC in BC patients. Transresv is a non-flavonoid polyphenol phytochemical compound emanated from natural sources and is abundantly found in several plants, including red grapes, peanuts, and berries **[14]**.

Transresv spans a large spectrum of scientific research as a cardioprotective agent in preclinical studies by virtue of its diverse biological activities including antioxidant, anti-inflammatory, anti-apoptotic, anti-platelets, vasorelaxant together with exhibiting a mitochondrial biogenesis enhancing activity **[15, 16]**. Pharmaceutically, Transresv is a bioactive supplement that is well-tolerated in humans due to its high therapeutic efficacy, almost null side effects beside its cost-effectiveness and biocompatibility **[14, 17]**. To the best of our knowledge, there is a lack of clinical trials that investigate the potential cardioprotective effect of Transresv on DIC in BC patients. Therefore, the rationale of our study is to address this knowledge gap via concomitantly administering Transresv with AC chemotherapy protocol to open a new prophylactic avenue in the field of cardio-oncology.

2. PATIENT AND METHODS

Study Design, Setting and Time Frame

This study was a randomized controlled parallel-group trial (RCT) that enrolled Egyptian BC patients under DOX-based chemotherapy protocol. It was carried out at Clinical Oncology and Nuclear Medicine Department at Zagazig University Hospital, Egypt. This trial was conducted over a 12-month period, from May 2023 to May 2024. The study protocol was approved by Institutional Review Board (IRB), Zagazig University (Approval No.10167/19-12-2022, December 2022). All study procedures were carried out in accordance with the Declaration of Helsinki and guided by the Consolidated Standards of Reporting Trails (CONSORT) guidelines for RCTs. The patients who were willing to participate signed a written informed consent.

Study Participants

Female BC patients aged 18-60 years attending the chemotherapy unit at Zagazig University Hospital were recruited to assess their eligibility for the trial. The inclusion criteria were (1) BC patients scheduled to receive four cycles of AC chemotherapy; (2) LVEF ≥ 55% (3) Normal liver and kidney function. The exclusion criteria were (1) LVEF < 55%; (2) Impaired liver or kidney function; (3) History of cardiac diseases; (4) Pregnant or breast-feeding females; (5) Medications interacting with Transresv.

Sample Size Calculation

An a *priori* power analysis was conducted using G*Power (3.1.9.7) **[18]** before launching the trial to determine our sample size that could detect the practical significant difference between AC and AC Transresv groups. The power analysis was based on a medium effect size (Cohen's d of 0.79) driven from a previous study catching our primary endpoint of measuring mitochondrial gene expression of electron transport chain (ETC) complexes upon resveratrol supplementation **[19]**. Based on this effect size, significance level (α) of 0.05, and statistical power (1 - β) at 0.08, the calculated sample size was 54 participants.

Randomization and Blinding

The patients were allocated randomly using the computer random number generator software with a 1:1 allocation ratio.

The allocation sequence was concealed in sequentially numbered, opaque, and sealed envelopes. The participants, investigators, and the statisticians were all blinded to the intervention assignment until the end of the trial. A total of 54 patients were equally divided into two equal groups: **(i)** ***Control group (AC group)*** in which 27 patients received four AC cycles, each of which was 3 weeks apart in a dose of 60 mg/m^2 Adriamycin (Adricin vial; hikma pharmaceutical company, Germany) with 600 mg/m^2 Cytoxan (Endoxan powder; Baxter pharmaceutical company, Germany) via IV infusion for 1 hour; **(ii)** ***Intervention group (AC/Transresv group)*** in which 27 patients were supplemented with oral Transresv (Ultra-Resveratrol ® capsules; Swiss Bioenergetics Company, UK) in a dose of 150 mg/day in concomitant with the four AC cycles and continued for 3 weeks after the end of the 4th cycle covering a total intervention period of 84 days.

Data Collection

All patients' data were collected in a case record form (CRF) that was comprised of demographic characteristics, full patient history, reproductive attributes, BC characteristics, immunohistochemical characteristics, TNM classification, staging of BC, and LVEF from baseline conducted echocardiography.

Study Procedures

From each patient in both groups, ~5 ml venous blood sample was collected before the 1st AC cycle (pre- interventional blood sample) and another blood sample was collected 3 weeks after the end of the 4th AC cycle (post-interventional blood sample). A 3-ml portion of the whole blood sample was left to clot at room temperature in a red-top non-heparinized tube, then it was centrifuged at 2000 rpm over 10 min for separation of serum. Aliquots of sera were stored and frozen at -80°C until assessing the biochemical parameters. While the other 2-ml of the whole blood sample was stored in a purple-top heparinized tube and frozen at -80°C until measuring the gene expression encoding mitochondrial enzymes (NADH dehydrogenase and cytochrome c oxidase subunits (MT-CO I, II, III)) by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Laboratory Biochemical Analysis

Serum biochemical parameters were assessed following the manufacturer's instructions and included: (i) Cardiac enzymes [cardiac troponin I (cTnI) and Creatine kinase cardiac muscle isoenzyme (CK-MB)] using the immunofluorescence assay (IFA) kits; (ii) The inflammatory biomarker C-reactive protein (CRP) using ELISA kit; (iii) Oxidative stress biomarkers [Malondialdehyde (MDA) and total superoxide dismutase (t-SOD)] using ELISA kits.

Reverse Transcription Quantitative PCR (RT-qPCR)

The expression of mitochondrial genes (mt-mRNA encoding NADH dehydrogenase and MT-CO I, II, and III subunits) was determined via three main consequent steps; RNA extraction, then reverse transcription followed by qPCR reaction **[20, 21]**. Total RNA was extracted from the whole blood sample using easy-red™ total RNA extraction kit (iNtRON Biotechnology, Inc. USA). Then quantification of the extracted RNA concentration was performed using the Quantus™ Fluorometer (Promega Corporation, Madison, USA). Afterwards, the extracted RNA was treated by DNase for purification. This purified RNA was converted to cDNA in a reverse transcription reaction using cDNA synthesis kit (ABT 2X RT Mix).

Finally, the qPCR reaction was prepared using PCR master mix (ABT 2X qPCR SYBR mix**)** and forward/reverse primers (Diagen, Altındag, Ankara, Turkey) **(Table 1)**. This PCR reaction mixture was transferred to the RT-qPCR apparatus (QuantStudio, thermoFisher scientific, Real-Time PCR system, UK). At the end of the PCR run, the obtained amplification plots were analysed to determine the relative gene expression of studied genes via a specialized software in which the Ct of each target gene was normalized relative to a standard mRNA reference gene (β-actin; housekeeping gene) using the comparative Ct ($2^{-\Delta\Delta Ct}$) method **[22]**. Afterward, the results were expressed as the mean fold change of each target gene after the 4th AC cycle compared to the baseline values and compared to the control group.

Table 1. Primer sequences and PCR thermal profile of the target genes and the reference gene (β-actin)

Target gene	Primer sequences	PCR thermal profile
NADH dehydrogenase	**(F)** 5'-CACATCTACCATCACCCTCT-3' **(R)** 5' -GTCATGATGGCAGGAGTAAT-3'	40 cycles: 30s at 95 °C, 40s at 50°C, 1 min at 72 °C
MT-CO I	**(F)** 5'-CCG TCCTAATCACAG CAGTCCTA-3' **(R)** 5'-TGA GGT TGC GGT CTG TTA GTAGT-3'	40 cycles: 30s at 95 °C, 40s at 55°C, 1 min at 72 °C
MT-CO II	**(F)** 5'-CCG CCATCA TCC TAG TCC TCAT-3' **(R)** 5'- GATCGTTGACCTCGTCTG TTATGT-3'	40 cycles: 30s at 95 °C, 40s at 55°C, 1 min at 72 °C

MT-CO III	**(F)** 5'-ACG GCATCTACG GCTCAA CA-3' **(R)** 5'-TGGCGG ATG AAGCAG ATAGTGA-3'	40 cycles: 30s at 95 °C, 40s at 55°C, 1 min at 72 °C
β-actin	**(F)** 5' -GTGGCATCCACGAAACTACC-3' **(R)** 5' -CAGGGCAGTGATCTCCTTCT-3'	40 cycles: 30s at 95 °C, 40s at 57°C, 1 min at 72 °C

Study Outcomes

The primary outcomes were the mean change in the serum levels of cardiac biomarkers (cTnI and CK-MB), LVEF and mitochondrial gene expression of ETC complexes. The secondary outcomes were the mean change in serum CRP, MDA, and t-SOD.

Statistical Analysis

The study data were analyzed using Statistical Package for Social Sciences (SPSS version 25.0). Numerical data were described and expressed as Mean ± SD, whereas the categorical data were described and expressed as frequency and percentage. Shapiro-Wilk test was used to check the normality of distribution among numerical data. For the normally distributed variables, paired *t*-test was used to compare two dependent variables while unpaired *t*-test was used to compare two independent variables. For the categorical data, Chi-squared test (χ^2) was used. P-value of ≤ 0.05 is considered statistically significant. For RT-qPCR analysis, statistical comparisons were performed on the normalized ΔCt values, calculated as the difference between Ct of target gene and Ct of β-actin reference gene.

3. RESULTS

A CONSORT flow chart **(Fig. 1)** illustrates the study design in which a total of 80 BC patients were assessed for eligibility. After screening, 26 patients were excluded; 17 did not meet the inclusion criteria, and 9 declined participation. Finally, a total of 54 patients completed the study; 27 (50%) constituted the AC group and 27 (50%) constituted the AC/Transresv group.

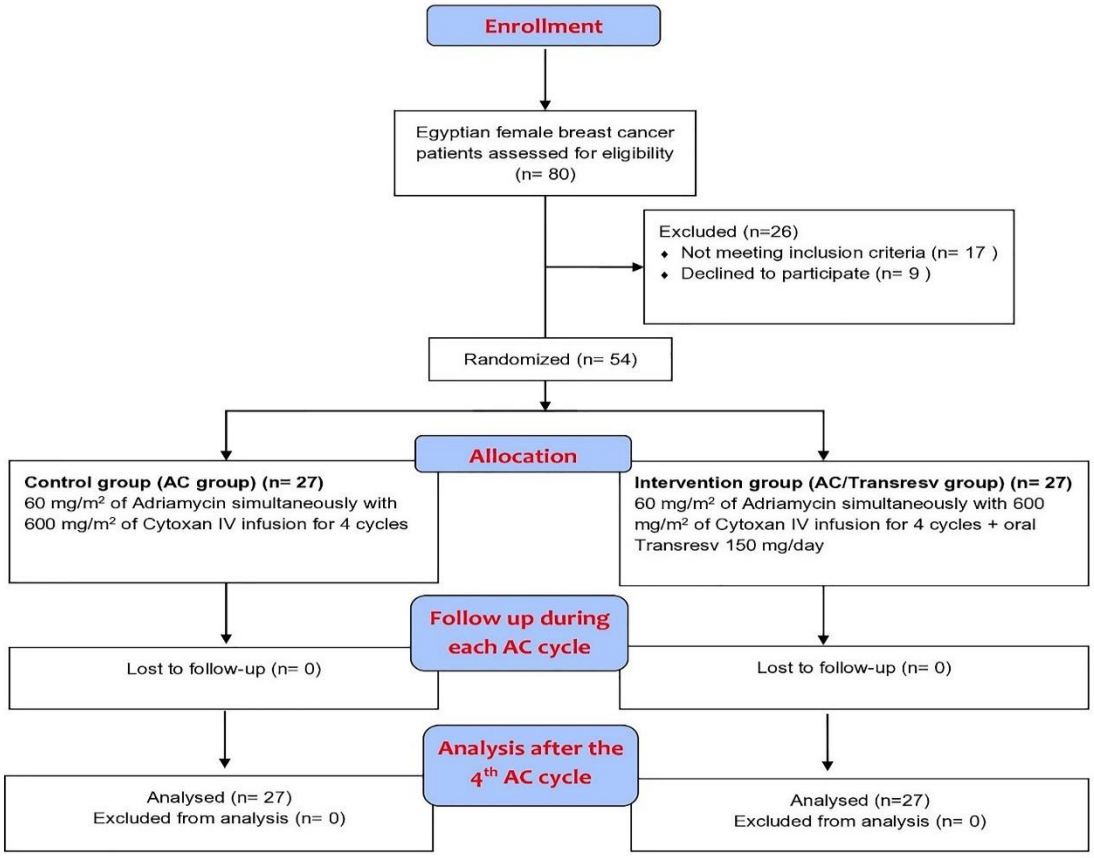

Fig. 1 CONSORT flow chart of enrollment, allocation, follow up, and data analysis

Results of Baseline Characteristics in The Enrolled BC patients

There was no significant difference (P > 0.05) between AC and AC/Transresv groups regarding baseline characteristics of BC patients **(Table 2),** BC characteristics **(Fig. 2)**, and immunohistochemical characteristics of BC **(Table 3)**.

Table 2 Baseline characteristics of BC patients in the AC and AC/Transresv groups (n = 54)

Variables	**Category**	**AC group (n = 27)**	**AC/Transresv group (n = 27)**	***t*/X^2**	***P*-value**
Age (years)		45.63 ± 10.81	46.89 ± 8.57	0.474	0.637
Marital status	Virgin	1 (1.9%)	1 (1.9%)	0.001	1.00
	Married	26 (48.1%)	26 (48.1%)		
Residency	Rural	4 (7.4%)	9 (16.7%)	2.533	0.111
	Urban	23 (42.6%)	18 (33.3%)		
Educational status	Illiterate	11 (20.4%)	8 (14.8%)	0.731	0.393
	Educated	16 (29.6%)	19 (35.2%)		
Occupational status	Manual job	19 (35.2%)	15 (27.8%)	1.271	0.260
	Office job	8 (14.8%)	12 (22.2%)		
Medical history (systemic diseases)	Yes	2 (3.7%)	4 (7.4%)	0.750	0.386
	No	25 (46.3%)	23 (42.6%)		
Medication history (Contraceptives)	Yes	11 (20.4%)	7 (13.0%)	1.333	0.248
	No	16 (29.6%)	20 (37.0%)		
Family history (Breast/ovarian cancer)	Yes	7 (13.0%)	10 (18.5%)	0.773	0.379
	No	20 (37.0%)	17 (31.5%)		
Screening mammogram	Yes	13 (24.1%)	15 (27.8%)	0.297	0.586
	No	14 (25.9%)	12 (22.2%)		
Special habits (Smoking or alcoholism)	Yes No	2 (3.7%) 25 (46.3%)	1 (1.9%) 26 (48.1%)	0.353	0.552
Menstruation status	Pre-menopausal	20 (37.0%)	16 (29.6%)	1.33	0.248
	Post-menopausal	7 (13.0%)	11 (20.4%)		
Parity status	Nullipara	6 (11.1%)	7 (13.0%)	0.101	0.750
	Multipara	21 (38.9%)	20 (37.0%)		
History of breast-feeding	Yes	21 (38.9%)	21 (38.9%)	0.00	1.000
	No	6 (11.1%)	6 (11.1%)		

Descriptive and inferential statistics of the baseline characteristics of BC patients in both groups. Normally distributed numerical variables are expressed as mean ± SD while categorical variables are expressed as frequency and percentage. *t*: An unpaired *t*-test compares normally distributed numerical variables between the two studied groups. X^2:

Chi-square test compares categorical variables. AC: Adriamycin-Cytoxan; Transresv: Transresveratrol. *P*-value ≤ 0.05 is considered statistically significant.

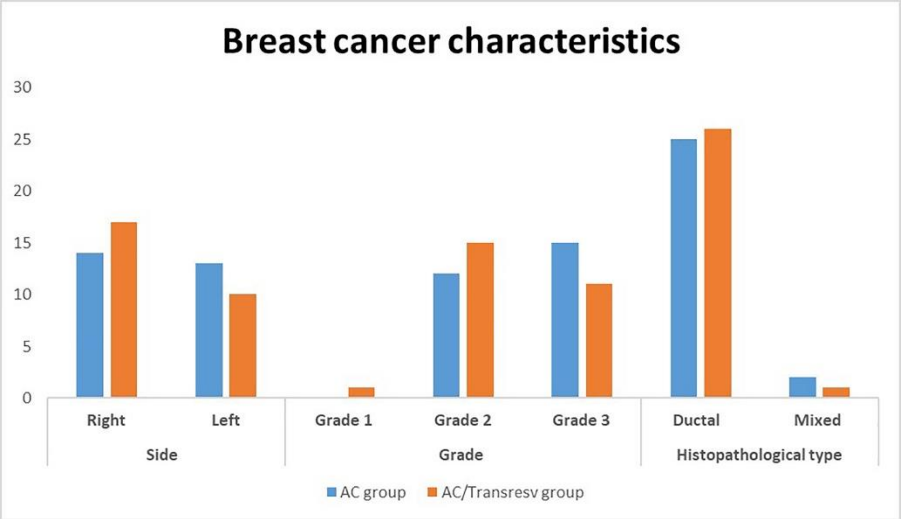

Fig. 2 Bar chart of BC characteristics in the AC and AC/Transresv groups

Table 3 Immunohistochemical characteristics of BC in the AC and AC/Transresv groups (n = 54)

Variables	Category	AC group (n = 27)	AC/Transresv group (n = 27)	X^2	*P*-value
ER	Positive	22 (40.7%)	22 (40.7%)	0.00	1.000
	Negative	5 (9.3%)	5 (9.3%)		
PR	Positive	17 (31.5%)	19 (35.2%)	0.333	0.564
	Negative	10 (18.5%)	8 (14.8%)		
HER2	Positive	5 (9.3%)	6 (11.1%)	0.114	0.735
	Negative	22 (40.7%)	21 (38.9%)		
Ki-67	< 20%	21 (38.9%)	17 (31.5%)	1.42	0.233
	≥ 20%	6 (11.1%)	10 (18.5%)		

Descriptive and inferential statistics of immunohistochemical characteristics of BC in both groups. These categorical variables are expressed as frequency and percentage. X^2: Chi-square test. ER: Estrogen receptors; PR: progesterone receptors; HER2: Human epidermal growth factor receptor 2; Ki-67: Marker of proliferation (Kiel 67 protein. *P*-value ≤ 0.05 is considered statistically significant.

Results of TNM Classification and Staging of BC

There was no significant difference (P > 0.05) in the TNM classification and staging of BC between the two studied groups, except for the T category, in which AC group was significantly higher (P = 0.042) compared to AC/Transresv group **(Table 4)**.

Table 4 TNM classification and staging of BC of the AC and AC/Transresv groups (n = 54)

	Category	AC group (n = 27)	AC/Transresv group (n = 27)	X^2	*P*-value
TNM classification	T1	11 (20.4%)	5 (9.3%)	8.18	0.042*
	T2	14 (25.9%)	12 (22.2%)		
	T3	2 (3.7%)	7 (13.0%)		
	T4	0 (0.0%)	3 (5.6%)		
	N0	10 (18.5%)	7 (13.0%)	2.47	0.480
	N1	11 (20.4%)	9 (16.7%)		
	N2	3 (5.6%)	4 (7.4%)		
	N3	3 (5.6%)	7 (13.0%)		
	M0	27 (50.0%)	26 (48.1%)	1.02	0.313
	M1	0 (0.0%)	1 (1.9%)		
Staging of BC	Stage I	4 (7.4%)	2 (3.7%)	8.17	0.226
	Stage II A	10 (18.5%)	5 (9.3%)		
	Stage II B	7 (13.0%)	5 (9.3%)		
	Stage III A	3 (5.6%)	5 (9.3%)		
	Stage III B	0 (0.0%)	3 (5.6%)		
	Stage III C	3 (5.6%)	6 (11.1%)		
	Stage IV	0 (0.0%)	1 (1.9%)		

Descriptive and inferential statistics of TNM classification and staging in both groups. These categorical variables are expressed as frequency and percentage. X^2: Chi-square test. TNM: Tumor, Lymph Nodes, Metastasis; Stage I (T1, N0, M0), Stage II A (T0, N1, M0) or (T1, N1, M0) or (T2, N0, M0), Stage II B (T2, N1, M0) or (T3, N0, M0), Stage III A (T0, N2, M0) or (T1, N2, M0) or (T2, N2, M0) or (T3, N1, M0) or (T3 N2, M0), Stage III B (T4, any N, M0), Stage III C (any T, N3, M0), Stage IV (any T, any N, M1). *P*-value ≤ 0.05 is considered statistically significant. *: A statistically significant *p*-value between the two studied groups.

Transresv Attenuated Cardiomyocyte Injury

There was a significant increase (P < 0.001) in the mean serum cTnI levels with a percentage change of +80% in the AC group after the 4th AC cycle compared to the baseline values before the 1st cycle. Meanwhile, the AC/Transresv group showed a significant increase (P < 0.001) with a lower percentage change of +23%. However, a non-significant change in the mean serum CK-MB was revealed in both AC and AC/Transresv groups (P = 0.72 and P = 0.23, respectively) after the 4th AC cycle compared to the baseline values **(Table 5)**.

A statistical comparison of the mean percentage change in serum cTnI levels between the two studied groups after the 4th AC cycle showed a significant decrease (P < 0.001) in the AC/Transresv group relative to the AC group. While the statistical comparison of the mean percentage change in serum CK-MB levels revealed a non-significant difference (P = 0.304) between the AC and AC/Transresv groups (**Fig. 3)**.

Table 5 Serum levels of cTnI and CK-MB before the 1st and after the 4th AC cycle and their percentage changes in both groups (mean ± SD)

Parameters	Groups	Before 1st AC cycle	After 4th AC cycle	t^a	*P*-value	Mean Percentage Change

cTnI (ng/ml)	AC	0.10 ± 0.001	0.18 ± 0.05	6.936	< 0.001#	+0.80 ± 0.059
	AC/Transresv	0.10 ± 0.001	0.12 ± 0.017	6.643	< 0.001#	+0.23 ± 0.017
***t*^b^**						4.774
***P*-value**						< 0.001*
CK-MB (U/L)	AC	8.80 ± 1.68	8.93 ± 1.70	-1.87	0.72	+0.02 ± 0.004
	AC/Transresv	9.87 ± 2.02	9.93 ± 2.00	-1.21	0.23	+0.01 ± 0.003
***t*^b^**						1.041
***P*-value**						0.304

Descriptive and inferential statistics of serum cTnI and CK-MB in both groups. Values are normally distributed and expressed as mean ± SD. t^a: A paired *t*-test compares the mean serum cTnI and CK-MB levels before the 1st and after the 4th AC cycle within the groups. t^b: An unpaired *t*-test compares the mean percentage changes of these two parameters after the 4th cycle between the two groups. cTnI: Cardiac troponin I; CK-MB: Creatine kinase-MB (Cardiac muscle isoenzyme). *P*-value ≤ 0.05 is considered statistically significant. #: A statistically significant *p*-value between the baseline values and those after the 4th AC cycle within the group. *****: A statistically significant *p*-value of mean percentage change between the two studied groups.

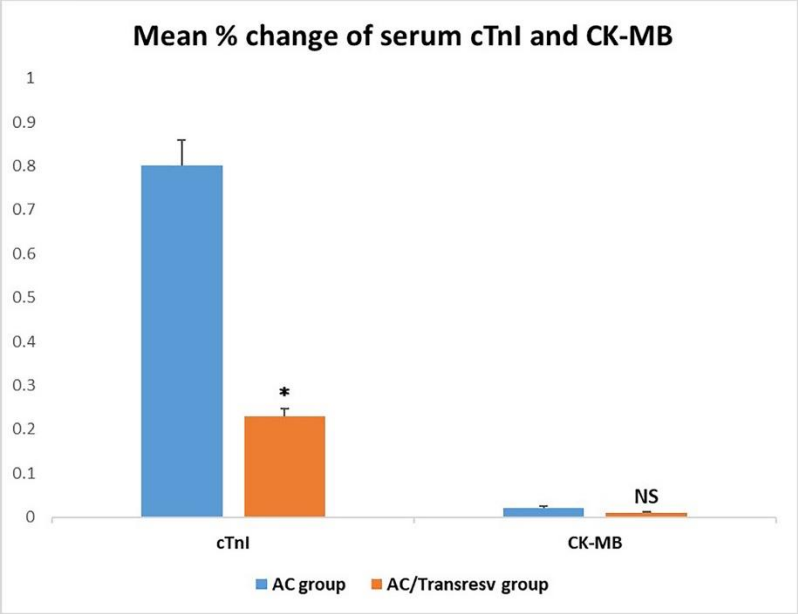

Fig. 3 Bar chart of a statistical comparison of the mean percentage change in serum cTnI and CK-MB levels between the AC and AC/Transresv groups after the 4th AC cycle.*: A statistically significant p-value between the two studied groups. NS: a non-significant p-value between the two studied groups

Transresv Preserved Cardiac Contractile Function

Table 6 depicted a significant decrease (P < 0.001) in the mean LVEF with a percentage change of -26% in the AC group after the 4th cycle compared to the baseline values. In the AC/Transresv group, the mean LVEF also showed a significant decrease (P < 0.001) but with a lower percentage change of -7%.

A statistical comparison of the mean percentage change of LVEF between the two groups after the 4th AC cycle showed a

significant increase (P < 0.001) in the AC/Transresv group compared to the AC group **(Fig. 4)**.

Table 6 LVEF before the 1st and after the 4th AC cycle and its mean percentage changes in both groups (mean ± SD)

Parameter	Groups	Before 1st AC cycle	After 4th AC cycle	t^a	*P*-value	Mean Percentage Change
LVEF	AC	0.65 ± 0.04	0.48 ± 0.06	14.34	< 0.001#	-0.26 ± 0.09
	AC/Transresv	0.64 ± 0.04	0.60 ± 0.04	6.338	< 0.001#	-0.07 ± 0.04
t^b						9.509
***P*-value**						< 0.001*

Descriptive and inferential statistics of LVEF in both groups. Values are normally distributed and expressed as mean ± SD. t^a: A paired *t*-test compares the mean LVEF before the 1st and after the 4th AC cycle within the groups. t^b: An unpaired *t*-test compares the mean percentage change between the two groups. *P*-value ≤ 0.05 is considered statistically significant. #: A statistically significant *p*-value between the baseline values and those after the 4th AC cycle within the group. *: A statistically significant *p*-value of mean percentage change between the two studied groups.

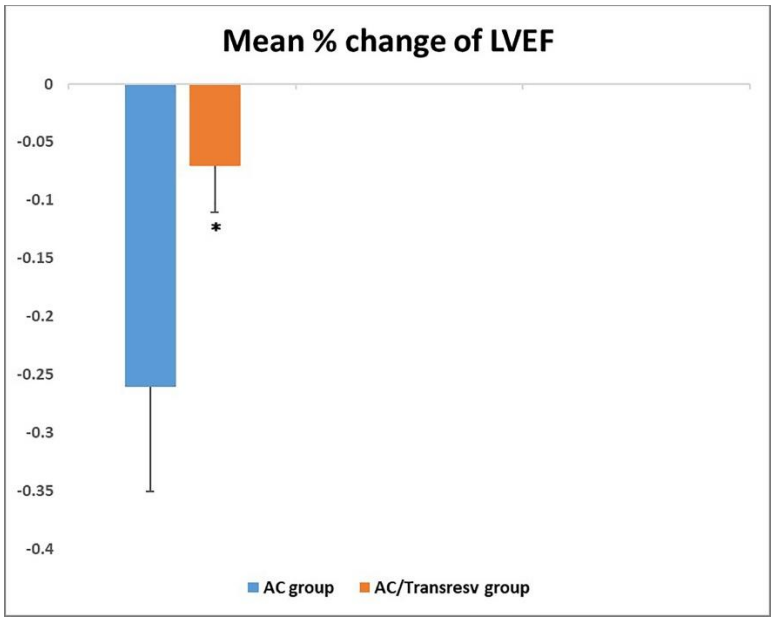

Fig. 4 Bar chart of a statistical comparison of the mean percentage change of LVEF between the AC and AC/Transresv groups after the 4th AC cycle. *: A statistically significant p-value between the two studied groups

Transresv Promoted Gene Expression of mRNA Encoding Mitochondrial Enzymes

The relative gene expression of mRNA encoding mitochondrial NADH dehydrogenase and MT-CO I, II, and III subunits in each group were represented in the terms of fold change after normalization with β-actin **(Table 7)**. In the AC group, these genes were significantly down regulated (P = 0.001, 0.004, 0.002, and 0.001) with ~ (-11, -13, -8, and -9 fold changes, respectively) after the 4th AC cycle compared to baseline values **(Fig. 5)**. Whereas, in the AC/Transresv group, the expression of NADH dehydrogenase and MT-CO I, II, and III subunits were significantly down regulated (P = 0.023, 0.019, 0.030, and 0.015) but with lower fold changes of ~ (-2, -3, -2 ,and -4, respectively) after the 4th AC cycle compared to baseline values **(Fig. 6)**.

A statistical comparison of the mean fold change of mRNA gene expression of NADH dehydrogenase and MT-CO I, II, and III between the two groups after the 4th AC cycle showed a significant higher levels (P = 0.008, 0.032, 0.023, and 0.039, respectively) in the AC/Transresv group compared to the AC group **(Table 7)**.

Table 7 Relative gene expression of mRNA encoding mitochondrial NADH dehydrogenase and MT-CO I, II, and III subunits in the terms of mean fold change before the 1st and after the 4th AC cycle in both groups

Gene expression	Groups	Before 1st AC cycle	After 4th AC cycle	t^a	*P*-value
NADH dehydrogenase	AC	1.033 ± 0.27	0.09 ± 0.01	9.032	0.001#
	AC/Transresv	1.05 ± 0.21	0.51 ± 0.08	3.027	0.023#
t^b					-3.510
P-value					0.008*
MT-CO I	AC	1.04 ± 0.14	0.08 ± 0.02	5.831	0.004#
	AC/Transresv	1.057 ± 0.29	0.39 ± 0.09	3.187	0.019#
t^b					-2.766
P-value					0.032*
MT-CO II	AC	1.007 ± 0.14	0.12 ± 0.05	7.431	0.002#
	AC/Transresv	1.065 ± 0.27	0.49 ± 0.01	2.827	0.030#
t^b					-2.731
P-value					0.023*
MT-CO III	AC	1.025 ± 0.16	0.11 ± 0.01	7.873	0.001#
	AC/Transresv	1.099 ± 0.27	0.28 ± 0.17	3.368	0.015#
t^b					-2.612
P-value					0.039*

Descriptive and inferential statistics of relative gene expression of mRNA encoding NADH dehydrogenase and MT-CO I, II, III subunits in both groups. Values are normally distributed and expressed as mean ± SD. t^a: A paired *t*-test compares the fold changes before the 1st and after the 4th AC cycle within the groups. t^b: An unpaired *t*-test compares the fold changes after the 4th AC cycle between the two groups. NADH: nicotinamide adenine dinucleotide and hydrogen; MT-CO: Cytochrome c oxidase subunits I, II, III. *P*-value ≤ 0.05 is considered statistically significant. #: A statistically significant *p*-value between the baseline values and those after the 4th AC cycle within the group. *: A statistically significant *p*-value of mean fold change between the two studied groups.

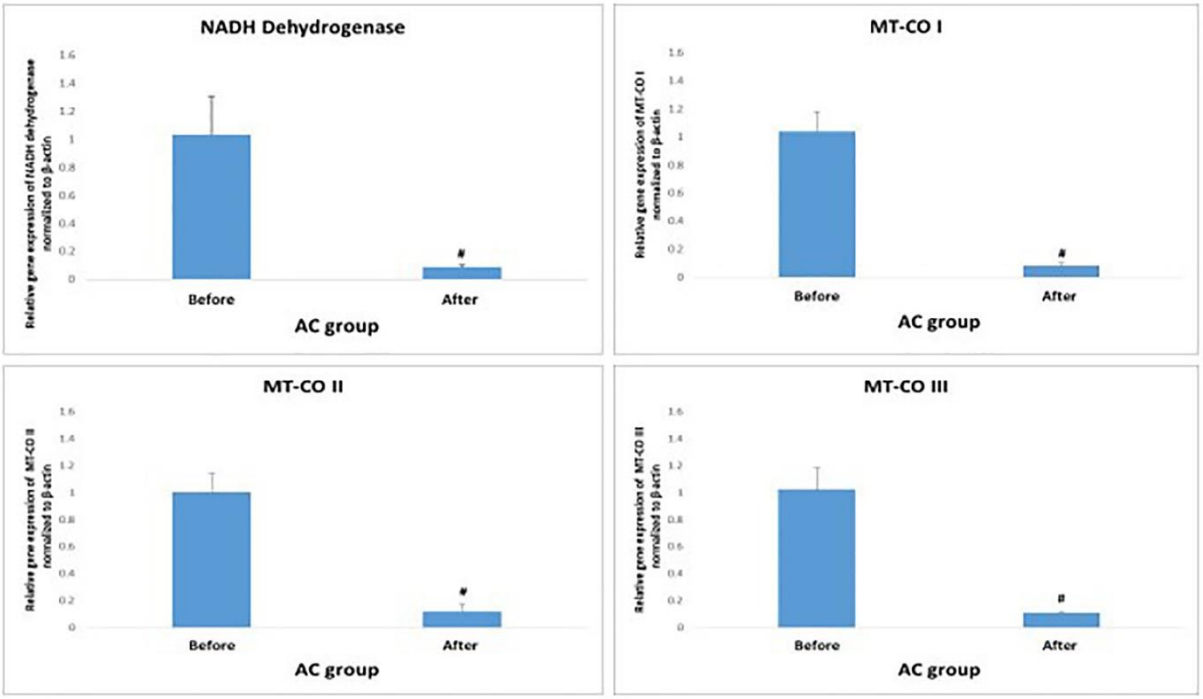

Fig. 5 Bar chart of relative gene expression of mRNA encoding NADH dehydrogenase and MT-CO I, II, III subunits in the AC group after the 4th cycle compared to the baseline values in the terms of fold change after normalization with β-actin. #: A statistically significant *p*-value before the 1st and after the 4th AC cycle within the group

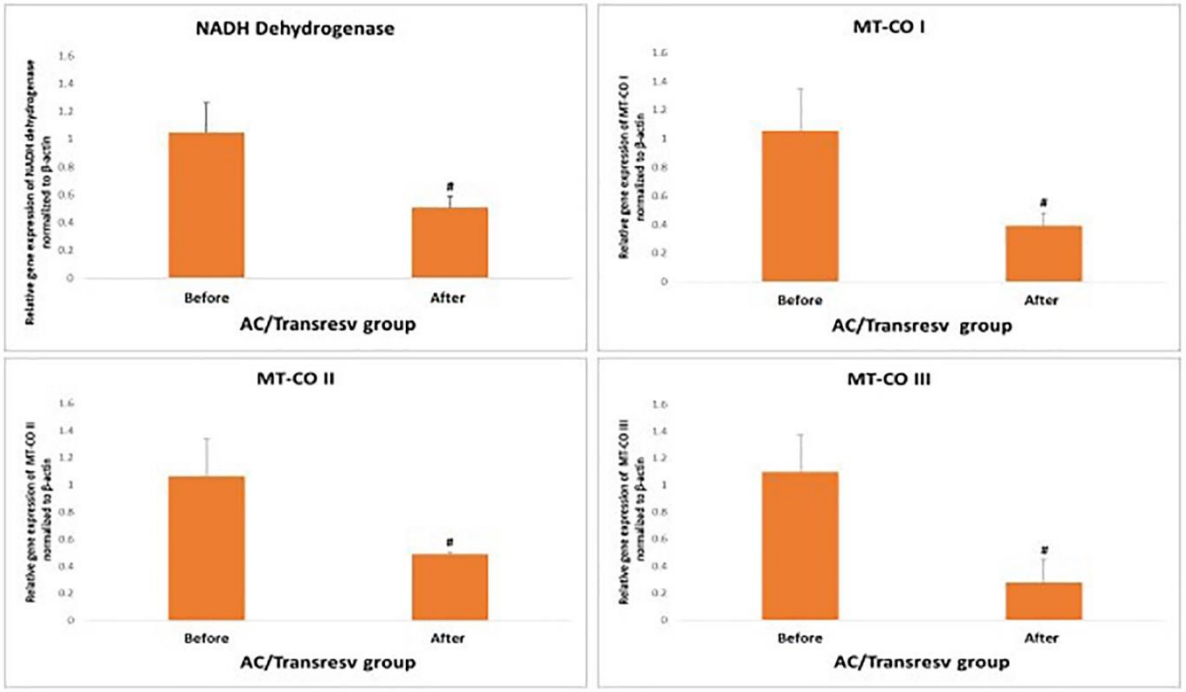

Fig. 6 Bar chart of relative gene expression of mRNA encoding NADH dehydrogenase and MT-CO I, II, III subunits in the AC/Transresv group after the 4th AC cycle compared to the baseline value in the terms of fold change after normalization with β-actin. #: A statistically significant *p*-value before the 1st and after the 4th AC cycle

Transresv Improved The Anti-inflammatory and Antioxidant Circulatory Capacities

In the AC group, the mean serum CRP and MDA increased significantly (P < 0.001) with percentage changes of +70% and +84%, respectively, while t-SOD activity decreased significantly (P < 0.001) with a percentage change of -23% after the 4th AC cycle compared to the baseline values. While, in the AC/Transresv group, the mean serum CRP and MDA revealed a statistical significant increase (P = 0.02 and P < 0.001, respectively) with lower percentage changes of +23% and +34% after the 4th cycle compared to the baseline values. The t-SOD activity showed a statistical decrease (P < 0.001) with a lower percentage change of -6% after the 4th AC cycle compared to baseline values **(Table 8)**.

A statistical comparison of the mean percentage change of serum levels of CRP, MDA, and t-SOD after the 4th AC cycle between the two groups revealed a statistical significant decrease in CRP and MDA (P < 0.001, P = 0.001, respectively) with a statistical significant increase in t-SOD (P = 0.035) in the AC/Transresv group compared to the AC group **(Fig. 7)**.

Table 8 Serum levels of CRP, MDA, and t-SOD before the 1st and after the 4th AC cycle and their mean percentage changes in both groups (mean ± SD)

Parameters	Groups	Before 1st AC cycle	After 4th AC cycle	t^a	*P*-value	Mean Percentage Change
CRP (ng/ml)	AC	17.14 ± 4.96	28.50 ± 8.37	10.127	< 0.001#	+0.71 ± 0.170
	AC/Transresv	16.30 ± 5.85	18.91 ± 5.86	-3.420	0.02#	+0.23 ± 0.033
t^b						4.799
P-value						< 0.001*
MDA (nmol/ml)	AC	84.90 ± 31.20	165.31 ± 24.08	-8.646	< 0.001#	+0.84 ± 0.067
	AC/Transresv	82.41 ± 20.19	106.89 ± 15.85	-14.209	< 0.001#	+0.34 ± 0.021
t^b						3.656
P-value						0.001*
t-SOD (U/ml)	AC	112.40 ± 13.27	84.44 ± 20.14	5.199	< 0.001#	-0.23 ± 0.170
	AC/Transresv	115.79 ± 27.54	107.98 ± 25.25	4.157	< 0.001#	-0.06 ± 0.120
t^b						0.939
P-value						0.035*

Descriptive and inferential statistics of serum CRP, MDA, and t-SOD in both groups. Values are normally distributed and expressed as mean ± SD. t^a: A paired *t*-test compares the mean serum levels of CRP, MDA, and t-SOD before the 1st and after the 4th AC cycle within the groups. t^b: An unpaired *t*-test compares the mean percentage changes of these three parameters after the 4th cycle between the two groups. CRP: C-reactive protein; MDA: Malondialdehyde; t-SOD: total superoxide dismutase. A *p*-value ≤ 0.05 is considered statistically significant. #: A statistically significant *p*-value between the baseline values and those after the 4th AC cycle within the group. *: A statistically significant *p*-value of mean percentage change between the two studied groups.

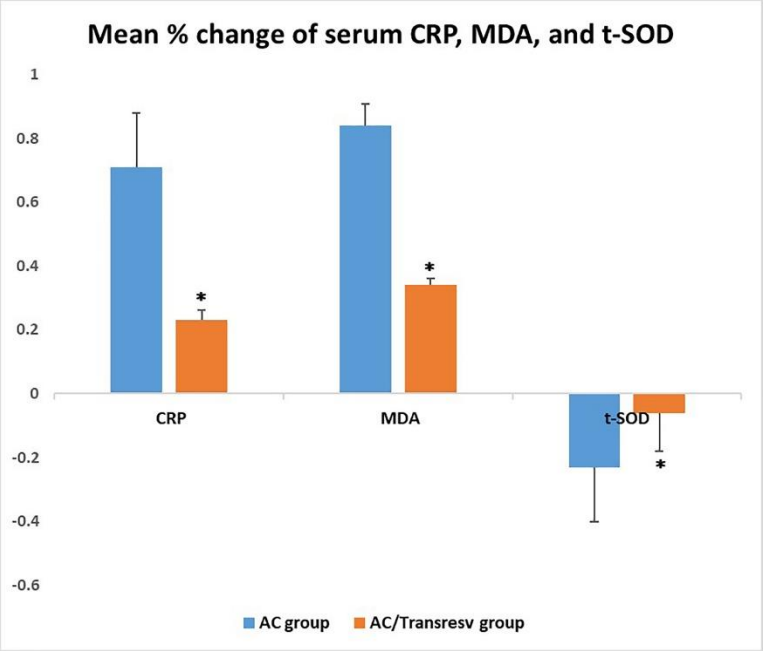

Fig. 7 Bar chart of a statistical comparison of the mean percentage change in serum CRP, MDA, and t-SOD levels between the AC and AC/Transresv groups after the 4th AC cycle.*: A statistically significant p-value between the two studied groups

4. DISCUSSION

Conspicuously, protecting the heart from DIC remains a conundrum that has not been solved yet, however, emerging hypotheses suggest that natural polyphenolic compounds may help in overcoming this DOX-related comorbidity **[23]**. In that issue, the present work emphasizes the prospect of Transresv as a cardioprotective pharmaceutical supplement and probes the likely molecular mechanisms implicated in its protective potential.

Our results revealed no statistically significant differences between AC and AC/Transresv groups regarding the baseline characteristics except T category in the TNM classification of BC. Although no significant difference was found, this result is still important because it is a good indicative of our proper random allocation and it also ensured that any observed effects are due to Transresv intervention itself without any confounding factors. Moreover, our trial was a single-center study in which variability between groups was nil.

In this study, to confirm the development of DIC, we have intentionally measured the serum levels of cTnI and CK-MB. The statistically non-significant difference of CK-MB in each studied group after the 4th AC cycle and between the two groups might largely attributed to the less sensitivity, specificity, and abundance of this enzyme in the myocardium [24]. On the other hand, the elevated serum cTnI level in the AC group after the 4th AC cycle was mostly indicated the development of DIC in our BC patients. Its elevation might be due to the sustained release of this cardiac enzyme as a result of cardiac myofibrils degradation under the influence of DOX-provoked ROS generation that ultimately leads to cardiomyocytes necrosis and apoptosis [25].

Importantly, Transresv could substantially reduce myocardial damage as evidenced by the observed lower serum levels of cTnI in the AC /Transresv group relative to that of AC group. This result may highlight the mitigating effect of Transresv against DIC in a tangible way via improving this deranged cardiac injury index. Actually, resveratrol has a well-known anti-apoptotic effect mediated by its modulating impact on multiple molecular signaling pathways that are known to protect the heart from various cardiotoxic stressors. One of such signaling pathways that is activated by resveratrol is PI3K/Akt/GSK-3β pathway which is a pro-survival signaling cascade implicated in the expression of marked anti-apoptotic proteins and inactivation of the pro-apoptotic ones **[26, 27]** . Another resveratrol eminent anti-apoptotic effect is via increasing Sirtuin-1 (SIRT-1) expression which reduces p53 acetylation resulting in suppression of p53 pro-apoptotic activity in the myocardial tissues **[27]**.

Interestingly, resveratrol inhibition of cardiomyocytes apoptosis was evidenced by the downregulation of caspase-3 and BAX expression **[27]**. Through the aforementioned anti-apoptotic mechanisms, resveratrol could preserve the cardiac cell

integrity and consequently attenuate DOX-evoked rise of cTnI in the BC patients.

Another major finding in this trial was the significant reduction in LVEF by ~ (-26%) in the AC group and mildly by ~ (-7%) in the AC/Transresv group after the 4th AC cycle compared to the baseline value. The decline of LVEF pointed mostly to the development of DIC in the AC group because the cut-off value that considered a primary diagnostic criterion of DIC is 10% absolute decrease in LVEF or a decline to <50% compared to the baseline value **[28]**.

The decreased level of LVEF in the AC group is primarily attributed to DOX-evoked oxidative stress, where ROS negatively impede the myocardial calcium handling that eventually led to cardiac remodeling and impaired contractility **[29]**. Moreover, DOX brings about mitochondrial damage that leads to reduction of ATP production in the cardiomyocytes with further contractile dysfunction **[30]**.

Additionally, a recent clinical study elucidated DOX-fostered myocardial fibrotic changes that diminish myocardial contractility and systolic performance **[31]**.

By the end of 84 days of Transresv co-supplementation, our results revealed a significant improvement of LVEF in the AC/Transresv group compared to the AC group that gave an indication about the Transresv capability in preserving cardiac contractility. This finding was in accordance with a RCT conducted on heart failure patients in whom resveratrol supplementation for 3 months attenuated the significant decline in the LVEF **[32]**.

Actually, Transresv could enhance the LVEF through preventing myocardial fibrotic remodeling via exerting anti-inflammatory effects and inhibiting some pro-fibrotic intracellular signaling pathways including TGF-β/SMAD and PI3K/AKT/mTOR Pathways **[14]**. Also, Transresv was found to upregulate the expression of mitofusin-2 (MFN2), a protein on the outer mitochondrial membrane that indirectly supports mitochondrial dynamics by enabling mitochondrial fusion and maintaining mitochondrial integrity. MFN2 also modulates some key signaling pathways, including peroxisome proliferator-activated receptor- γ coactivator (PGC-1α), AMP-activated protein kinase (AMPK), and SIRT1 which are known to promote mitochondrial biogenesis that consequently increase ATP generation which in turn improves the myocardial contractile function **[33]**.

The notion frequently emphasized in prior literature that mitochondrial dysfunction underlies DIC served as the basis for our measurement of mitochondrial gene expression of NADH dehydrogenase (Mitochondrial complex I) and MT-CO I, II, III subunits (Mitochondrial complex IV). Intriguingly, the decline of mRNA expression of these genes in the AC group after the 4th cycle compared to the baseline value implied that DOX markedly downregulates the expression of genes encoding vital enzymes of the mitochondrial ETC that in turn disrupts the cardiac mitochondrial bioenergetics. Our results were aligned with **Lebrecht, Setzer, Ketelsen, Haberstroh and Walker [34]** who found a down regulation of these mitochondrial ETC enzymes in chronic DOX cardiomyopathy rat model.

The proposed mechanism of DOX-triggered downregulation of these key mitochondrial-related transcripts is the fact that mitochondria are the primary targets of DOX where its cationic charge attracts the anionic charge of cardiolipin, a phospholipid abundant in the inner mitochondrial membrane forming a DOX-cardiolipin complex which accepts one electron from NADH at the expense of NADH dehydrogenase leading to inhibition of this mitochondrial enzyme **[35]**. By inhibiting this enzyme, DOX impairs the flow of electrons to ubiquinone (CoQ) of the ETC and hence the mitochondrial ATP production is compromised, with decreased energy production in the high-energy demands' cardiac cells leading to their eventual injury **[36]**.

Of particular interest, the downregulation of MT-CO I, II, and III subunits could be attributed to the direct inhibitory effect of DOX on these vital components of ETC **[37]**. In addition, DOX-induced ROS generation results in indirect oxidative damage of these subunits that involved in mitochondrial OXPHOS process **[38]**.

The results of Transresv co-administration revealed a relative improvement in the gene expression of NADH dehydrogenase which mildly decreased (-2 fold change) in the AC/Transresv group compared to the marked decrease (-11 fold change) in the AC group. Consistent with our results, **Desquiret-Dumas, Gueguen, Leman, Baron, Nivet-Antoine, Chupin, Chevrollier, Vessières, Ayer, Ferré, Bonneau, Henrion, Reynier and Procaccio [39]** demonstrated that resveratrol directly stimulates NADH dehydrogenases which trigger NADH oxidation with subsequent electron transfer across the mitochondrial ETC, thereby boosting mitochondrial respiration and cellular ATP levels. Resveratrol stimulation of this enzyme also increases NAD^+ / NADH ratio that was suggested to be the hidden hand behind resveratrol-induced activation of sirtuins (SIRT1 and SIRT3), a NAD^+-dependent deacetylases that deemed as fundamental elements in modulating numerous transcription factors and co-regulators involved in mitochondrial quality control of cardiomyocytes **[33, 40]**.

Multiple intracellular signaling pathways were modulated by resveratrol in a sirtuin-dependent manner including SIRT1/AMPK, SIRT1/PGC-1α, and SIRT1/NLRP3 that collectively exert cardioprotective effects against DIC by suppressing inflammation, oxidative stress, promoting mitochondrial biogenesis, and enhancing cell survival **[41]**. **Ruan, Dong, Patel, Duan, Wang, Wu, Cao, Pu, Lu and Shen [42]** demonstrated that SIRT1 activation could overcome

cardiomyocyte apoptosis by inhibiting mitogen-activated protein kinases (p38 MAPK) phosphorylation and caspase-3 activation **[42]**. In a remarkable way, resveratrol could also modulate mitochondrial autophagy of cardiomyocytes during oxidative stress conditions via stimulating SIRT1/SIRT3-Parkin and SIRT1/SIRT3-FoxO pathways **[33]**. Another resveratrol-targeted pathway that was implicated in cardiac mitochondrial biogenesis is AMPK/PGC-1α/SIRT3 signaling that regulates gene expression of numerous proteins constituting the mitochondrial ETC **[43]**.

It is remarkable that resveratrol has an imprint on mitochondrial ETC complexes not only via fostering mitochondrial biogenesis but also via promoting mitochondrial dynamics through upregulation of mRNA expression of vital regulatory proteins involved in mitochondrial fusion and fission via Sirt1/Sirt3-Mfn2 pathway **[33]**.

Intriguingly, our results also revealed an improvement in the gene expression of MT-CO I, II, and III subunits which slightly decreased (-3, -2, and -4 fold change, respectively) in the AC/Transresv group compared to the marked decrease (-13, -8, and -9 fold change) in the AC group.

The proposed mechanism beyond this improvement is the resveratrol-enhanced SIRT3 level that directly deacetylates and activates complex IV subunits thus combating OXPHOS deficiency **[44]**. It was also documented that resveratrol upregulates the expression of nuclear-encoded subunits of MT-CO through AMPK /SIRT1/PGC-1α signaling pathway **[45]**.

As a secondary outcome, this trial involved analysis of CRP in which the mean serum level increased markedly in the AC group after the 4th AC cycle compared to the baseline value. This providing an evidence of the pro-inflammatory effect of DOX **[46]**. The CRP elevation is mediated via DOX redox cycling that is the mainstay of activating nuclear factor kappa B (NF-κB), a crucial transcription factor that activates many pro-inflammatory genes encoding for cytokines synthesis including TNF-α, IL-1β, and IL-6 **[47]**. In particular, IL-6 mediates hepatic synthesis of CRP via activating a transcription factor called signal transducer and activator of transcription 3 (STAT3) leading to increased transcription of CRP gene with subsequent synthesis and release into the circulation **[48]**.

On the contrary, the mild relative increase of serum CRP in the AC/Transresv group compared to its marked increase in the AC group pointed to the anti-inflammatory effect of this phytochemical supplement that exerted by inhibiting diverse signaling pathways which regulate inflammation and cytokine production including NF-κB pathway and MAPK pathways **[49]**. Moreover, resveratrol inhibits the expression of cyclooxygenases (COX1, COX2) which are rate limiting enzymes in the production of prostanoids **[50]**. These previously outlined anti-inflammatory mechanisms of resveratrol might be the hidden hand behind mitigating the inflammatory cascades proceeded within the myocardium. Our result was consistent with a meta-analysis of RCTs in which resveratrol reduced the high-sensitivity CRP (hs-CRP) in various inflammatory disorders **[51]**.

In this study, DOX not only evoked inflammation but also induced redox imbalance that was evidenced by the marked elevation of the mean serum MDA with a substantial decline of the serum antioxidant enzyme t-SOD in the AC group after the 4th AC cycle. This provided compelling evidence of disrupted oxidative homeostasis that might be a contributing factor in the DIC development in our BC patients.

Notably, the answerable clue regarding the elevated MDA level after DOX administration was the lipid peroxidation imprint and oxidant effect of this cytotoxic drug as a result of its redox-reduction reaction ***[52]***. While the reduced t-SOD activity might be attributed to the DOX capability of down regulating the transcription of endogenous antioxidant enzymes **[53]** and impairing the Nrf2–Keap1–ARE signaling pathway leading to insufficient antioxidant defense **[54]**. This makes the heart tissue to be more vulnerable and susceptible to DOX-associated oxidative stress with subsequent cardiotoxicity.

Obviously, upon co-administration of Transresv, a mild relative rise of serum MDA with a mild relative decrease of t-SOD level were observed, a beneficial result that pointed to the partial restoration of the oxidative hemostasis in the AC/Transrev group compared to the AC group. This was attributed to the direct free radical scavenging capability of Transresv owing to the polyhydroxyl (–OH) groups attached to its phenolic rings which donate electrons to superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) resulting in formation of relatively stable inert structures [55]. Also, resveratrol indirectly reduces ROS that are generated from DOX-insulted mitochondria via activating SIRT1-based pathways with subsequent restoration of mitochondrial dynamics and biogenesis **[43]**. Another antioxidant effect of resveratrol is via activating the Nrf2–Keap1–ARE signaling pathway in which nuclear factor erythroid 2–related factor 2 (Nrf2) increases the transcription and expression of antioxidant genes encoding for SOD, catalase (CAT), and Glutathione Peroxidase (GPx) thereby boosting the endogenous antioxidant defense **[56]**.

5. CONCLUSION

This RCT affirms the cardioprotective potency of Transresv against DIC via scrutinizing the mitochondrial targeting signature by modulating the gene expression of mRNA encoding mitochondrial ETC complexes. Furthermore, the anti-inflammatory imprint of Transresv coupled with its capacity to reduce ROS overproduction were the secret codes beyond

restoring redox homeostasis and abrogating the cardiomyocytes contractile dysfunction that deemed as a concerning aspect in all over DOX history. From our perspective, Transresv constituted a promising prophylactic avenue for BC patients under DOX-based chemotherapy protocols, being able to circumvent such life-threatening cardiotoxicity.

LIST OF ABBREVIATIONS

AC	Adriamycin-Cytoxan
ACS	American Cancer Society
AMPK	Adenosine monophosphate-activated protein kinase
ARE	Antioxidant response element
ATP	Adenosine triphosphate
BC	Breast cancer
CAT	Catalase
CK-MB	Creatine kinase cardiac muscle isoenzyme
CONSORT	Consolidated standards of reporting trails
CoQ	Ubiquinone
CRF	Case record form
CRP	C-reactive protein
cTnI	Cardiac troponin I
DIC	DOX-induced cardiotoxicity
DOX	Doxorubicin
ER	Estrogen receptors
ETC	Electron transport chain
FoxO	Forkhead box transcription factors
GPx	Glutathione Peroxidase
GSK-3β	Glycogen synthase kinase-3 beta
HER2	Human epidermal growth factor receptor 2
hs-CRP	high-sensitivity C-reactive protein
IFA	Immunofluorescence Assay
IRB	Institutional Review Board
Keap1	Kelch-like ECH-associated protein 1
Ki-67	Marker of proliferation (Kiel 67 protein)
LVEF	Left ventricular ejection fraction
MAPK	Mitogen-activated protein kinases
MDA	Malondialdehyde

MFN2	Mitofusin-2
MT-CO	Mitochondrial cytochrome c oxidase
NADH	Nicotinamide adenine dinucleotide and hydrogen
NF-κB	Nuclear factor kappa B
NLRP3	NLR family pyrin domain containing 3
Nrf2	Nuclear factor erythroid 2–related factor 2
OXPHOS	Oxidative phosphorylation
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator 1alpha
PR	Progesterone receptors
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SIRT / sirt	Sirtuin
STAT3	Signal transducer and activator of transcription 3
TNM	Tumor, Lymph Nodes, Metastasis
Top IIβ	Topoisomerase IIβ
Transresv	Transresveratrol
t-SOD	total superoxide dismutase

Acknowledgement

We sincerely acknowledge the valuable assistance provided by the hospital staff of the Clinical Oncology and Nuclear Medicine Department, Zagazig University, Egypt. We also extend our appreciation to all breast cancer (BC) volunteers for their participation in the study.

CRediT authorship contribution statement

Rana M. Bendary, Nabila Hefzi, Hanan M. Elnahas: Conceptualization. **Rana M. Bendary**: Investigation, Resources, Writing – original draft. **Omar Y. El-Azzazy:** Investigation, Data curation, Validation, Formal analysis. **Nabila Hefzi**: Investigation, Methodology, Project administration. **Hanan M. Elnahas**: Writing – review & editing, Supervision. All authors read and agreed to the published version of the manuscript.

Data availability

The datasets generated and analyzed during the current study are not publicly available as individual privacy could be compromised but are available from the corresponding author on reasonable request.

Statements and Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding sources

The authors declare that no funds, grants, or other support were received during the conduction of this trial.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. The protocol approval was granted by the ethics committee of research (Institutional Review Board; IRB) at Faculty of Medicine, Zagazig University (Approval No.10167/19-12-2022, December 2022).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publication

The authors affirm that the manuscript does not contain any individual person's data in any form

REFERENCES

[1] ACS, Key Statistics for Breast Cancer, (2025).

[2] C. Belger, C. Abrahams, A. Imamdin, S. Lecour, Doxorubicin-induced cardiotoxicity and risk factors, International journal of cardiology. Heart & vasculature 50 (2023) 101332.

[3] F.A. Tadesse, A.A. Leminie, Effects of Adriamycin-Cytoxan chemotherapy on hematological and electrolyte parameters among breast cancer patients, Frontiers in Oncology 13 (2023) 1103013.

[4] K.J.-A.R. Dubey., Doxorubicin, 2023. https://www.ncbi.nlm.nih.gov/books/NBK459232/. (Accessed 2025).

[5] ACS, What You Need to Know About Cardiovascular Disease and Cancer, 2023. https://pressroom.cancer.org/releases?item=1187. (Accessed 10/6 2024).

[6] S.M. Swain, F.S. Whaley, M.S. Ewer, Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials, Cancer: Interdisciplinary International Journal of the American Cancer Society 97(11) (2003) 2869-2879.

[7] G. Curigliano, D. Cardinale, S. Dent, C. Criscitiello, O. Aseyev, D. Lenihan, C.M. Cipolla, Cardiotoxicity of anticancer treatments: Epidemiology, detection, and management, CA: a cancer journal for clinicians 66(4) (2016) 309-325.

[8] B.I. Bodai, P. Tuso, Breast cancer survivorship: a comprehensive review of long-term medical issues and lifestyle recommendations, The Permanente Journal 19(2) (2015) 48.

[9] B. Kalyanaraman, Teaching the basics of the mechanism of doxorubicin-induced cardiotoxicity: Have we been barking up the wrong tree?, Redox Biology 29 (2020) 101394.

[10] A. Avagimyan, N. Pogosova, L. Kakturskiy, M. Sheibani, A. Challa, E. Kogan, F. Fogacci, L. Mikhaleva, R. Vandysheva, M. Yakubovskaya, A. Faggiano, S. Carugo, O. Urazova, B. Jahanbin, E. Lesovaya, S. Polana, K. Kirsanov, Y. Sattar, A. Trofimenko, T. Demura, A. Saghazadeh, G. Koliakos, D. Shafie, A. Alizadehasl, A. Cicero, J.P. Costabel, G. Biondi-Zoccai, G. Ottaviani, N. Sarrafzadegan, Doxorubicin-related cardiotoxicity: review of fundamental pathways of cardiovascular system injury, Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology 73 (2024) 107683.

[11] K.B. Wallace, Doxorubicin-induced cardiac mitochondrionopathy, Pharmacology & toxicology 93(3) (2003) 105-115.

[12] C.-Y. Kong, Z. Guo, P. Song, X. Zhang, Y.-P. Yuan, T. Teng, L. Yan, Q.-Z. Tang, Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death, International journal of biological sciences 18(2) (2022) 760.

[13] A. Birk, W. Chao, C. Bracken, J. Warren, H. Szeto, Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis, British journal of pharmacology 171(8) (2014) 2017-2028.

[14] R. Gál, R. Halmosi, F. Gallyas, M. Tschida, P. Mutirangura, K. Tóth, T. Alexy, L. Czopf, Resveratrol and beyond: the effect of natural polyphenols on the cardiovascular system: a narrative review, Biomedicines 11(11) (2023) 2888.

[15] V.A. Brown, K.R. Patel, M. Viskaduraki, J.A. Crowell, M. Perloff, T.D. Booth, G. Vasilinin, A. Sen, A.M. Schinas, G. Piccirilli, K. Brown, W.P. Steward, A.J. Gescher, D.E. Brenner, Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis, Cancer research 70(22) (2010) 9003-11.

[16] S. Galiniak, D. Aebisher, D. Bartusik-Aebisher, Health benefits of resveratrol administration, Acta biochimica Polonica 66(1) (2019) 13-21.

[17] S. Seyyedebrahimi, H. Khodabandehloo, E. Nasli Esfahani, R. Meshkani, The effects of resveratrol on markers of oxidative stress in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial, Acta diabetologica 55(4) (2018) 341-353.

[18] F. Faul, E. Erdfelder, A.-G. Lang, A. Buchner, G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences, Behavior Research Methods 39(2) (2007) 175-191.

[19] V.W. Dolinsky, K.J. Rogan, M.M. Sung, B.N. Zordoky, M.J. Haykowsky, M.E. Young, L.W. Jones, J.R. Dyck, Both aerobic exercise and resveratrol supplementation attenuate doxorubicin-induced cardiac injury in mice, American Journal of Physiology-Endocrinology and Metabolism 305(2) (2013) E243-E253.

[20] N. Nagai, Y. Mano, H. Otake, T. Shibata, E. Kubo, H. Sasaki, Changes in mitochondrial cytochrome c oxidase mRNA levels with cataract severity in lens epithelia of Japanese patients, Molecular Medicine Reports 19(6) (2019) 5464-5472.

[21] M. Sarkar, S. Das, A. Bandyopadhaya, K. Ray, K. Chaudhuri, Upregulation of human mitochondrial NADH dehydrogenase subunit 5 in intestinal epithelial cells is modulated by Vibrio cholerae pathogenesis, FEBS letters 579(16) (2005) 3449-3460.

[22] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nature protocols 3(6) (2008) 1101-1108.

[23] I. Koss-Mikołajczyk, V. Todorovic, Natural Products Counteracting Cardiotoxicity during Cancer Chemotherapy: The Special Case of Doxorubicin, a Comprehensive Review, 22(18) (2021).

[24] D. Cardinale, M.T. Sandri, Role of biomarkers in chemotherapy-induced cardiotoxicity, Progress in cardiovascular diseases 53(2) (2010) 121-129.

[25] A.H.B. Wu, Release of cardiac troponin from healthy and damaged myocardium, Frontiers in Laboratory Medicine 1(3) (2017) 144-150.

[26] D. Yu, J. Xiong, Y. Gao, J. Li, D. Zhu, X. Shen, L. Sun, X. Wang, Resveratrol activates PI3K/AKT to reduce myocardial cell apoptosis and mitochondrial oxidative damage caused by myocardial ischemia/reperfusion injury, Acta Histochemica 123(5) (2021) 151739.

[27] T. Li, Z. Chen, Y. Zhou, H. Li, J. Xie, L. Li, Resveratrol pretreatment inhibits myocardial apoptosis in rats following coronary microembolization via inducing the PI3K/Akt/GSK-3β signaling cascade, Drug Design, Development and Therapy (2021) 3821-3834.

[28] J.L. Zamorano, P. Lancellotti, D. Rodriguez Muñoz, V. Aboyans, R. Asteggiano, M. Galderisi, G. Habib, D.J. Lenihan, G.Y. Lip, A.R. Lyon, T. Lopez Fernandez, D. Mohty, M.F. Piepoli, J. Tamargo, A. Torbicki, T.M. Suter, J.L. Zamorano, V. Aboyans, S. Achenbach, S. Agewall, L. Badimon, G. Barón-Esquivias, H. Baumgartner, J.J. Bax, H. Bueno, S. Carerj, V. Dean, Ç. Erol, D. Fitzsimons, O. Gaemperli, P. Kirchhof, P. Kolh, P. Lancellotti, G.Y. Lip, P. Nihoyannopoulos, M.F. Piepoli, P. Ponikowski, M. Roffi, A. Torbicki, A. Vaz Carneiro, S. Windecker, 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC), European journal of heart failure 19(1) (2017) 9-42.

[29] A. Foster, D. Greensmith, The effects of doxorubicin on calcium handling and contractility in sheep ventricular myocytes; role of oxidative stress, Physiology 38(S1) (2023) 5732119.

[30] D. Montaigne, X. Marechal, S. Preau, R. Baccouch, T. Modine, G. Fayad, S. Lancel, R. Neviere, Doxorubicin induces mitochondrial permeability transition and contractile dysfunction in the human myocardium, Mitochondrion 11(1) (2011) 22-26.

[31] J.M. Aldana, N.C. Mariaca, S.R. Uvera, Kiosk 5R-TA-05 - Impact of Edema and Myocardial Fibrosis on Systolic Function in Patients with Cardiotoxicity Due to Chemotherapy, Journal of Cardiovascular Magnetic Resonance 26 (2024) 100681.

[32] R. Gal, L. Deres, O. Horvath, K. Eros, B. Sandor, P. Urban, Resveratrol Improves Heart Function by Moderating Inflammatory Processes in Patients with Systolic Heart Failure, 9(11) (2020).

[33] M. Zheng, Y. Bai, X. Sun, R. Fu, L. Liu, M. Liu, Z. Li, X. Huang, Resveratrol reestablishes mitochondrial quality control in myocardial ischemia/reperfusion injury through Sirt1/Sirt3-Mfn2-Parkin-PGC-1α pathway, Molecules 27(17) (2022) 5545.

[34] D. Lebrecht, B. Setzer, U.-P. Ketelsen, J.r. Haberstroh, U.A. Walker, Time-dependent and tissue-specific accumulation of mtDNA and respiratory chain defects in chronic doxorubicin cardiomyopathy, Circulation 108(19) (2003) 2423-2429.

[35] B.B. Wu, K.T. Leung, E.N. Poon, Mitochondrial-Targeted Therapy for Doxorubicin-Induced Cardiotoxicity, 23(3) (2022).

[36] P.S. Rawat, A. Jaiswal, A. Khurana, J.S. Bhatti, U. Navik, Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management, Biomedicine & Pharmacotherapy 139 (2021) 111708.

[37] K.G. Cheung, L.K. Cole, B. Xiang, K. Chen, X. Ma, Y. Myal, G.M. Hatch, Q. Tong, V.W. Dolinsky, Sirtuin-3 (SIRT3) protein attenuates doxorubicin-induced oxidative stress and improves mitochondrial respiration in H9c2

cardiomyocytes, Journal of Biological Chemistry 290(17) (2015) 10981-10993.

[38] N. Wenningmann, M. Knapp, A. Ande, T.R. Vaidya, S. Ait-Oudhia, Insights into doxorubicin-induced cardiotoxicity: molecular mechanisms, preventive strategies, and early monitoring, Molecular pharmacology 96(2) (2019) 219-232.

[39] V. Desquiret-Dumas, N. Gueguen, G. Leman, S. Baron, V. Nivet-Antoine, S. Chupin, A. Chevrollier, E. Vessières, A. Ayer, M. Ferré, D. Bonneau, D. Henrion, P. Reynier, V. Procaccio, Resveratrol induces a mitochondrial complex I-dependent increase in NADH oxidation responsible for sirtuin activation in liver cells, The Journal of biological chemistry 288(51) (2013) 36662-75.

[40] B. Osborne, G.J. Cooney, N. Turner, Are sirtuin deacylase enzymes important modulators of mitochondrial energy metabolism?, Biochimica et Biophysica Acta (BBA) - General Subjects 1840(4) (2014) 1295-1302.

[41] F.B. Tabrizi, F. Yarmohammadi, A.W. Hayes, G. Karimi, The modulation of SIRT1 and SIRT3 by natural compounds as a therapeutic target in doxorubicin-induced cardiotoxicity: a review, Journal of biochemical and molecular toxicology 36(1) (2022) e22946.

[42] Y. Ruan, C. Dong, J. Patel, C. Duan, X. Wang, X. Wu, Y. Cao, L. Pu, D. Lu, T. Shen, SIRT1 suppresses doxorubicin-induced cardiotoxicity by regulating the oxidative stress and p38MAPK pathways, Cellular Physiology and Biochemistry 35(3) (2015) 1116-1124.

[43] Y.-g. Li, W. Zhu, J.-p. Tao, P. Xin, M.-y. Liu, J.-b. Li, M. Wei, Resveratrol protects cardiomyocytes from oxidative stress through SIRT1 and mitochondrial biogenesis signaling pathways, Biochemical and biophysical research communications 438(2) (2013) 270-276.

[44] L.F. Tu, L.F. Cao, Y.H. Zhang, Y.L. Guo, Y.F. Zhou, W.Q. Lu, T.Z. Zhang, T. Zhang, G.X. Zhang, H. Kurihara, Sirt3-dependent deacetylation of COX-1 counteracts oxidative stress-induced cell apoptosis, The FASEB Journal 33(12) (2019) 14118-14128.

[45] L. Chodari, M. Dilsiz Aytemir, P. Vahedi, M. Alipour, S.Z. Vahed, S.M.H. Khatibi, E. Ahmadian, M. Ardalan, A. Eftekhari, Targeting mitochondrial biogenesis with polyphenol compounds, Oxidative Medicine and Cellular Longevity 2021(1) (2021) 4946711.

[46] C.Y. Kong, Z. Guo, P. Song, X. Zhang, Y.P. Yuan, T. Teng, L. Yan, Q.Z. Tang, Underlying the Mechanisms of Doxorubicin-Induced Acute Cardiotoxicity: Oxidative Stress and Cell Death, International journal of biological sciences 18(2) (2022) 760-770.

[47] N. Sumneang, P. Tanajak, T.T. Oo, Toll-like receptor 4 inflammatory perspective on doxorubicin-induced cardiotoxicity, Molecules 28(11) (2023) 4294.

[48] D.N. Ngwa, A. Pathak, A. Agrawal, IL-6 regulates induction of C-reactive protein gene expression by activating STAT3 isoforms, Molecular immunology 146 (2022) 50-56.

[49] L.-M. Zhong, Y. Zong, L. Sun, J.-Z. Guo, W. Zhang, Y. He, R. Song, W.-M. Wang, C.-J. Xiao, D. Lu, Resveratrol inhibits inflammatory responses via the mammalian target of rapamycin signaling pathway in cultured LPS-stimulated microglial cells, PloS one 7(2) (2012) e32195.

[50] M.M. Poulsen, K. Fjeldborg, M.J. Ornstrup, T.N. Kjær, M.K. Nøhr, S.B. Pedersen, Resveratrol and inflammation: Challenges in translating pre-clinical findings to improved patient outcomes, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1852(6) (2015) 1124-1136.

[51] A.M. Gorabi, S. Aslani, D. Imani, B. Razi, T. Sathyapalan, A. Sahebkar, Effect of resveratrol on C-reactive protein: An updated meta-analysis of randomized controlled trials, Phytotherapy Research 35(12) (2021) 6754-6767.

[52] P.L. Dulf, M. Mocan, C.A. Coadă, D.V. Dulf, R. Moldovan, I. Baldea, A.D. Farcas, D. Blendea, A.G. Filip, Doxorubicin-induced acute cardiotoxicity is associated with increased oxidative stress, autophagy, and inflammation in a murine model, Naunyn Schmiedebergs Arch Pharmacol 396(6) (2023) 1105-1115.

[53] H. Zhu, S. Sarkar, L. Scott, I. Danelisen, M.A. Trush, Z. Jia, Y.R. Li, Doxorubicin redox biology: redox cycling, topoisomerase inhibition, and oxidative stress, Reactive oxygen species (Apex, NC) 1(3) (2016) 189.

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

[55] S.A. Abdelsalam, K. Renu, H.A. Zahra, B.M. Abdallah, E.M. Ali, V.P. Veeraraghavan, K. Sivalingam, L. Ronsard, R.B. Ammar, D.S. Vidya, Polyphenols mediate neuroprotection in cerebral ischemic stroke—An update, Nutrients 15(5) (2023) 1107.

[56] Z. Ungvari, Z. Bagi, A. Feher, F.A. Recchia, W.E. Sonntag, K. Pearson, R. De Cabo, A. Csiszar, Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2, American Journal of Physiology-Heart and Circulatory Physiology 299(1) (2010) H18-H24.