



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_157_26

Synergistic effect of resveratrol and curcumin against doxorubicin-induced cardiac injury: Network pharmacology, molecular mechanisms and *in-vivo* evidenceGaurav Borse¹, Raosaheb Ghegade², Mahesh Ghaisas¹, Vaibhav Shinde³, Ravindra Kulkarni⁴, Hemant Kamble⁵, Amol Muthal^{1✉}¹Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune, Maharashtra, 411038, India²Department of Pharmacognosy, Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik, 422005, India³Department of Pharmacognosy, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune, Maharashtra, 411038, India⁴Department of Pharmaceutical Chemistry, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune, Maharashtra, 411038, India⁵Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Maharashtra, 412211, India

ABSTRACT

Objective: To evaluate the cardioprotective effects of resveratrol and curcumin, individually and in combination, against doxorubicin-induced cardiac toxicity and explore the underlying mechanisms *via* network pharmacology.

Methods: *In silico* target prediction, enrichment analyses, and Cytoscape network modeling were performed to investigate shared molecular targets and pathways among resveratrol, curcumin and doxorubicin. Male Wistar rats ($n=6/\text{group}$) received oral treatments of vehicle, nebivolol (5 mg/kg), resveratrol (20 mg/kg), curcumin (100 mg/kg), resveratrol plus curcumin for 28 d, with cardiotoxicity induced *via* doxorubicin (2.5 mg/kg, *i.p.*) on days 7 and 14. Cardioprotection was evaluated using electrocardiographic, hemodynamic, biochemical, RT-PCR, and histopathological assays.

Results: Network analysis revealed four common targets (NFE2L2/Nrf2, TNF, CYP3A4, and MAPT) with significant protein-protein interaction enrichment ($P=0.0196$), implicating modulation of redox balance, inflammation, and xenobiotic metabolism. Concomitant therapy with resveratrol and curcumin significantly mitigated doxorubicin-induced cardiac injury by suppressing relative heart weight, reducing myocardial infarction area, reversing electrocardiographic and conduction abnormalities, attenuating dyslipidemia, and lowering serum CK-MB, LDH, and troponin I leakage ($P < 0.05$). Furthermore, combination treatment was more effective in restoring cardiac antioxidants, decreasing lipid peroxidation, downregulating mRNA expressions of *TNF- α* and *NF- κ B*, upregulating *Nrf2* mRNA expression, and improving myocardial architecture compared with individual monotherapies.

Conclusions: Concomitant therapy with resveratrol and curcumin

confers robust cardioprotection against doxorubicin-induced cardiotoxicity in rats, likely *via* coordinated activation of Nrf2-mediated antioxidant defenses and suppression of TNF- α /NF- κ B-driven inflammation, supporting their potential for future investigation as adjunct therapies during anthracycline treatment.

KEYWORDS: Cardiotoxicity; Resveratrol; Curcumin; Doxorubicin; Oxidative stress; Inflammation; Network pharmacology

Summary

Question: Can the combination treatment of resveratrol and curcumin protect against doxorubicin-induced cardiotoxicity?

Findings: Co-treatment with resveratrol and curcumin significantly attenuated myocardial necrosis, normalized electrocardiographic intervals, and restored cardiac antioxidant enzymes in rats with doxorubicin-induced cardiotoxicity, showing better cardioprotection than monotherapy with either agent.

Meaning: Combination therapy may serve as an effective adjunct strategy to prevent anthracycline-induced myocardial damage.

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How to cite this article: Borse G, Ghegade R, Ghaisas M, Shinde V, Kulkarni R, Kamble H, et al. Synergistic effect of resveratrol and curcumin against doxorubicin-induced cardiac injury: Network pharmacology, molecular mechanisms and *in-vivo* evidence. Asian Pac J Trop Biomed 2026; 16(9): 376–388.

Article history: Received 23 March 2026; Revision 8 May 2026; Accepted 23 July 2026; Available online 6 August 2026

1. Introduction

Doxorubicin (DOX) is a cornerstone anthracycline extracted from *Streptomyces* species and is used in cancer chemotherapy; it remains widely administered because of its broad antitumor activity and high efficacy against both solid and hematological malignancies[1]. Despite these therapeutic values, its clinical use is constrained by a well-recognized and dose-related risk of cardiotoxicity, which can compromise treatment continuity and long-term cardiovascular health in cancer survivors[2]. DOX-induced cardiotoxicity has become more clinically relevant, as it may induce a spectrum of side effects, from subclinical myocardial injury and transient electrocardiographic abnormalities to progressive cardiomyopathy and overt heart failure[3]. It is a multifactorial process rather than a consequence of a single lesion and has been extensively studied in both clinical and preclinical studies[4]. The central events of this toxicity include the generation of reactive oxygen species (ROS), redox cycling, and iron-associated free radical formation, which exceed myocardial antioxidant defenses and induce oxidative injury[5]. These changes facilitate lipid peroxidation at the cellular and mitochondrial membranes, compromise membrane integrity, alter ion handling, and disrupt the bioenergetic physiology of cardiac cells[6]. In addition to oxidative stress, mitochondrial dysfunction and inflammatory signaling are major mediators of DOX-induced myocardial injury.

Among naturally derived bioactive compounds, resveratrol, a polyphenolic stilbene, has attracted considerable attention as a promising cardioprotective agent[7]. Resveratrol is present in grapes, berries, and several medicinal plants found in India[8] and has been thoroughly investigated for its antioxidant, anti-inflammatory, anti-apoptotic, and vasoprotective effects[7]. Resveratrol alleviates oxidative stress by neutralizing ROS and reactive species, enhancing intrinsic antioxidant mechanisms, and modulating redox-sensitive signaling pathways linked to mitochondrial integrity and cellular viability[9]. It may also inhibit the expression of inflammatory mediators and reduce myocardial DNA damage and apoptosis, thereby restoring the structural integrity and preserving cardiac function[9]. Experimental research suggests that resveratrol may protect against several biochemical and histopathological alterations linked to DOX-induced cardiotoxicity, supporting its continued assessment in preclinical models[9]. Curcumin, the principal bioactive polyphenol of *Curcuma longa* (turmeric), is another extensively studied natural flavonoid with substantial pharmacological significance in reducing inflammation and acting as an antioxidant[10,11]. Its cardioprotective therapeutic potential derives from its antioxidant, anti-inflammatory, and cytoprotective effects, including reduced lipid peroxidation, suppression of pro-inflammatory signaling, preservation of endogenous antioxidant systems, and modulation of apoptosis and cellular stress response pathways[12]. In experimental models of DOX-induced injury,

curcumin has been shown to reduce myocardial oxidative damage, decrease the inflammatory burden, and improve the biochemical indicators of cardiac cell injury[12,13]. These effects are particularly relevant in anthracycline-induced cardiotoxicity, where oxidative imbalance and inflammatory responses are tightly interconnected[13]. However, despite concerns regarding curcumin bioavailability in translational contexts, the preclinical research supporting curcumin's protective role in cardiac injury remains sufficiently strong to justify its continued investigation as an adjunctive protective strategy[14].

The simultaneous or concomitant administration of resveratrol and curcumin is particularly noteworthy because of the potential of these phytoconstituents to function through overlapping, yet synergistic mechanisms[15]. Both compounds have demonstrated antioxidant and anti-inflammatory effects; however, their effects vary through different mechanisms of action or molecular pathways in redox homeostasis, mitochondrial preservation, inflammatory signaling, and cell death regulation[16]. Thus, their synergistic application may provide more extensive protection against the varied pathophysiology of DOX-induced cardiac cell damage than either agent employed separately[16]. This combination therapy offers additive or synergistic protection by simultaneously decreasing the free radical burden, inhibiting lipid peroxidation, maintaining mitochondrial function, and alleviating inflammatory and apoptotic pathways[9,12]. This comprehensive strategy is attractive for a model of cardiotoxicity resulting from multiple interacting pathways. Concurrently, rigorous experimental evaluation is crucial, as the magnitude of the cumulative advantage cannot be deduced from the known effects of individual substances. While resveratrol and curcumin have each shown encouraging cardioprotective effects in experimental studies, the benefit of their combined administration has not been clearly established in a well-standardized preclinical model. Given the multifactorial basis of anthracycline-induced myocardial injury, a combination strategy targeting oxidative stress, inflammation, mitochondrial dysfunction, and cardiomyocyte death may offer a more effective protective profile than monotherapy. Based on this, investigating the combined effect of resveratrol and curcumin in a validated Wistar rat model of DOX-induced cardiotoxicity is scientifically justified and pharmacologically relevant. Thus, the present study aimed to evaluate the cardioprotective activity of concomitant administration of resveratrol and curcumin against DOX-induced cardiac toxicity in Wistar rats.

2. Methods

2.1. *In silico* studies

2.1.1. Target prediction and gene set enrichment analysis

To determine the potential protein targets of the chosen compound, target forecasting and gene set functional enrichment analyses were

performed using the BindingDB and SuperPred online platforms with a probability threshold of 0.7 or higher. Gene IDs were retrieved from the UniProt database (<https://www.uniprot.org/>) for the predicted protein targets[17]. To examine the interactions between proteins, a collection of proteins was entered into the STRING v.11.0 database, which is a resource for the retrieval of interacting genes/proteins (<https://string-db.org/>).

2.1.2. Network analysis

Enrichment analysis revealed that the protein targets were associated with DOX and cardiotoxicity pathways. A datasheet was created to detail the interactions between resveratrol and curcumin, the protein targets were mapped, and an interaction network was generated using Cytoscape 3.10.3 software. The network was evaluated using a directed graph to capture the direction of the interactions. The topological parameter “edge count” was used to assess the level of connectivity among nodes. In the network, node sizes were adjusted from “low values to small sizes” and node colors ranged from “low values to blue and high values to green”, both based on edge count topological parameters[18].

2.1.3. Pathway analysis

Statistical analysis of gene pathway databases, including the Kyoto Encyclopedia of Genes and Genomes (KEGG; www.kegg.jp/)[19] and DisGeNet[20] databases, was used to identify functional processes linked to subgroups of genes within the sets of differentially expressed genes. ShinyGO v0.741 was utilized to conduct an overrepresentation analysis, enabling a comparison of outcomes through various computational methods[21]. The KEGG PATHWAY Database (<https://www.kegg.jp/kegg/pathway.html>) was used to identify and map target genes associated with the nuclear factor erythroid 2-related factor 2 (Nrf2) and tumor necrosis factor- α (TNF- α).

2.2. In vivo study

2.2.1. Animals and ethical approval

Adult male Wistar rats weighing 175–250 g were procured from Cryst Bio Solutions Pvt. Ltd., Pune. A sample size of $n=6$ per group was determined based on institutional CCSEA resource optimization guidelines and established historical protocols for rodent model cardiotoxicity evaluation to ensure adequate statistical power while minimizing animal use[22]. Following acquisition, the animals were housed in polypropylene cages under typical laboratory conditions, which included a 12-h light/dark cycle, a temperature of $(25 \pm 1)^\circ\text{C}$, and relative humidity of 45%–55%. The rats were fed a commercial pellet meal from Nav Maharashtra Chakan Oil Mills Ltd. in Pune and provided free access to water. The Institutional Animal Ethics Committee (IAEC) of Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, approved all experimental

procedures, which were conducted in compliance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals and in accordance with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. IAEC approval number PCP/IAEC/2024/4R-40 was officially granted on December 13, 2024, to conduct the study.

2.2.2. Experimental design

The animals were randomly divided into six groups of six rats each: Group I (vehicle control) received only the vehicle 1% carboxymethylcellulose (CMC, 10 mg/kg, *p.o.*), Group II (DOX control) received vehicle (1% CMC, 10 mg/kg, *p.o.*), Group III (nebivolol group) received nebivolol (5 mg/kg, *p.o.*) to serve as a positive control, Group IV (resveratrol group) received resveratrol (BLD Pharmatech, India Pvt. Ltd., India; purity $\geq 98\%$; 20 mg/kg in 1% CMC, *p.o.*), Group V (curcumin group) received curcumin (BLD Pharmatech, India Pvt. Ltd., India; purity $\geq 98\%$; 100 mg/kg in 1% CMC, *p.o.*), and Group VI (resveratrol and curcumin combination group) received a combination of resveratrol (20 mg/kg, *p.o.*) and curcumin (100 mg/kg, *p.o.*). The doses were selected based on previously reported studies[9,13]. The vehicle and DOX control groups received an identical 1% CMC vehicle. The rats received the respective treatments for 28 d, and cardiotoxicity was induced by intraperitoneal administration of DOX (Samarth Life Sciences Pvt. Ltd., India) in two equal injections of 2.5 mg/kg on days 7 and 14, with a total cumulative exposure of 5 mg/kg.

2.2.3. Analysis of hemodynamic and electrocardiogram (ECG) parameters

Electrodes were placed on the right foreleg (negative electrode), left foreleg (positive electrode), and right hind leg (neutral electrode) of the anesthetized rats to measure the ECG. An eight-channel Power Lab system (LabChart 7.3, AD Instrument Pty Ltd., Bella Vista, New South Wales, Australia) was used to record hemodynamic parameters, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP), as well as electrocardiographic changes, including the heart rate, RR, QRS, QT, QTc, and PR intervals, and ST segment level.

2.2.4. Serum parameters

Blood was collected using the retro-orbital plexus technique, and serum was separated by centrifugation using an Eppendorf cryocentrifuge (model no. 5810; Eppendorf, Hamburg, Germany) maintained at 4°C and run at a speed of 7000 rpm for 15 min. On days 0, 15, and 28, serum levels of cardiac troponin-I (cTn-I), lactate dehydrogenase (LDH), creatine kinase isoenzyme MB (CK-MB), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) were measured using commercial reagent kits (Accurex Biomedical

Pvt. Ltd., Mumbai, India and Pathozyme Diagnostics, India) and a spectrophotometer (UV-visible spectrophotometer, Jasco V-530, Tokyo, Japan).

2.2.5. Biochemical and molecular analyses

At the end of the study (day 29), all animals were euthanized by cervical dislocation, and cardiac tissue was immediately isolated and preserved at -80°C for biochemical analysis. 2,3,5-Triphenyltetrazolium chloride (TTC) staining was performed on one portion of the cardiac tissue to assess the myocardial infarction. Another portion of the cardiac tissue was homogenized using 0.1 M Tris-HCl buffer (pH 7.4), and the homogenate supernatant was used to estimate total protein, superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), and catalase (CAT). The expression levels of nuclear factor kappa-light-chain-enhancer of activated B cells (*NF- κ B*), *TNF- α* , and *Nrf2* were measured using reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Briefly, total ribonucleic acid (RNA) was isolated from the tissue using a lysis and spin-column protocol (Biotools B & M Labs, Spain), followed by on-column deoxyribonucleic acid (DNA) digestion and purification. This RNA was then used to synthesize complementary DNA (cDNA), which was amplified *via* RT-PCR using specific primers (Supplementary Table) with β -actin as an internal control. The resulting amplicons were separated by agarose gel electrophoresis, visualized with ethidium bromide, and documented using a gel-imaging system. Finally, gene expression was semi-quantitatively analyzed using a gel documentation system (Alpha Innotech Inc., USA) and ImageJ software (Version 1.33, USA) by calculating the densitometric ratio of target mRNA to β -actin.

2.2.6. Histopathological analysis

Another portion of the cardiac tissue samples was preserved in a fixative solution (10% formalin) and processed for 12 h using paraffin, xylene, and isopropyl alcohol for light microscopy (Motic BA). Hematoxylin and Eosin (H&E) and Masson's trichrome staining were used to prepare paraffin-embedded tissue sections, which were cut to a thickness of 5 μm . Under a light microscope (40 $\times$ and 100 $\times$), the tissue sections were qualitatively evaluated by an observer blinded to the groups for architectural damage, signs of inflammation, congestion, edema, and necrosis. No automated scoring software was used in this study.

2.3. Statistical analysis

All results were statistically analyzed using GraphPad Prism software (GraphPad Software, La Jolla, California, USA), version 8.0.2. Two-way repeated-measures analysis of variance (ANOVA), followed by Tukey's multiple range test, was used to analyze biochemical results. One-way ANOVA, followed by Tukey's multiple range test, was employed to assess the statistical

significance of all remaining results. Values are presented as mean $\pm$ SEM. Statistical significance was set at $P < 0.05$.

3. Results

3.1. *In silico* studies

Network pharmacology analysis identified a limited but meaningful overlap among the target profiles of resveratrol, curcumin, and DOX. The intersection analysis showed 33 unique targets for resveratrol, 54 for curcumin, and 127 for DOX, with shared regions comprising three targets between resveratrol and curcumin, ten between resveratrol and DOX, eight between curcumin and DOX, and four targets common to all three. This intersection indicates the presence of a small but relevant shared molecular space through which resveratrol and curcumin may influence the pathways associated with DOX-induced toxicity.

This network pattern was consistent with a polypharmacological profile and supports the involvement of multiple pathways related to oxidative stress, inflammatory signaling, cellular stress adaptation, and xenobiotic handling, rather than a single-target mechanism (Figure 1A).

The PPI network showed significant connectivity among the four core proteins, with six observed interactions compared to two expected interactions (PPI enrichment, $P = 0.0196$). This supports the presence of a functionally coordinated target set in which NFE2L2 is linked to antioxidant defense, TNF with inflammatory signaling, CYP3A4 with xenobiotic metabolism, and MAPT with broader structural or regulatory functions (Figure 1B). These *in silico* findings support a multi-target, multi-pathway framework for the potential modulatory role of resveratrol and curcumin against DOX-induced toxicity, particularly through pathways related to oxidative stress, inflammation, and xenobiotic metabolism.

KEGG pathway analysis showed enrichment in pathways associated with linoleic acid metabolism, steroid hormone biosynthesis, retinol metabolism, drug metabolism by cytochrome P450, metabolism of xenobiotics by cytochrome P450, protein processing in the endoplasmic reticulum, and chemical carcinogenesis, such as ROS, indicating a coordinated involvement of lipid metabolism, detoxification, oxidative stress handling, and adaptation to chemical injury. Although not all enriched pathways are cardiac-specific, their collective profiles support biologically relevant processes linked to systemic toxic stress and cytoprotection (Figure 1C).

3.2. *In-vivo* study

3.2.1. Cardiac tissue morphology and TTC staining

Gross cross-sectional examination and TTC staining revealed severe myocardial damage in DOX-treated rats, as evidenced by structural distortion, irregular ventricular cavities, and prominent

pale infarcted regions. In contrast, the vehicle control group maintained a regular cardiac architecture and uniform red staining, indicating a healthy, viable myocardium. Monotherapy with resveratrol or curcumin partially preserved cardiac morphology and reduced the extent of infarcted areas compared to the DOX control group. However, the combination of resveratrol and curcumin afforded the highest degree of cardioprotection, maintaining section integrity and myocardial viability more effectively than single-agent treatments (Supplementary Figure 1A-B).

3.2.2. Body weight and heart weight

DOX administration induced significant systemic and cardiac

toxicity, characterized by a marked reduction in final body weight and a significant increase in absolute heart weight and heart-to-body weight ratio compared with the vehicle control group ($P < 0.05$). These changes indicate the induction of cardiac hypertrophy and edema alongside general physiological decline. Treatment with resveratrol or curcumin alone significantly attenuated these alterations ($P < 0.05$) compared to the DOX control group. Notably, the combination of resveratrol or curcumin demonstrated a superior protective effect ($P < 0.05$) compared to their respective monotherapies, resulting in the most pronounced normalization of gravimetric parameters, with values closely approaching those of the vehicle control (Supplementary Figure 1C-E). No animal mortality

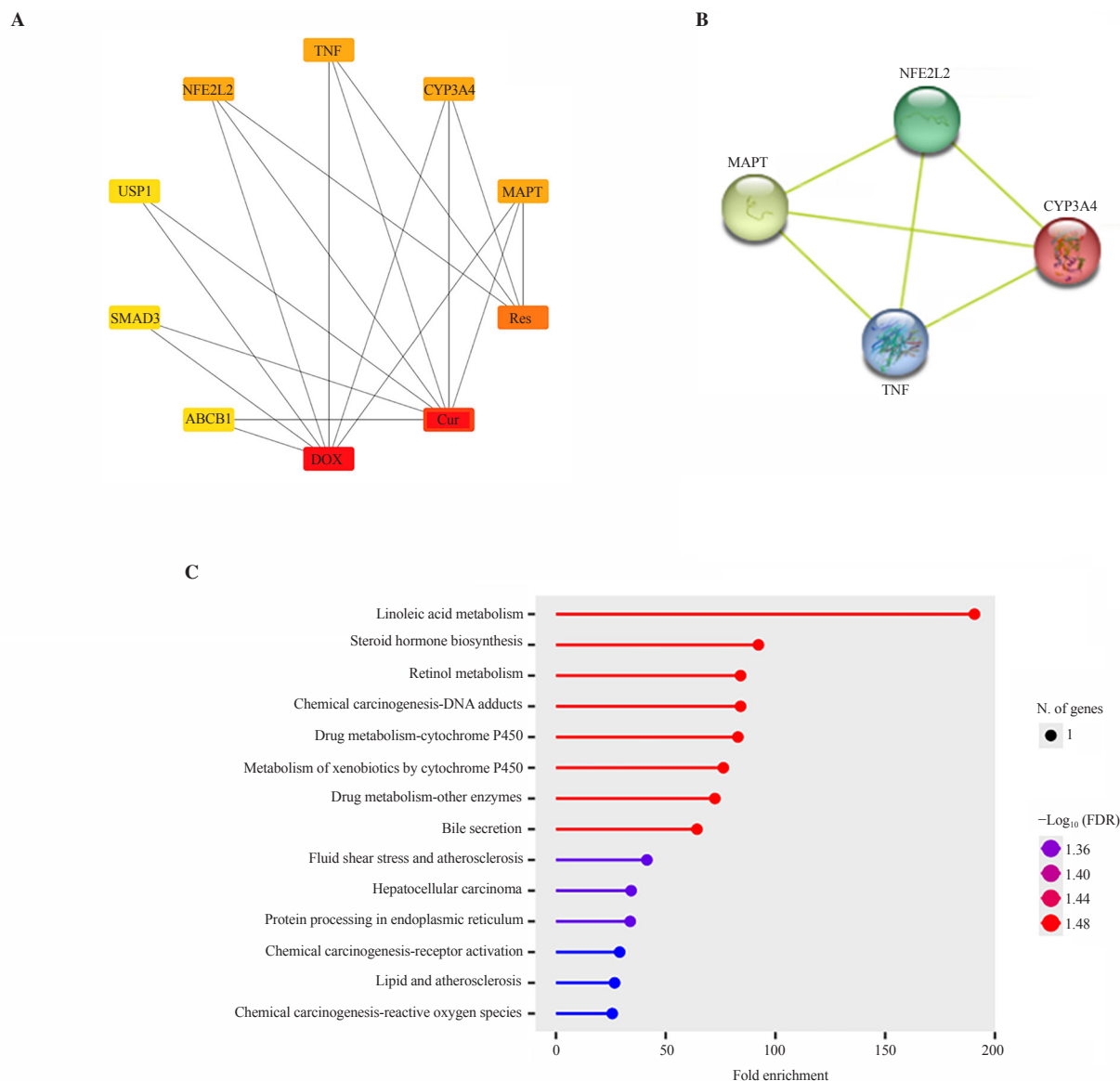


Figure 1. Network representation of resveratrol, curcumin, protein targets, and molecular pathways involved in doxorubicin (DOX)-induced cardiotoxicity. (A) Compound-target network illustrating the interactions between DOX, resveratrol and curcumin, along with their protein targets. (B) A STRING protein-protein network analysis is presented with nodes. (C) Functional enrichment of differentially expressed genes in response to resveratrol and curcumin treatment, represented as a lollipop graph on a $-\log_{10}(P)$ scale, based on Metascape enrichment analysis of differentially expressed genes-associated pathways. Cur: curcumin; Res: resveratrol; FDR: false discovery rate.

was observed throughout the 28-day protocol duration.

3.2.3. Hemodynamic and electrocardiographic parameters

DOX exposure resulted in marked hemodynamic disturbances and progressive deterioration of cardiac electrical activity (Figure 2). Compared to the vehicle control, DOX-treated rats exhibited significant elevations in SBP, DBP, and MABP, along with a decline in heart rate and substantial prolongation of RR, PR, QRS, QT, QTc, and ST intervals ($P < 0.05$). Although single-agent treatments with resveratrol

or curcumin significantly mitigated these conduction and pressure abnormalities ($P < 0.05$) from day 15 onwards compared to the DOX control, the combination of resveratrol and curcumin was more effective ($P < 0.05$) than the individual components. This combination regimen provided the most consistent protection, restoring the heart rate and conduction intervals to near-normal levels on day 28 (Table 1).

3.2.4. Serum CK-MB, LDH, and cTn-I levels

The induction of myocardial necrosis by DOX was confirmed by

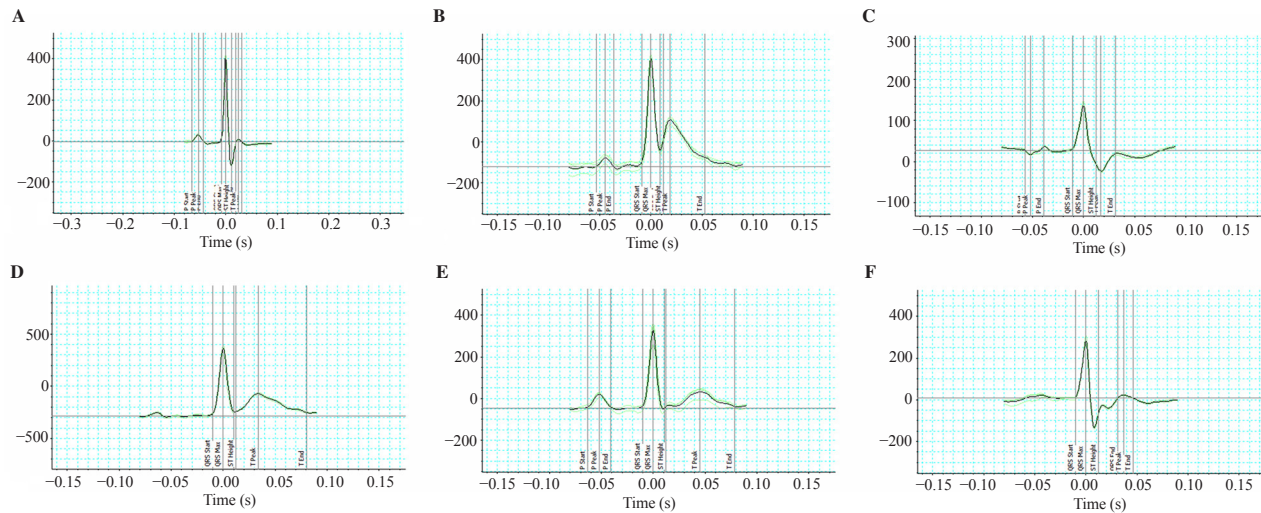


Figure 2. Effects of resveratrol and curcumin on DOX-induced alterations in electrocardiographic tracking in rats. Representative images of hearts from rats subjected to vehicle control (A), DOX control (B), DOX + nebivolol (C), DOX + resveratrol (D), DOX + curcumin (E), and DOX + resveratrol + curcumin (F) treatments.

Table 1. Effects of resveratrol and curcumin on doxorubicin-induced electrocardiographic and hemodynamic alterations.

Parameter	Days	Vehicle control	DOX control	DOX + nebivolol (5 mg/kg)	DOX + resveratrol (20 mg/kg)	DOX + curcumin (100 mg/kg)	DOX + resveratrol (20 mg/kg) + curcumin (100 mg/kg)
Heart rate (bpm)	0	313.67 ± 4.72	313.67 ± 5.04	307.33 ± 3.21	311.67 ± 2.32	318.50 ± 5.23	319.67 ± 2.67
	15	315.33 ± 4.40	266.83 ± 4.71 [#]	308.33 ± 4.90 [§]	294.67 ± 3.31 [§]	291.67 ± 4.63 [§]	299.67 ± 2.96 [*]
	28	316.17 ± 5.02	236.83 ± 3.74 [#]	309.67 ± 2.69 [§]	280.33 ± 4.81 [§]	288.33 ± 4.88 [§]	304.00 ± 4.15 [*]
RR interval (ms)	0	150.50 ± 2.74	150.83 ± 3.20	150.83 ± 4.99	148.67 ± 5.23	149.83 ± 5.13	150.33 ± 3.49
	15	148.33 ± 3.57	190.67 ± 2.65 [#]	175.67 ± 3.84 [§]	169.83 ± 4.04 [§]	165.33 ± 4.19 [§]	157.83 ± 5.21 [*]
	28	154.33 ± 3.69	206.17 ± 2.46 [#]	171.17 ± 4.00 [§]	171.50 ± 3.39 [§]	174.67 ± 3.94 [§]	159.83 ± 4.89 [*]
PR interval (ms)	0	52.33 ± 1.48	53.50 ± 1.61	53.83 ± 1.28	53.83 ± 2.01	53.33 ± 1.28	54.00 ± 1.67
	15	52.33 ± 1.20	70.50 ± 1.54 [#]	65.67 ± 1.73 [§]	63.17 ± 1.35 [§]	63.67 ± 1.52 [§]	59.17 ± 1.75 [*]
	28	54.00 ± 0.97	75.50 ± 1.65 [#]	60.00 ± 1.29 [§]	62.83 ± 0.91 [§]	63.17 ± 1.56 [§]	57.17 ± 1.32 [*]
QRS interval (ms)	0	14.50 ± 0.99	13.67 ± 1.05	14.67 ± 1.05	14.83 ± 1.05	14.50 ± 0.96	15.17 ± 0.83
	15	14.33 ± 0.99	27.67 ± 0.99 [#]	23.83 ± 1.19 [§]	24.00 ± 0.93 [§]	23.83 ± 0.70 [§]	22.00 ± 0.73 [*]
	28	14.33 ± 0.67	32.33 ± 0.80 [#]	24.17 ± 0.75 [§]	23.00 ± 0.93 [§]	22.67 ± 0.67 [§]	17.83 ± 0.79 [*]
QT interval (ms)	0	46.00 ± 1.48	47.17 ± 1.51	44.00 ± 1.67	45.83 ± 1.49	44.17 ± 1.40	44.17 ± 1.83
	15	48.33 ± 1.82	72.67 ± 1.45 [#]	59.83 ± 1.28 [§]	58.00 ± 2.02 [§]	56.83 ± 1.25 [§]	52.50 ± 1.72 [*]
	28	47.67 ± 1.05	88.17 ± 1.35 [#]	63.33 ± 1.94 [§]	65.67 ± 1.15 [§]	66.33 ± 1.87 [§]	62.83 ± 0.79 [*]
QTc interval (ms)	0	156.00 ± 3.24	153.00 ± 3.12	157.33 ± 4.26	157.00 ± 5.73	153.17 ± 4.51	157.00 ± 4.85
	15	157.33 ± 5.00	188.17 ± 4.29 [#]	174.50 ± 4.03 [§]	169.83 ± 4.80 [§]	167.17 ± 3.89 [§]	159.83 ± 4.46 [*]
	28	161.17 ± 3.80	208.83 ± 4.00 [#]	177.67 ± 4.19 [§]	172.67 ± 2.26 [§]	171.33 ± 4.53 [§]	163.17 ± 3.15 [*]
ST interval (ms)	0	20.17 ± 0.79	20.67 ± 0.21	19.67 ± 0.67	20.67 ± 0.92	20.00 ± 0.52	20.33 ± 0.55
	15	20.50 ± 0.67	31.67 ± 0.71 [#]	25.00 ± 0.73 [§]	26.00 ± 0.58 [§]	24.50 ± 0.56 [§]	22.33 ± 0.76 [*]
	28	21.17 ± 0.79	36.17 ± 0.83 [#]	26.67 ± 0.80 [§]	28.00 ± 0.73 [§]	27.00 ± 0.86 [§]	25.33 ± 0.55 [*]
SBP (mmHg)	28	112.30 ± 1.31	157.50 ± 1.38 [#]	118.70 ± 1.17 [§]	123.70 ± 1.05 [§]	119.50 ± 0.67 [§]	119.00 ± 0.97 [*]
DBP (mmHg)	28	88.00 ± 1.34	124.30 ± 0.92 [#]	92.17 ± 0.54 [§]	97.33 ± 0.92 [§]	98.50 ± 0.99 [§]	93.67 ± 0.92 [*]
MABP (mmHg)	28	69.33 ± 1.20	106.00 ± 0.82 [#]	72.50 ± 0.22 [§]	81.67 ± 0.99 [§]	80.17 ± 0.48 [§]	73.67 ± 0.92 [*]

Data are expressed as mean ± SEM ($n=6$) and analyzed using two-way ANOVA, followed by Tukey's multiple range test. ^{*} $P < 0.05$ compared to the DOX control group, [#] $P < 0.05$ compared to the vehicle control group, and [§] $P < 0.05$ compared to the combination group. DOX: doxorubicin; SBP: systolic blood pressure; DBP: diastolic blood pressure; MABP: mean arterial blood pressure.

a significant and time-dependent increase in serum cardiac injury markers, CK-MB, LDH, and cTn-I, compared to the vehicle control group ($P < 0.05$). Treatment with resveratrol or curcumin alone significantly reduced the leakage of these enzymes into the systemic circulation ($P < 0.05$) relative to the DOX control. Combination treatment with resveratrol and curcumin conferred a greater effect ($P < 0.05$) than the individual treatments, with CK-MB and LDH levels returning to values nearly close to those in the vehicle control group on day 28 (Table 2).

3.2.5. Serum lipid profile

DOX administration disrupted lipid homeostasis, resulting in a significant increase in TC, TG, LDL-C, and VLDL-C levels and a

concurrent decline in HDL-C levels compared to the vehicle control ($P < 0.05$). While individual treatments with resveratrol or curcumin significantly improved the lipid profile ($P < 0.05$) compared to the DOX control, the combination of resveratrol and curcumin yielded the most robust restoration of lipid profiles. This dual-treatment approach was significantly more effective ($P < 0.05$) in lowering atherogenic lipids and maximizing HDL levels than the respective monotherapies (Table 2).

3.2.6. Oxidative stress markers

Biochemical analysis of cardiac tissue indicated that DOX induced severe oxidative stress, characterized by a significant reduction in total protein and antioxidant enzymes (SOD, GSH, and CAT) and a sharp increase in MDA compared to the vehicle control ($P < 0.05$).

Table 2. Effects of resveratrol and curcumin on serum CK-MB, LDH, and cardiac troponin I levels, as well as lipid profile in rats with doxorubicin-induced cardiotoxicity.

Parameter	Days	Vehicle control	DOX control	DOX + nebivolol (5 mg/kg)	DOX + resveratrol (20 mg/kg)	DOX + curcumin (100 mg/kg)	DOX + resveratrol (20 mg/ kg) + curcumin (100 mg/kg)
Serum CK-MB (IU/L)	0	22.04 ± 0.56	22.12 ± 0.58	22.59 ± 0.92	21.95 ± 0.69	22.48 ± 0.59	22.53 ± 0.64
	15	21.97 ± 0.53	29.96 ± 0.57 [#]	26.84 ± 1.05 ^{*s}	24.78 ± 0.58 ^{*s}	25.18 ± 0.64 ^{*s}	23.44 ± 0.66 [*]
	28	22.05 ± 0.59	30.43 ± 0.63 [#]	25.14 ± 1.05 ^{*s}	24.49 ± 0.58 ^{*s}	24.64 ± 0.50 ^{*s}	22.14 ± 0.68 [*]
Serum LDH (mg/dL)	0	278.20 ± 3.35	280.43 ± 3.06	277.25 ± 3.36	275.70 ± 3.09	279.06 ± 2.94	277.53 ± 3.14
	15	278.95 ± 3.50	354.32 ± 3.25 [#]	320.29 ± 3.38 ^{*s}	317.80 ± 2.83 ^{*s}	321.97 ± 3.25 ^{*s}	285.97 ± 3.33 [*]
	28	278.46 ± 2.75	369.93 ± 3.51 [#]	309.81 ± 3.42 ^{*s}	307.70 ± 3.08 ^{*s}	309.80 ± 3.54 ^{*s}	274.88 ± 3.03 [*]
Serum LDL-C (mg/dL)	0	68.28 ± 3.12	69.32 ± 3.20	69.23 ± 3.37	68.26 ± 3.35	68.95 ± 3.21	68.24 ± 3.49
	15	68.07 ± 3.37	94.97 ± 3.25 [#]	83.42 ± 3.08 ^{*s}	84.87 ± 3.23 ^{*s}	85.89 ± 3.21 ^{*s}	74.89 ± 3.34 [*]
	28	67.98 ± 3.23	101.25 ± 3.18 [#]	72.81 ± 3.01 ^{*s}	78.47 ± 3.22 ^{*s}	78.46 ± 3.30 ^{*s}	70.01 ± 3.35 [*]
Serum HDL-C (mg/dL)	0	23.95 ± 1.09	23.27 ± 1.11	23.66 ± 1.08	23.53 ± 1.17	23.40 ± 1.14	23.92 ± 1.14
	15	24.13 ± 1.08	17.92 ± 1.15 [#]	21.70 ± 1.21 ^{*s}	21.17 ± 1.28 ^{*s}	21.04 ± 1.16 ^{*s}	23.67 ± 1.16 [*]
	28	24.01 ± 1.23	15.90 ± 1.18 [#]	22.59 ± 1.29 ^{*s}	19.79 ± 1.08 ^{*s}	19.71 ± 1.10 ^{*s}	23.82 ± 1.17 [*]
Serum TC (mg/dL)	0	99.27 ± 3.92	100.13 ± 3.82	99.14 ± 3.95	99.41 ± 3.99	99.61 ± 3.80	99.33 ± 3.96
	15	99.14 ± 4.04	165.00 ± 3.90 [#]	147.17 ± 3.88 ^{*s}	143.41 ± 4.00 ^{*s}	145.05 ± 4.05 ^{*s}	125.12 ± 3.94 [*]
	28	99.01 ± 3.76	185.32 ± 3.81 [#]	124.85 ± 4.02 ^{*s}	119.37 ± 3.94 ^{*s}	125.15 ± 3.86 ^{*s}	103.92 ± 3.91 [*]
Serum TG (mg/dL)	0	59.08 ± 1.82	60.11 ± 1.87	60.34 ± 1.80	60.24 ± 1.92	60.08 ± 1.80	60.02 ± 1.99
	15	59.42 ± 1.86	88.16 ± 1.99 [#]	81.27 ± 1.99 ^{*s}	80.01 ± 1.95 ^{*s}	80.50 ± 1.88 ^{*s}	75.00 ± 1.88 [*]
	28	59.46 ± 1.74	100.22 ± 1.92 [#]	79.81 ± 1.68 ^{*s}	80.78 ± 1.90 ^{*s}	78.69 ± 1.89 ^{*s}	67.00 ± 1.81 [*]
Serum VLDL-C (mg/dL)	0	19.85 ± 1.06	20.03 ± 1.05	19.83 ± 1.07	19.88 ± 1.08	19.92 ± 1.04	19.87 ± 1.07
	15	19.83 ± 1.09	33.00 ± 1.06 [#]	29.43 ± 1.06 ^{*s}	28.68 ± 1.08 ^{*s}	29.01 ± 1.09 ^{*s}	25.02 ± 1.07 [*]
	28	19.80 ± 1.03	37.06 ± 1.04 [#]	24.97 ± 1.09 ^{*s}	23.87 ± 1.07 ^{*s}	25.03 ± 1.05 ^{*s}	20.78 ± 1.06 [*]
Serum cTn-I (pg/mL)	28	0.93 ± 0.08	4.39 ± 0.09 [#]	1.26 ± 0.08 ^{*s}	1.74 ± 0.07 ^{*s}	1.97 ± 0.07 ^{*s}	1.23 ± 0.08 [*]

Data are expressed as mean ± SEM ($n=6$) and analyzed using two-way ANOVA (for serum CK-MB, LDH, LDL-C, HDL-C, TC, TG, and VLDL-C), or one-way ANOVA (for serum cTn-I) followed by Tukey's multiple range test. ^{*} $P < 0.05$ compared to the DOX control group, [#] $P < 0.05$ compared to the vehicle control group, and ^s $P < 0.05$ compared to the combination group. CK-MB: creatine kinase isoenzyme MB; HDL-C: high-density lipoprotein cholesterol; LDH: lactate dehydrogenase; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; VLDL-C: very-low-density lipoprotein cholesterol; cTn-I: cardiac troponin-I.

Table 3. Effects of resveratrol and curcumin on antioxidant markers in rats with doxorubicin-induced cardiotoxicity.

Parameter	Vehicle control	DOX control	DOX + nebivolol (5 mg/kg)	DOX + resveratrol (20 mg/kg)	DOX + curcumin (100 mg/kg)	DOX + resveratrol (20 mg/ kg) + curcumin (100 mg/kg)
Total protein (g/mL)	6.69 ± 0.31	1.78 ± 0.31 [#]	5.56 ± 0.31 ^{*s}	4.84 ± 0.31 ^{*s}	4.92 ± 0.31 ^{*s}	5.98 ± 0.31 [*]
SOD (U/mg protein)	10.01 ± 0.65	3.55 ± 0.60 [#]	8.10 ± 0.69 ^{*s}	8.75 ± 0.69 ^{*s}	7.90 ± 0.64 ^{*s}	9.35 ± 0.64 [*]
GSH (μmol/g tissue)	7.53 ± 0.40	1.92 ± 0.40 [#]	6.58 ± 0.37 ^{*s}	5.23 ± 0.62 ^{*s}	5.61 ± 0.64 ^{*s}	6.60 ± 0.38 [*]
MDA (nM/mg protein)	2.00 ± 0.20	9.80 ± 0.21 [#]	4.19 ± 0.20 ^{*s}	5.78 ± 0.38 ^{*s}	6.18 ± 0.40 ^{*s}	3.40 ± 0.21 [*]
CAT (U/mg protein)	41.49 ± 1.68	11.73 ± 1.48 [#]	35.50 ± 1.58 ^{*s}	29.63 ± 1.49 ^{*s}	32.92 ± 1.56 ^{*s}	40.23 ± 1.55 [*]

Data are expressed as mean ± SEM ($n=6$) and analyzed using one-way ANOVA, followed by Tukey's multiple range test. ^{*} $P < 0.05$ compared to the DOX control group, [#] $P < 0.05$ compared to the vehicle control group, and ^s $P < 0.05$ compared to the combination group. CAT: catalase; GSH: glutathione; MDA: malondialdehyde; SOD: superoxide dismutase.

Treatment with resveratrol or curcumin significantly boosted the antioxidant defense system and reduced lipid peroxidation ($P < 0.05$) compared to the DOX control. The combination of resveratrol and curcumin provided the most significant enhancement in antioxidant status and the lowest levels of MDA ($P < 0.05$), demonstrating a synergistic protective effect compared to either agent used alone (Table 3).

3.2.7. Cardiac *TNF- α* , *Nrf2*, and *NF- κ B* mRNA expressions

DOX administration triggered a pro-inflammatory and oxidative stress state, as evidenced by significantly upregulated mRNA expression of *TNF- α* and *NF- κ B* and downregulated expression of *Nrf2* compared to the vehicle control ($P < 0.05$). While monotherapies significantly attenuated DOX-induced changes in these mRNA expressions ($P < 0.05$) relative to the DOX control, combination treatment was even more effective ($P < 0.05$) (Figure 3).

3.2.8. Cardiac histology

Histopathological evaluation of the myocardium *via* H & E staining revealed severe architectural disturbances in the DOX control group compared to the vehicle control group. The vehicle control group exhibited regularly arranged myocardial fibers and preserved histology (Figure 4A), whereas the DOX control group showed marked structural disruption, including loss of normal fiber alignment and visible myocardial damage (Figure 4B). Treatment with nebivolol resulted in noticeable structural improvement, with better-defined fiber orientation and less myocardial damage than that in the DOX control (Figure 4C). Resveratrol monotherapy moderately attenuated the structural changes, showing

partial protection of myocardial tissue (Figure 4D). Curcumin administration demonstrated relatively superior preservation of myocardial organization among the individual treatments, with enhanced fiber continuity compared to the DOX control (Figure 4E). Combination treatment with resveratrol and curcumin produced the most robust restoration of cardiac histology in this study. This group showed a remarkably higher degree of structural integrity and near-complete preservation of myocardial continuity than the corresponding single-agent treatments, closely resembling the pristine histological pattern of vehicle control rats (Figure 4F).

Masson's trichrome staining further confirmed this pattern of injury and protection. The DOX control group exhibited pronounced structural derangement and fibrotic remodeling, consistent with myocardial injury (Figure 4H), which differed from the normal myocardial structure of the vehicle control group (Figure 4G). The nebivolol treatment group showed substantial attenuation of these changes and improved tissue organization compared to the disease control group (Figure 4I). Rats receiving resveratrol monotherapy showed a discernible decrease in structural abnormalities compared to those in the DOX control group (Figure 4J). Curcumin treatment further mitigated these changes, showing refined tissue organization and stronger preservation of myocardial architecture compared to resveratrol monotherapy (Figure 4K). The resveratrol and curcumin combination group demonstrated the most notable protection against structural remodeling (Figure 4L). These sections showed strikingly clearer preservation of myocardial architecture with minimal remodeling compared to both the DOX control and the respective monotherapy groups.

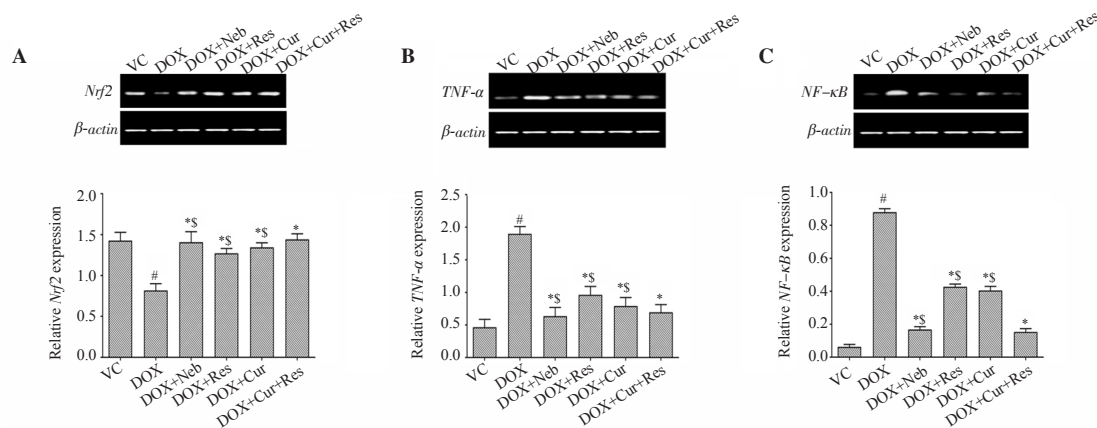


Figure 3. Effects of resveratrol and curcumin on DOX-induced alterations in the mRNA expression levels of *Nrf2* (A), *TNF- α* (B), and *NF- κ B* (C). Data are expressed as mean $\pm$ SEM ($n=6$) and analyzed using one-way ANOVA, followed by Tukey's multiple range test. β -actin is used as an internal control. ^{*} $P < 0.05$ compared to the DOX control group, [#] $P < 0.05$ compared to the VC group, and ^{\$} $P < 0.05$ compared to the combination group. VC: vehicle control; Neb: nebivolol; Res: resveratrol; Cur: curcumin. *Nrf2*: nuclear factor erythroid 2-related factor 2; *TNF- α* : tumor necrosis factor- α ; *NF- κ B*: nuclear factor kappa-light-chain-enhancer of activated B cells.

4. Discussion

DOX is a highly effective anthracycline extracted from *Streptomyces* species; however, its therapeutic effectiveness is often limited by cumulative dose-related cardiac injury. DOX-induced cardiotoxicity primarily results from oxidative stress, mitochondrial dysfunction, and activation of inflammatory and cell death pathways, leading to irreversible cardiac damage. Although no definitive treatment exists, strategies such as iron chelation with dexrazoxane, pharmacological agents, and non-pharmacological interventions such as exercise have shown potential in reducing cardiac injury[23]. The present study demonstrated that DOX caused cardiotoxicity in Wistar rats, which was manifested at the structural, functional, biochemical, molecular, and histological levels. In contrast, treatment with resveratrol and

curcumin reduced the severity of this damage, with the combination regimen showing the most pronounced protective effect. This synergistic effect is mediated through the simultaneous modulation of oxidative stress, inflammatory signaling, and lipid metabolism pathways.

DOX reduces body weight, reflecting systemic toxicity and metabolic disturbances during treatment[24]. The observed decline in final body weight in the DOX control group was consistent with the systemic burden of anthracycline toxicity and may reflect worsening general physiological conditions, reduced metabolic efficiency, and progressive organ dysfunction. In the present study, the attenuation of body weight loss by monotherapies and combination treatment indicated the restoration of systemic physiological stability alongside cardiac protection. DOX causes cardiac remodeling, characterized

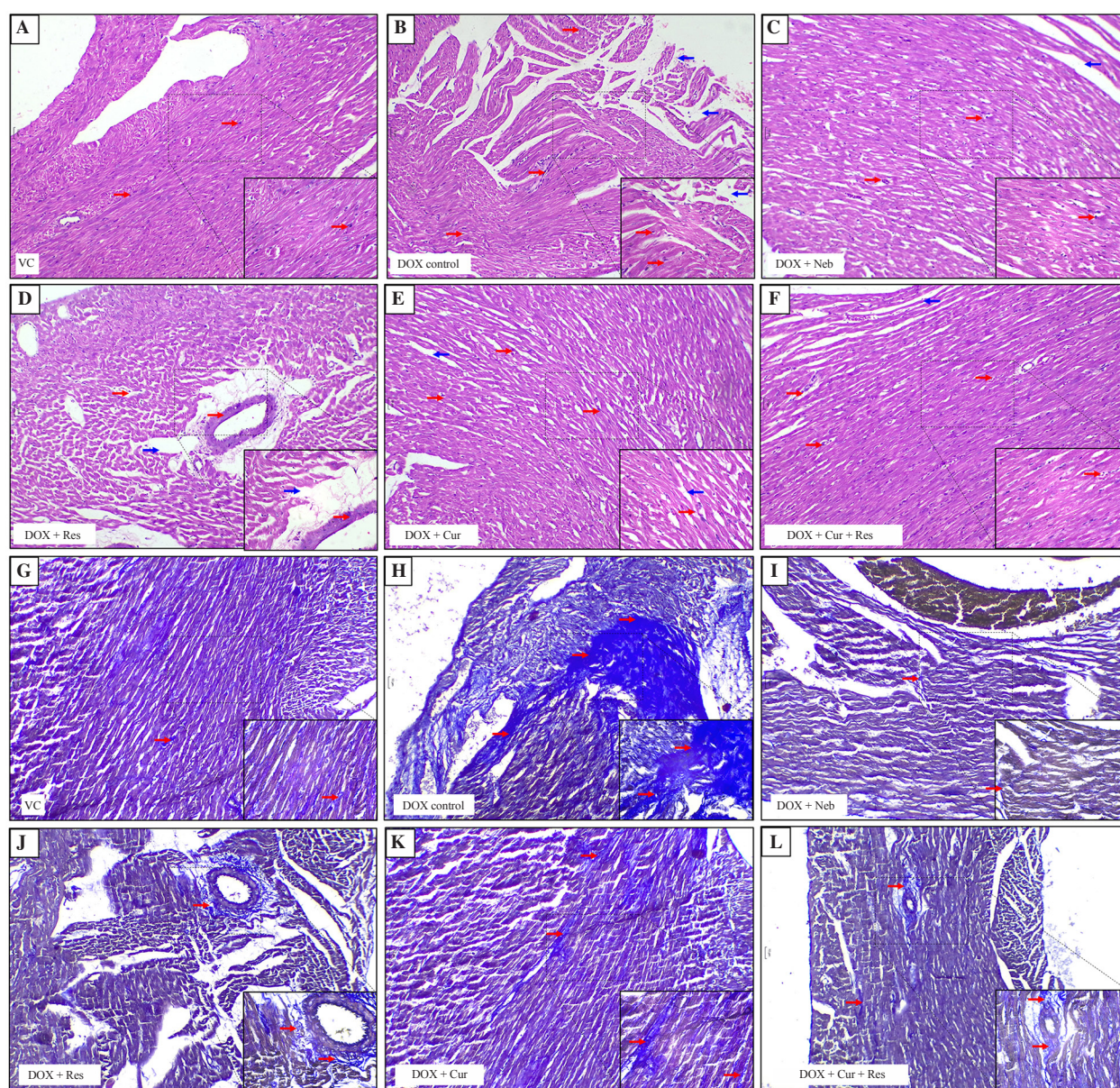


Figure 4. Effects of resveratrol and curcumin on DOX-induced alterations in cardiac histology of rats stained with hematoxylin and eosin (A-F) and Masson's trichrome staining (G-L) (magnification: 40× and 100×). Inflammatory infiltration (red arrow) and necrosis (blue arrow).

by inflammatory cascades, fibrosis, and vascular and valvular changes, ultimately leading to cardiomyopathy and deterioration of cardiac structure and function[25,26]. Furthermore, the increases in absolute heart weight and in the heart weight-to-body weight ratio in the DOX control group were compatible with cardiac enlargement secondary to edema, inflammatory infiltration, or pathological remodeling. This physiological impact was further confirmed by alterations in the cardiac electrical activity. DOX triggers profound changes, including increased systolic and diastolic blood pressure and heart rate alterations, partly mediated by histamine release and secondary catecholamine effects[27]. Electrocardiographically, DOX alters parameters such as increased P-wave duration and T-wave amplitude and prolongation of RR, QRS, QTc, ST, and PR intervals, indicating disturbed electrical conduction and cardiac injury[27]. In this study, sub-acute DOX exposure triggered systemic hemodynamic stress manifested as secondary hypertension and bradycardia. This pressure elevation likely stems from acute vascular remodeling, endothelial stiffening, and altered compensatory responses. However, combination treatment with resveratrol and curcumin effectively reduced these cardiac indices and restored cardiac electrical activity. Furthermore, resveratrol has been noted to prolong the effective refractory period and reduce ventricular arrhythmias by inhibiting various potassium and sodium currents.

The induction of myocardial necrosis by DOX was confirmed by a significant increase in serum cardiac injury markers, specifically CK-MB, LDH, and cTn-I levels. Elevated serum CK-MB and LDH levels indicate the severity of myocardial injury[28,29], whereas increased cTn-I levels specifically indicate myocardial cell necrosis and are associated with impaired left ventricular function[22]. Treatment with resveratrol and curcumin significantly reduced the leakage of these enzymes, likely by protecting the integrity of the cell membrane. Furthermore, the significant reduction in serum markers of myocardial necrosis by combination treatment suggests that the dual-phytoconstituent approach preserves cardiomyocyte membrane integrity more effectively than monotherapy. This was supported by the histopathological and TTC staining results, which showed that the combination group maintained structural integrity and myocardial viability, closely resembling the healthy architecture of the vehicle control. A recent meta-analysis reported that resveratrol significantly reduced the leakage of cardiac injury markers CK-MB and LDH in animal models, confirming its cardioprotective role against myocardial infarction and ischemia/reperfusion damage[30]. Researchers have suggested that the combination of resveratrol and curcumin exerts a potent synergistic effect by significantly enhancing the cytotoxicity and pro-apoptotic activity of these phytochemicals against colorectal cancer cells compared to their individual treatments[31].

DOX alters the lipid profile by impairing peroxisome proliferator-activated receptor α , which disrupts the expression of genes

involved in fatty acid transport, lipolysis, and lipid oxidation, leading to elevated circulating lipid levels and impaired cardiac lipid metabolism. This lipid metabolic disturbance causes lipotoxicity in the heart, contributing to oxidative stress and cardiac dysfunction during DOX treatment[32]. Additionally, DOX-containing chemotherapy regimens are associated with increased levels of TG, TC, and LDL-C in patients[33]. In the present study, DOX administration in rats disrupted lipid homeostasis, contributing to secondary cardiovascular strain. This strain resulted in a significant increase in TC, TG, LDL-C, and VLDL-C levels, with a concurrent decrease in HDL-C levels. This dyslipidemia is indicative of altered lipid metabolism associated with myocardial infarction. However, combination therapy of resveratrol and curcumin robustly lowers atherogenic lipids (LDL-C, VLDL-C, and TC) and increases cardioprotective HDL-C levels. This comprehensive metabolic regulation, highlighted in the KEGG pathway analysis (*e.g.*, linoleic acid metabolism and steroid hormone biosynthesis), suggests that the benefits of resveratrol and curcumin extend beyond direct antioxidant effects to a broader systemic cytoprotection.

DOX-induced cardiotoxicity is a multifactorial process in which the central events are the generation of ROS, redox cycling, and iron-associated free radical formation, which exceed myocardial antioxidant defenses. Excessive ROS generation leads to mitochondrial damage, DNA injury, and cardiomyocyte death. Oxidative stress disrupts mitochondrial bioenergetics and triggers cell death pathways, culminating in dose-dependent cardiomyopathy and heart failure[34]. Antioxidant agents have shown potential in mitigating DOX-induced cardiotoxicity by reducing ROS levels and protecting cardiac tissue integrity[35]. Our findings confirm that while monotherapies significantly improve antioxidant status, combination therapy resulted in the most pronounced restoration of endogenous antioxidants, such as SOD, GSH, and CAT, while minimizing lipid peroxidation (MDA levels). Resveratrol and curcumin exert these effects through polypharmacological behavior, altering the intricate biological networks underlying redox homeostasis. This was further confirmed by *in silico* network pharmacology analysis, which showed that resveratrol and curcumin target a shared molecular space, particularly the NFE2L2 (Nrf2) and TNF nodes.

TNF- α and NF- κ B are upregulated in DOX-induced cardiotoxicity, promoting inflammatory responses, oxidative stress, and subsequent cardiac injury[36]. NF- κ B activation enhances TNF- α expression, driving pro-inflammatory signaling pathways that exacerbate cardiac injury[36–38]. Conversely, Nrf2 functions as a key antioxidant transcription factor that mitigates DOX-induced oxidative stress, reduces cardiac dysfunction, and limits inflammation through upregulation of antioxidant enzymes[39]. Nrf2 deficiency worsens the cardiotoxic effects of DOX by increasing oxidative damage and inflammation, partly by failing to suppress NF- κ B and TNF- α signaling[39]. Thus, the interplay between TNF- α /NF- κ B-driven

inflammation and Nrf2-mediated antioxidative defense critically influences the extent of DOX-induced cardiotoxicity and oxidative stress. The downstream crosstalk between these axes is pivotal, as it activates Nrf2 transcriptional products that suppress NF- κ B nuclear translocation by maintaining low intracellular ROS pools and preventing the phosphorylation-mediated breakdown of the structural inhibitor[40,41]. In the present study, DOX triggered a pro-inflammatory state, as evidenced by the upregulated mRNA expression of *TNF- α* and *NF- κ B* and downregulated expression of the antioxidant regulator *Nrf2*. Combination therapy markedly upregulated *Nrf2* mRNA, a master regulator of the antioxidant response, and suppressed *TNF- α* and *NF- κ B*. This dual action effectively breaks the interconnected cycle of oxidative imbalance and inflammatory response typically observed in anthracycline-induced injury. Zamanian *et al.* highlighted that resveratrol and curcumin act as dual modulators to suppress lung cancer progression by inhibiting the NF- κ B signaling pathway and reducing the production of pro-inflammatory cytokines such as TNF- α [42]. The present study also supports the findings of previous studies, wherein concomitant administration of resveratrol and curcumin downregulated the expression of pro-inflammatory cytokines *via* the inhibition of the NF- κ B signaling pathway.

In the present study, biochemical and molecular findings of DOX-induced cardiotoxicity were also confirmed by histological examination. H&E and Masson's trichrome staining revealed that DOX caused marked structural disruption, fiber misalignment, and fibrotic remodeling. However, the combination of resveratrol and curcumin provided the most robust restoration of cardiac histology compared to other treatments. This group showed near-complete preservation of myocardial continuity, closely resembling the pristine pattern observed in vehicle control rats.

Clinical and human studies have indicated that the combination of resveratrol and curcumin shows promise as a complementary approach in cancer treatment because of their synergistic anticancer effects and relatively favorable safety profiles. Resveratrol and curcumin, both natural polyphenols, have demonstrated the potential to enhance the efficacy of chemotherapeutic agents such as DOX by sensitizing cancer cells and reducing drug resistance, partly by modulating the molecular pathways involved in apoptosis and oxidative stress[43]. Clinical trials with curcumin have revealed its safety at doses of up to 12 g/day for extended periods, although its poor bioavailability remains a challenge that various formulations aim to overcome[44]. While human studies on the combined use of resveratrol and curcumin are limited, evidence suggests that their use as adjuvants can mitigate oxidative stress and inflammation induced by chemotherapy, enhancing anticancer effects and reducing side effects. Nonetheless, caution and thorough risk-benefit analysis are necessary due to variable bioavailability and potential interactions,

underscoring the need for more clinical research to establish optimal dosing and safety before recommending routine adjunctive use alongside DOX in cancer therapy[45].

Several limitations must be noted regarding the current study. First, echocardiography, the gold standard for functional cardiac evaluation of *in-vivo* left ventricular ejection fraction and cardiac hypertrophy, was unavailable at our testing facility. Second, histopathological scoring was strictly qualitative and lacked automated software quantification workflows. Finally, while our 28-day sub-acute animal model provides robust structural, biochemical, and molecular evidence of protection, these rodent outcomes cannot be directly extrapolated to human clinical oncology timelines without extensive long-term pharmacokinetic, toxicity, and translational safety evaluations.

In conclusion, DOX-induced cardiotoxicity in rats is driven by oxidative stress, inflammation, and disturbances in lipid metabolism, thereby causing cardiac damage. Treatment with resveratrol and curcumin, especially in combination, significantly improved antioxidant defense, reduced inflammation, normalized lipid profile, and preserved cardiac structure and function. This synergistic effect highlights the potential of this combination therapy as an effective strategy for preventing DOX-induced cardiotoxicity, warranting further translational validation and controlled clinical investigation.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

Medical writing support for the development of this manuscript, under the direction of the authors, was provided by Yonnova Scientific Consultancy Pvt. Ltd., following the Good Publication Practice guidelines.

Funding

The study received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

AM and GB conceived and designed the study. GB, RG, VS, and RK performed the data collection and experimental work. MG and HK conducted the data analysis and interpretation, including the network pharmacology and statistical evaluations. GB and AM drafted the initial manuscript. RG, MG, VS, RK, and HK provided critical revision of the article for important intellectual content. All authors gave final approval of the version to be published.

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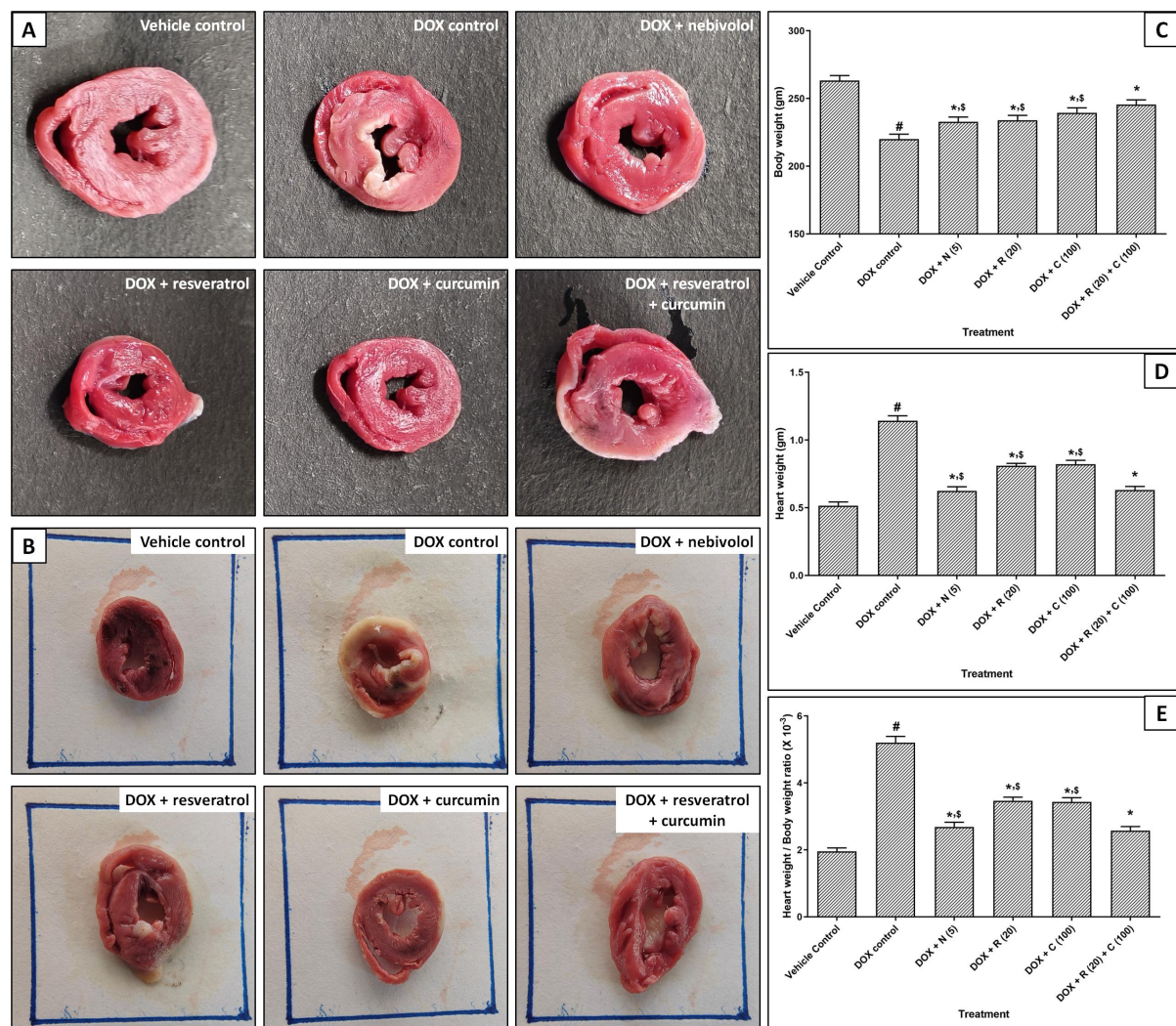
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Edited by Liang Q, Tan BJ

Supplementary Table. Primer sequences used for RT-PCR analysis.

Gene	Sequence		Size (bp)
	Forward primer	Reverse primer	
<i>Nrf2</i>	CCTCACCTCTGCTGCCAGT	GGGAGGAATTCTCCGGTCTC	316
<i>NF-κB</i>	CCGTTATGTA TGTGAAGG	AGAGTCCAGG ATTATAGC	338
<i>TNF-α</i>	AAGCCTGTAGCCCATGTTGT	CAGATAGATGGGCTCATACC	295
<i>β-actin</i>	GCCATGTACGTAGCCATC	GAACCGCTCATTGCCGAT	375



Supplementary Figure 1. Effects of resveratrol and curcumin on DOX-induced alterations in (A) cardiac morphology, (B) the infarcted area in 2,3,5-triphenyltetrazolium chloride-stained heart slices, (C) body weight, (D) absolute heart weight, and (E) relative heart weight in rats. Data are expressed as mean $\pm$ SEM ($n=6$) and analyzed using one-way ANOVA, followed by

Tukey's multiple range test. * $P < 0.05$ compared to the DOX control group, # $P < 0.05$ compared to the vehicle control group, and $^{\$}P < 0.05$ compared to the combination group.