Journal of Applied Pharmaceutical Research
Volume 12 Issue 6, Year of Publication 2024, Page 37 – 56
DOI: https://doi.org/10.69857/joapr.v12i6.673

Check for updates

Review Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ***ISSN: 2348 – 0335***

DOXORUBICIN-INDUCED CARDIOTOXICITY: AN UPDATE ON THE MOLECULAR MECHANISM, BIOMARKERS AND MANAGEMENT

Mohd Amaan[1], Radha Goel[1]*, Surovi Paul[1], Iqbal Danish[2]

Article Information
Received: 31st July 2024
Revised: 4th October 2024
Accepted: 2nd November 2024
Published: 31st December 2024

Keywords
Doxorubicin, apoptosis, reactive oxygen species

ABSTRACT
Background: Doxorubicin (DOX) is a widely used chemotherapeutic agent that is effective against various solid tumors and hematologic malignancies. However, its clinical application is severely limited by dose-dependent cardiotoxicity, which affects nearly 26% of patients. **Objective:** This review focuses on recent insights into the molecular mechanisms of DOX-induced cardiotoxicity, particularly highlighting the roles of oxidative stress and mitochondrial dysfunction. **Methods:** We have reviewed and retrieved the relevant information by probing the main keywords in online databases (PubMed, Scopus, Science Direct and Web of Science, *etc*.). Screening of relevant literature was done to pick suitable content based on the pharmacological profile of DOX. Key biomarkers such as troponins, brain natriuretic peptides (BNP), and atrial natriuretic peptides (ANP) are crucial for early detection of cardiac injury. The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), mediated by enzymes like NADPH oxidase and mitochondrial cytochrome c, is central in triggering apoptosis and cardiomyocyte damage. Furthermore, DOX's impact extends to other organs, notably the liver and kidneys, contributing to systemic toxicity. **Conclusion**: This review synthesizes current strategies to mitigate DOX-induced cardiotoxicity, including applying antioxidants, liposomal DOX formulations, and emerging nanocarrier technologies designed to enhance therapeutic selectivity. Looking ahead, integrating personalized medicine approaches and developing innovative therapeutic interventions hold promise for balancing DOX's antitumor efficacy with a reduced risk of cardiotoxicity. By addressing critical gaps in our understanding, this review highlights the need for integrative approaches combining biomarker discovery and targeted therapies to optimize patient outcomes and guide future research directions.

INTRODUCTION

DOX, a licensed chemotherapy medication, has shown significant therapeutic potential due to its effectiveness and effectiveness [1]. The medication, a class I anthracycline, is known for its harmful effects on human noncancerous cells; this

[1]*Department of Pharmacology, Lloyd Institute of Management & Technology, Plot No.11, Knowledge Park-II, Greater Noida, Uttar Pradesh 201306, India*
[2]*Department of Pharmaceutics, Lloyd School of Pharmacy, Plot No.-3, Knowledge, Park-II, Greater Noida,, Uttar Pradesh 201306, India*
For Correspondence: **radhamit2006@gmail.com**

restricts the ability to stop the spread of infection and target rapidly dividing cells over several decades. It contains glyconic and aglyconic moieties with a tetracyclic ring, quinine-hydroquinone, methoxy substituent, and carbonyl group. This is made up of a compound known as 3-amino-2,3,4-trideoxy-L-fucosyl [2] (Figure 1)

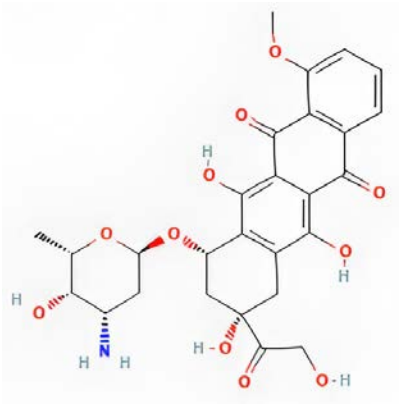

Figure1: Doxorubicin structure

DOX, a potent chemotherapeutic drug approved by the FDA, has demonstrated significant treatment potential in its unaltered form [3]. The drug, a nonselective class I anthracycline, has been recognized for effectively combating rapidly proliferated cells and slowing disease progression; however, its toxicity to human body non-cancerous cells is minimal. It consists of aglycon and glycon components, with the sugar part linked by a glycosidic bond. Studies show that while DOX achieves high efficacy in treating various malignancies, it is associated with a 26-36% risk of cardiotoxicity at cumulative doses above 550 mg/m², compared to lower rates observed with epirubicin and daunorubicin. Understanding these adverse effects is crucial for enhancing the safety of chemotherapeutic regimens [4].

Mechanism of action

By intercalating the medication between the double-helical DNA's nucleotides and inhibiting topoisomerase II [Top II], DOX prevents malignant cells from proliferating [5]. DOX inhibits cell division in non-cancerous cells by creating free radicals, leading to toxicity. This imbalance promotes oxidative stress, causing nitric oxide [NO] to react with these radicals, resulting in reactive nitrogen species [RNS] [6]. DOX is converted into DOX through enzymatic or non-enzymatic methods, with the NADPH reductase enzyme or a ferrous- DOX complex formation [7]. Both processes result in the production of reactive oxygen species [ROS] and reactive nitrogen species [RNS], which in turn cause lipid peroxidation and DNA damage and ultimately cause both malignant and non-malignant cells to undergo apoptosis [8]. The detrimental effects of DOX on numerous organs have been explained based on several factors, the most important of which is considered to be oxidative stress. The massive production of ROS and RNS leading to lipid peroxidation, the dysregulation of the electron transport chain which impairs energy status, and the expression of genes that regulate antioxidants are among the numerous factors identified contributing to the oxidative stress induced by DOX. These elements push the cells in the off-target areas towards necrosis and apoptosis [9]. One of the primary mechanisms contributing to DOX-induced cardiotoxicity involves the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

The enzyme NADPH oxidase plays a crucial role in this process by facilitating the electron transfer that generates superoxide radicals. These radicals are converted into more reactive species, leading to oxidative stress. Mitochondria-specific proteins, particularly cytochrome c, are critical in mediating the apoptotic pathway. Upon exposure to DOX, oxidative damage to the mitochondria results in the release of cytochrome c into the cytosol, which activates the caspase cascade, ultimately driving cellular apoptosis. Recent studies indicate that the accumulation of ROS and RNS induces lipid peroxidation and DNA damage and disrupts mitochondrial membrane potential, further exacerbating cardiomyocyte death. This oxidative damage is a key factor in the progression of DOX-induced cardiomyopathy, highlighting the need for targeted therapies to mitigate ROS/ RNS generation while preserving the drug's anticancer efficacy [10].

A drug's ability to cause toxicity is entirely dependent on the dosage used. Once DOX dosages exceed 300 mg/m² in children and 400–700 mg/m² in adults, the likelihood of cardiotoxicity increases at an exponential rate. The medication DOX has a dual nature and manifests cardiotoxicity in two distinct phases: an acute phase that starts 2-4 days after drug administration and a chronic phase that starts later and is characterized by left ventricular dysfunction, arrhythmia, congestive heart failure, and cardiomyopathy [11].

DOX-mediated toxicity in the heart

DOX toxicity, a common side effect of anthracycline drugs, includes cardiotoxicity, acute vomiting, nausea, gastrointestinal issues, baldness, and neurological disturbances, often causing

hallucinations and light-headedness. Following early clinical assessments, phase II and III investigations conducted in the 1970s demonstrated these effects [12]. DOX, lacking specific targeting to tumors, can influence the growth of other cell types, causing suppressed immunity, increased infection susceptibility, and delayed healing. The potentiality of these actions varies based on the dosage and the patient's bone marrow regeneration capability [13]. When administered consistently into small veins, DOX can cause phlebosclerosis, necrosis, cellulitis, thrombophlebitis, and joint movement limitation due to painful induration, necrosis, and extravasations in local tissues or organs, causing tissue hardening.

Oxidative Stress: DOX's redox cycling at NADPH dehydrogenase produces excessive ROS, leading to lipid peroxidation.

Mitochondrial Damage: Cytochrome c release compromises the mitochondrial membrane potential, promoting apoptosis [14].

Biomarkers: Elevated BNP and troponins correlate with early myocardial damage, aiding in the timely diagnosis of cardiotoxicity [14].

Toxicity in the heart

Cardiotoxicity is a common type of DOX-induced toxicity, and there are several reasons why [Figure 2]. The medication DOX, which is used to treat heart diseases, needs to be used in moderation because it can change the structure of cardiomyocytes and result in cardiac hypertrophy. The leading causes of this toxicity are the genes brain natriuretic peptide [BNP] and atrial natriuretic peptide [ANP], which are similarly influenced by the effects of DOX on the heart muscle's mitochondria. Changes in mitochondrial protein expression amplify the redox cycling of NADH dehydrogenase and DOX. TLR4, or toll-like receptor 4, increases TNF-a by reducing ROS levels. Higher ROS levels trigger the apoptotic cascade, which allows mitochondria to release cytochrome c.

A lifetime deposition of DOX near 500 mg/m2 increases cardiomyopathy risk, often leading to 20 % congestive heart failure in patients [15]. As previously indicated, iron oxidation and the production of oxygen-free radicals are the molecular mechanisms underlying this process. Because DOX is known to impact several biomarkers, measuring troponins and specific natriuretic peptides [proBNP and DNP] may assist in the early detection of DOX-induced cardiotoxicity [16]. Administering antioxidants has not effectively reduced toxicity levels and research on specific iron chelators has produced contradictory or unfavorable findings. This suggests that DOX-induced cardiotoxicity may be caused by mechanisms other than ROS and iron [17]. Studies have looked into the activation of p53 and p300 degradation and the downregulation of GATA binding protein 4 [GATA-4]. These proteins are impacted by DOX administration, resulting in the apoptosis of cardiomyocytes [18-21]. GATA-4, a recognized transcription factor, functions as a component of survival for postnatal and distinct cardiac muscle cells. Furthermore, it has been demonstrated to activate the anti-apoptotic gene Bcl-xL.

DOX, a potent antineoplastic agent, is primarily used for dose-limited administration due to its cardiotoxic effects, including arrhythmias, hypotension, and electrocardiographic alterations, with the frequency of these effects varying widely [22- 24], which disappear once the treatment ceases [25]. Chronic effects of DOX are observed in just 1.7% of patients, and 50% of them die. DOXs lead to cause dose-dependent cardiotoxicity, potentially resulting in congestive heart failure. In almost 4% of patients, cumulative doses between 500 and 550 mg/m² were administered, 18% received doses between 551-600 mg/m², and 36% received doses over 601 mg/m² [26]. 630 patients with a combined diagnosis of lung and breast cancer were examined [27]. Studies indicate that approximately 26% of patients may develop DOX-related congestive heart failure [CHF] when exposed to a cumulative dose of 550 mg/m². Furthermore, research documents that chronic administration of DOX to mice over 7 weeks led to cardiac hypertrophy [28]. These findings were reinforced by in vitro studies indicating that DOX induces hypertrophy in primary neonatal rat cardiomyocytes.

Animal models are crucial in preclinical research for understanding cardiac disorder onset and propagation in living organisms. Experimental studies in animals, especially rodents, evaluate new diagnostic and therapeutic cardiac drugs before clinical testing. However, their ability to predict clinical efficacy is debated due to their inability to represent human disease accurately. Most successful agents found in animal models do not translate to human trials due to differences in physiology and molecular target homology. While animal models remain a valuable source of in vivo information, other translational alternatives may eventually replace the link between in vitro studies and clinical applications.

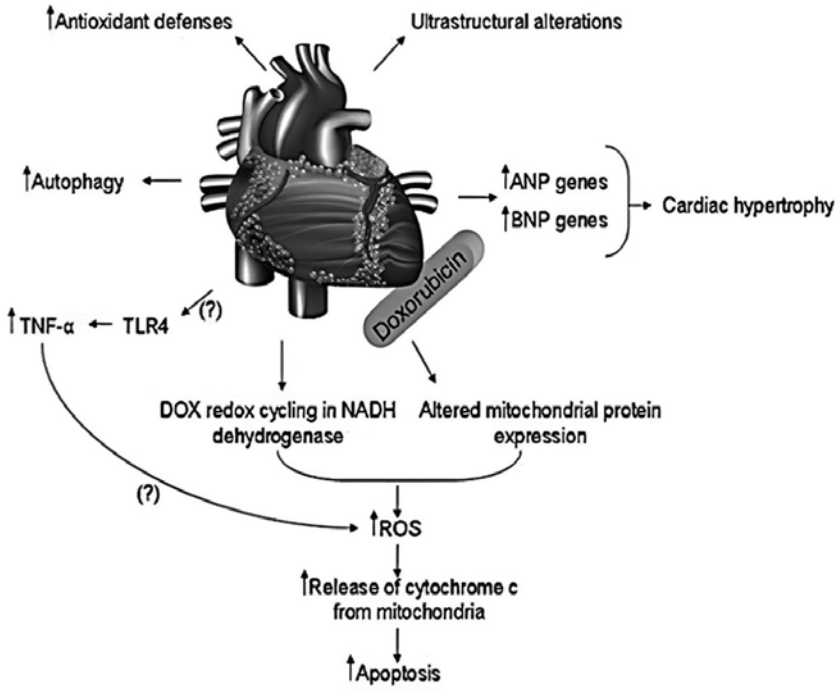

Figure 2: DOX, an anticancer medication, causes cardiomyocytes to expand and undergo ultrastructural changes, leading to increased gene expression and mitochondrial damage

DOX redox cycling at NADH dehydrogenase intensifies reactive oxygen species [ROS] production and changes in mitochondrial protein expression. Toll-like receptors [TLR4] influence ROS levels, likely by raising TNF- levels. An increase in ROS levels activates the apoptotic cascade, and the autophagic process is activated due to mitochondrial deterioration and DNA damage caused by DOX [29] and in the myoblastic cell line H9c2 [30], which are also related to cytoskeletal alterations [31].

Zordosky and El-Kadi demonstrated that DOX leads to a notable increase in the expression of atrial natriuretic peptide [ANP] and brain natriuretic peptide [BNP] genes, both markers of cardiac hypertrophy. Furthermore, they noticed increased expression of cytochrome P [CYP] genes, which belong to a subfamily of monooxygenases and are in charge of the oxidative metabolism of endogenous and xenobiotic chemicals (Figure 3) [32]. Alterations in arachidonic acid metabolism, which have also been connected to heart failure and cardiac hypertrophy, may arise from activating these genes [33]. Acute DOX administration causes ultrastructural alterations in rat and mice cardiomyocytes, including interstitial edema, myocardial disarray, degeneration, and perinuclear vacuolation. These alterations also occur in mitochondria, leading to vacuolization, myelin deposition, membrane disruption, and organelle degeneration. Drug concentration affects how the myoblast cell line H9c2 changes. Low DOX concentrations cause alterations in fibrous structural proteins, mitochondrial depolarization, and cell shape changes. Nuclear swelling, extensive cytoplasm vacuolization, mitochondrial swelling, and other more significant cellular changes are facilitated by high doses [5–50 HM]. At the same time, low concentrations cause alterations in the nuclear lamina and cardiac myosin. DOX therapy patients experience alterations like myofibrillar loss, sarcoplasmic reticulum dilatation, and swollen mitochondria. The heart's vulnerability to DOX-induced toxicity is heightened by its interaction with cardiolipin, a critical constituent of the mitochondrial inner membrane [34]. Compared to most other tissues, heart cells have a higher density of mitochondria per unit volume [35]. The contentious presence of a heart-specific form of mitochondrial complex I, or NADH dehydrogenase, an enzyme that can start DOX redox cycling and consequently increase ROS production, may be the reason why the heart has lower levels of antioxidant defenses than other organs [36,37]. The heightened vulnerability of the heart to DOX-induced toxicity stems from various factors. Moreover, restoring cardiac function following DOX-induced injury is particularly challenging due to the non-dividing nature of cardiomyocytes

[38,39]. In summary, the heart exhibits a heightened vulnerability to injury induced by DOX, with its cardiotoxic effects stemming from a complex interplay of multiple mechanisms.

Molecular insights into the cardiotoxic effects of DOX

There are multiple ways that DOX operates, yet pinpointing which one predominantly drives its cardiac toxicity remains uncertain. Several studies support the idea that the administration of DOX might cause reactive oxygen species [ROS], which can then cause lipid peroxidation, abnormalities in calcium, and disruption of energy transfer. All of these factors may play a significant role in the development of heart failure [40-42]. Nonetheless, the precise biochemical pathways responsible for its toxicity remain incompletely understood. As illustrated in Figure 2, we outline the most plausible intracellular and signaling pathways that underlie cardiotoxicity in the following section.

Generation of reactive oxygen species [ROS]

Mitochondrial dysfunction is the origin of increased ROS and oxidative stress and is closely associated with DOX-induced cardiotoxicity. Exposure to DOX mainly affects cells' mitochondria. One important factor contributing to DOX accumulation in this particular cellular compartment is the high-affinity binding of DOX to cardiolipin in the inner mitochondrial membrane [43,44].

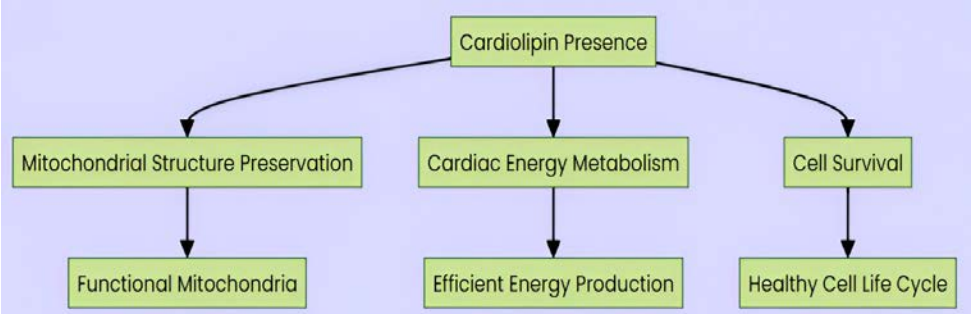

Figure 3: Cardiolipin's role in Mitochondrial function

The inner leaflet of the mitochondrial membrane contains a phospholipid called cardiolipin, essential for maintaining the mitochondria's structure, function, cardiac energy metabolism, and cell viability [45]. Oxidative stress is the primary mechanism via which the cardiolipin-bound DOX causes mitochondrial damage [46]. Complexes I, III, and IV in the electron transport chain [ETC], which depend on cardiolipin for maximum activity, are adversely affected by the electrostatic interaction between cardiolipin and DOX. Moreover, complex I have proven to be capable of catalyzing DOX's reduction to a semiquinone radical. When this species is reoxidized, the superoxide anion can be created by adding one electron to molecular oxygen [O_2]. This electron transport via DOX can further disrupt the ETC since it can form an even stronger bond with cardiolipin [47,48]. Because of its chemical makeup, which includes a quinone-containing tetracycline component, DOX is also readily reduced by endothelium nitric-oxide synthase into semiquinone [49]. As previously mentioned, several membrane-bound enzymes within mitochondria can aid in quinone's transition to the semi-quinone state. Among them, a subset of flavoprotein oxidoreductases participates in the redox quinone cycle of DOX. Notable examples include xanthine oxidase, The electron transport chain's complex I, sometimes referred to as nicotinamide adenine dinucleotide hydrogen [NADH] dehydrogenase, and NADPH/cytochrome P_{450}, which are both found in the endoplasmic reticulum [50]. Auto-oxidation returns the semi-quinone to its parent molecule by transferring electrons to molecular oxygen [O_2]. This process generates superoxide radicals, which further react to produce hydrogen peroxide [H_2O_2]. Under certain conditions, hydrogen peroxide can be transformed into highly reactive hydroxyl radicals in the presence of iron or copper ions. As a result, the buildup of DOX in mitochondria increases the generation of reactive nitrogen species [RNS] and reactive oxygen species [ROS] [51,52]. Reactive species cause lipid peroxidation, DNA and protein damage, mitochondrial DNA damage, ATP loss, cardiolipin peroxidation, and mitochondrial permeability transition [53,54]. A vicious loop may result from the interplay of ROS, mtDNA damage, and ETC, whereby elevated ROS levels deactivate the ETC and promote the production of further ROS.On the other hand, elevated ROS levels can damage mtDNA and inhibit ETC proteins, exacerbating mitochondrial malfunction and ROS

generation. When all these components are released from mitochondria, cytochrome c is released together with other apoptogenic factors, which starts the apoptotic process. Since mitochondria comprise up to 20–40% of the cellular volume of the heart tissue, which requires a lot of energy, cardiomyocytes will likely produce a large amount of free radicals in response to DOX through oxidative metabolism. The oxidative stress and mitochondrial dysfunction induced by DOX result in impaired contractility and arrhythmias in clinical settings. The downregulation of genes like GATA-4, which plays a protective role in cardiomyocytes, is directly associated with increased susceptibility to heart failure in chemotherapy patients [55]. In addition to ROS-mediated damage, DOX-induced cardiotoxicity involves immune system activation and calcium dysregulation. Studies have shown elevated intracellular calcium levels trigger apoptosis through calcineurin signaling, exacerbating cardiomyocyte death [56].

The Pathway of Cell Self-Destruction: Exploring Apoptosis Mechanisms

DOX triggers apoptosis through both intrinsic and extrinsic mechanisms. Human promyelocytic leukemia [HL60] cells treated with DOX showed caspase-3 protein activation, which led to cell death [57,58]. The study reveals an H2O2-dependent mechanism mediating apoptosis through PARP. DIC is primarily caused by increased mitochondrial membrane permeability and NOX activation, with the mitochondrial-dependent intrinsic route being a key player. The work in rat cardiomyoblasts [H9c2] emphasizes how the nuclear factor-kappa B [NFκB] signaling pathway controlled by NOX/ROS contributes to the induction of DOX-induced apoptosis, with ERK1/2 and MAK involved in the cascade, leading to cell death [59,60]. Similarly, the involvement of ERKs and p53 in facilitating DOX-induced apoptosis in H9c2 cells and cardiomyocytes has been elucidated. DOX facilitates cardiomyocyte apoptosis by engaging extrinsic pathway mediators such as death receptors, causing DOX-treated human induced pluripotent stem cell-derived cardiomyocytes [iPS-CMs] to overexpress death receptors [61]. Upon up-regulation, these death receptors [DRs] bind with their corresponding ligands, initiating the caspase cascade and culminating in apoptosis. Additionally, TNF-related apoptosis-inducing ligand [TRAIL] has been found to enhance drug-induced cardiotoxicity [DIC] further, indicating that serum levels of TRAIL might serve as predictive biomarkers for identifying populations at heightened risk for DIC [62]. Caspase inhibitors offer incomplete protection against cell death, prompting the discovery of a caspase-independent pathway driven by mitochondrial apoptosis-inducing factor [AIF]. Elevated ROS levels induced by DOX lead to heightened cathepsin B activity, triggering the release of AIF from mitochondria. AIF, in turn, induces DNA damage, enhances p53 expression, and activates PARP1, ultimately leading to caspase-independent apoptosis. In vivo experiments conducted on male Wistar rats demonstrated that acute drug-induced cardiotoxicity [DIC] involves apoptosis of cardiomyocytes [63]. DOX treatment did not increase TUNEL-positive cardiomyocyte percentages or cause apoptotic cells to decline [64] A clinical investigation revealed that DOX therapy could potentially hinder myocardial growth in pediatric cancer patients, leading to a slight elevation in left ventricular wall thickness attributed to the loss or injury of cardiac myocytes induced by DOX [65]. Similar findings were also documented in children experiencing DOX-induced congestive heart failure [CHF] [66]. Two adult patients with CHF showed a significant decrease in cardiac myocytes and degeneration after receiving a cumulative dose of over 700 mg/m2 post-mortem as per the clinical study [67].

Calcium Disturbance

Calcium disturbance or dysregulation of calcium is a known mechanism of cardiotoxicity from DOX [68]. Doxorubicinol, a hydroxyl metabolite of DOX, impacts calcium homeostasis through various mechanisms, including modulating the sarco/endoplasmic reticulum Ca^{2+} ATPase and sodium/ potassium exchanger on the sarcolemma [69,70]. The SR proteins in charge of calcium transport were shown to have different amounts of gene expression in a rabbit model of cardiomyopathy. A calcium imbalance and apoptosis study found that calcineurin, a calcium-dependent phosphatase, triggers apoptosis via Fas [71]. The study found that exposure to DOX in rat cardiac cells increased mitochondrial ROS, leading to increased cytosolic calcium levels and activation of NFAT, enhancing Fas-mediated cardiac cell death [72]. Additionally, the study revealed that CaMKII disrupts calcium balance by promoting SR calcium leakage [73,74]. After 15 weeks of chronic DOX treatment, mice develop cardiac malfunction, potentially resulting from a decreased [Ca^{2+}] I transient. Mechanical unloading raises [Ca^{2+}] levels I handling and contractility in rats with cardiomyopathy caused by DOX [75]. The study suggests that changes in Ca^{2+} handling in cardiac

myocytes precede heart dysfunction, with mitochondrial deoxyribonucleic acid depletion and associated ETC impairment being the primary mechanisms of calcium dysfunction compared to other mechanisms [76]. Despite the challenges, targeting calcium dysfunction remains a viable method for treating DOX-mediated cardiotoxicity [77,78].

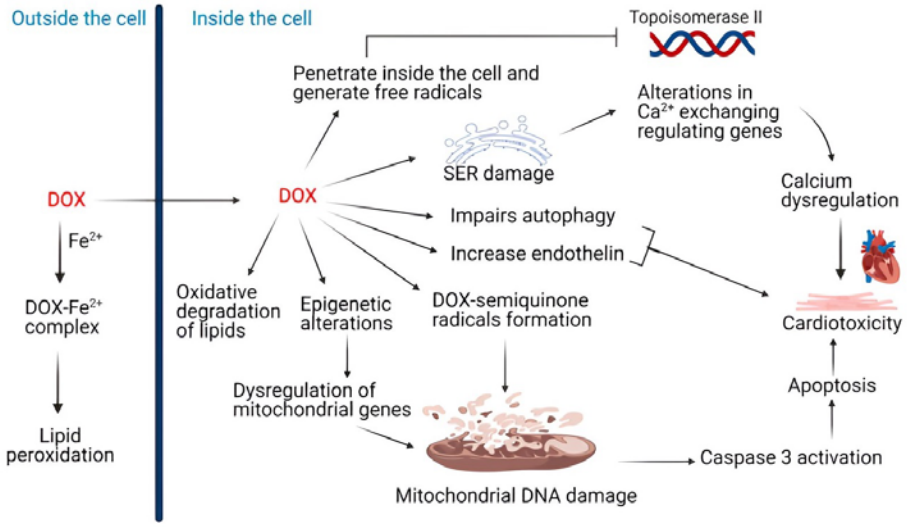

Figure 4: Diverse mechanisms of action underlying DOX-induced cardiotoxicity both intracellularly and extracellularly

Endothelin-1

Endothelin-1 [ET-1] is a powerful peptide known for its vasoconstrictive properties. It triggers multiple cellular responses in various cell types, including cardiomyocytes. These responses encompass vasoconstriction, inflammation, cell growth, proliferation, the generation of free radicals, and activation of platelets [79]. ET-1 plays a crucial role in the progression of vascular dysfunction, cardiovascular ailments, and drug-induced cardiotoxicity [DIC], notably in initiating dysfunction in the left ventricle [80]. DOX treatment increases plasma concentrations in patients and animal models of cardiomyopathy, affecting both acute and chronic studies [81-83]. Schwabe et al.'s study on the murine DOX cardiotoxicity model revealed that ET-1 receptor subunits ETA and ETB contribute to DIC, with ET-1 antagonists modulating signal transduction pathways [84]. The ET-1 receptor, specifically its ETA subunit, in primary neonatal rat cardiomyocytes, up-regulates MnSOD, demonstrating cytoprotective effects at an early DIC rescue phase.

Topoisomerase-II

DOX also targets topoisomerase-II as another cellular mechanism, causing DNA to experience single- and double-strand breaks [85]. Topoisomerase IIβ is the most prevalent isoform of topoisomerase between the two types found in adult cardiomyocyte mitochondria. It forms a ternary cleavage complex with DNA and DOX, which causes DNA double-strand breaks and consequent cell death-induced cardiotoxicity [DIC] has been confirmed through experiments utilizing cardiomyocyte-specific topoisomerase IIβ knockout mice [86]. The protective effects of dexrazoxane, the only medication licensed by the Food and Drug Administration [FDA] to prevent DOX-induced cardiotoxicity, further highlight the function of topoisomerase IIβ in mediating drug-induced cardiotoxicity [DIC] [87,88]. According to Deng et al., the results suggested that dexrazoxane prevented double-strand breaks instead of iron chelation by degrading topoisomerase IIβ. [89] This study marks the initial in vivo evidence showcasing the temporary reduction of topoisomerase IIβ by dexrazoxane, affirming earlier in vitro findings [90]. Due to the array of molecular mechanisms culminating in DOX-induced cardiotoxicity [DIC], its clinical application is restricted. Despite the identification of several mechanisms underlying DIC, the precise contribution of each remains incompletely understood. Oliveira et al. endeavored to discern the pathways responsible for acute and chronic DIC using a systems pharmacological approach [91]. According to system-based calculations, ETC inhibition is crucial in developing heart hypertrophy. This statement is consistent with previous independent studies on DOX-treated mice by pathway and biological analysis [92]. DOX-induced mtDNA damage is responsible for chronic cardiovascular disease, leading to irreversible mito-primary dysfunction at therapeutic doses.

Role of epigenetic alterations in DOX-induced cardiotoxicity

Mitochondrial metabolites are crucial cofactors for various enzymes involved in human biochemical pathways, including epigenetic modifications. S-adenosylmethionine (SAM) is the universal substrate for DNA and histone methylation, which may affect cardiomyocyte genomic chromatin. DNA methylation, histone modifications, and non-coding RNA expression have been found to play a role in DOX-induced cardiotoxicity. Histone modifications can lead to synergistic or antagonistic interactions with chromatin-associated proteins, resulting in dynamic switching between transcriptionally active and silent states. HDAC6, a histone deacetylase, was upregulated in DOX-treated primary rat cardiomyocytes and mice models, leading to deacetylation of α-tubulin. Inhibition of HDAC6 in mice showed a cardioprotective effect against DOX by restoring autophagic flux [93].

Role of epigenetics in doxorubicin-induced cardiotoxicity

Epigenetics is a natural process that involves heritable phenotypic changes in DNA base-pair sequences, causing gene function changes. These changes occur regularly due to age, environment, external factors, and disease state. DNA methylation and histone protein modification are two main epigenetic modifications in diseases and natural growth. These mechanisms induce phenotypic changes and control gene expression by blocking proteins that attach to the silencer region of the DNA. Every cell in the human body contains the same DNA molecule, which develops from individual cells at impregnation. Highly arranged epigenetic mechanisms facilitate normal human development and support stable expression regulation in diverse cell types. Regulation of these mechanisms involves proteins that begin, recite, and erase specific epigenetic alterations, defining where and when the transcriptional machine can approach the chief DNA sequences for regular growth and differentiation in embryos and fetuses. Some epigenetic marks work in whole human cells but run specific gene expression, such as DNA methylation at the CpG island, covalent alterations of histone proteins, and regulatory ncRNAs [94].

Measurement of DOX-induced cardiotoxicity biomarkers

Left ventricular ejection fraction [LVEF]

When the left ventricle contracts, the percentage of blood volume that it pumps is known as the ejection fraction or EF. Under typical circumstances, LV ejects between 50% and 70% of its capacity. Reduced left ventricular ejection fraction [LVEF] is a precursor to irreversible cardiomyopathy, which can develop following anthracycline therapy. When deciding how to treat DOX-mediated cardiotoxicity, LVEF is essential [95]. LVEF analysis is a dependable and repeatable way of identifying and diagnosing early cardiac abnormalities caused by several chemotherapeutics, including DOX. It utilizes techniques such as radionuclide angiocardiography, echocardiography, and Doppler echocardiography [96]. Changes in LVEF with DOX therapy may be caused by other non-cardiac diseases as well as by DOX itself. Consequently, LVEF assessment should be used with different strategies, such as tracking DOX and its metabolite plasma concentrations, to evaluate cardiac function in patients receiving DOX treatment. While troponins and BNP are well-established biomarkers for cardiotoxicity, recent studies emphasize the potential of novel imaging techniques, such as 123I-MIBG scintigraphy, for early detection. These biomarkers offer varying sensitivity levels, with troponin I showing higher specificity for myocardial injury than BNP [97].

Scintigraphy techniques and In vivo imaging

Various in vivo imaging and scintigraphic techniques have been reported.

(i) Imaging with Indium-111 [In-111] antimyosin,
(ii) Scintigraphy utilizing 123-labeled metaiodobenzylguanidine [123I-MIBG],
(iii) Utilization of Tc-99 annexin for scintigraphic imaging [98-100].

Three techniques use radiolabeled material to identify cardiac targets, and In-111 antimycin is used as an immuno-scintigraphic agent to visualize myocardial tissue and evaluate cardiac structures associated with myocardial cell damage**.** When irreparable injury to cardiac tissue results in the loss of the sarcolemma's integrity, the myocardium absorbs in-111-antimycin. Although it is no longer in commercial use, this immuno-scintigraphic method has shed light on the cardiotoxicity caused by chemotherapy. Sensitive and repeatable, 123I-MIBG scintigraphy can identify anomalies in myocardial adrenergic innervation before a decrease in left ventricular ejection fraction. LV myocardium can be seen with intravenous injection of 123I-MIBG, which initially has different cardiac concentrations depending on cardiac blood flow and accumulation in cardiac neurons. Four hours after injection, this concentration stabilizes, indicating particular cardiac neuron damage and norepinephrine uptake dysfunction [101]. In a study of patients with various neoplasms treated with

DOX, cardiac uptake of 123I-MIBG was decreased in a dose-dependent manner by DOX; this suggests that DOX has a cardiac adrenergic neurotoxic impact [102]. One helpful technique for identifying drug-related heart failure early on is 123I-MIBG scintigraphy; apoptotic cardiomyocytes can be seen using 99mTc-annexin scintigraphy. These methods have enhanced our knowledge of cardiac damage at the cellular and molecular levels. They have been found in cancer patients receiving anthracycline therapy, even though they are no longer employed to diagnose chemotherapy-induced cardiac dysfunction [103-106]. When proteases and sphingomyelinases are active, The extracellular layer of the cardiomyocyte membrane is where phosphatidylserine [PS] molecules migrate and the cell undergoes apoptosis. It is possible to image apoptotic cardiomyocytes at this stage with 99mTc-annexin V.

Table 1: Comparative Evaluation of Biomarkers for DOX-Induced Cardiotoxicity

Biomarker	Sensitivity	Specificity	Ease of Use	Clinical Utility
Cardiac Troponin I (cTnI)	High (Detects early myocardial injury)	High (Specific to myocardial tissue)	Moderate (Requires blood sample)	Widely used for early detection of cardiotoxicity; elevated levels correlate with myocardial damage and predict long-term outcomes.
Cardiac Troponin T (cTnT)	Moderate to High	High (Cardiac-specific)	Moderate	Useful in detecting chronic cardiotoxicity; cTnT levels increase with cumulative DOX dosage. High-sensitivity assays enhance early diagnosis, particularly in pediatric patients.
B-type Natriuretic Peptide (BNP)	Moderate	Moderate (Can be influenced by other conditions)	High (Simple blood test)	Effective for monitoring heart failure; elevated BNP levels correlate with left ventricular dysfunction. Recent research suggests combining BNP with imaging for enhanced sensitivity.
N-terminal proBNP (NT-proBNP)	Moderate to High	Moderate	High	Useful for early detection of diastolic dysfunction; more stable than BNP in circulation. New studies indicate its potential in detecting subclinical cardiac dysfunction in chemotherapy patients.
Echocardiography (LVEF)	Moderate	Low (Affected by loading conditions)	High (Non-invasive)	Commonly used to assess left ventricular function; can detect reductions in LVEF but may miss subclinical changes. Advanced techniques like strain imaging offer improved sensitivity.
123I-MIBG Scintigraphy	High	High	Low (Requires specialized equipment)	Provides early detection of myocardial adrenergic dysfunction before LVEF changes. Useful for patients with subclinical cardiotoxicity. Limited availability in routine practice.
99mTc-Annexin V Imaging	High (Detects apoptosis)	High	Low (Requires nuclear imaging)	Identifies apoptotic cardiomyocytes; sensitive for early stages of DOX-induced cardiotoxicity. Promising in research but not widely adopted clinically due to cost and accessibility.
Galectin-3	Moderate	Moderate	Moderate	Emerging biomarker for fibrosis and inflammation in heart failure. Shows promise in detecting early cardiac remodeling in cancer patients receiving DOX therapy.
High-Sensitivity C-Reactive Protein (hs-CRP)	Low to Moderate	Low (Non-specific inflammatory marker)	High	May indicate systemic inflammation related to DOX therapy but lacks specificity for cardiac damage. Potential use in combination with other biomarkers.

Apoptosis creates blebs and vesicles in the outer membrane layer of cardiomyocytes before morphological alterations. DNA fragmentation, cytoskeleton disintegration, cytoplasm volume reduction, and nuclear chromatin condensation are intracellular changes [107]. Animal models have shown elevated uptake of 99mTc-annexin V in the myocardium, with more prolonged exposure resulting in higher uptake. These findings align with histopathology and immunohistochemistry studies on DOX cardiotoxicity [108,109] and other toxicity indices. The TUNEL technique is an additional tool that facilitates the identification of advanced stages of apoptosis by identifying damage to DNA [110]. The method's restricted ability to identify late apoptotic cells makes it potentially unsuitable for early TDM of DOX-induced cardiotoxicity. Combining scintigraphy imaging with the TUNEL assay and 99mTc-annexin V might be more efficient.

Specific soluble cardiac biomarkers for clinical use

A useful predictor of drug therapy [TDM] for DOX therapy is the assessment of cardiac biomarkers in serum, such as cardiac troponin T and I, natriuretic peptide [ANP], and brain natriuretic peptide [BNP] [111]. In contrast to scintigraphic and imaging methods, serum amounts of cardiac-specific proteins released from damaged cardiomyocytes show myocardial damage in its early phases. Initially, cardiac troponin T was considered a potential biomarker [112]. According to the study, the absence of an acute increase in children limits the utility of cTnT as a prospective non-invasive cardiac biomarker for therapeutic use in DOX therapy [113-115]. A study in breast cancer patients showed that DOX treatment increases brain natriuretic peptide [BNP] levels and cTnT levels. Recent research indicates that 2D-STE, combined with cTnT levels, is superior to conventional electrocardiography in detecting cardiotoxicity early, thereby improving the effectiveness of DOX therapy treatment. Natriuretic peptides such as BNP were connected to compromised left ventricular diastolic performance in adult non-Hodgkin's lymphoma patients receiving DOX therapy [116, 117]. In pediatric cancer patients experiencing left ventricular ejection fraction [LVEF] dysfunction, concentrations of natriuretic peptides are elevated, primarily correlating with systolic function, unlike in adult patients. Nevertheless, monitoring LVEF continues to be the recommended approach for clinical diagnosis even when there is a correlation between natriuretic peptide concentrations and decreased LVEF and prognosis in DOX-induced heart failure [118].

Strategies for prevention of DOX-induced cardiotoxicity

Dose-Dependent administration

The study suggests that slow continuous administration of anthracycline [DOX] is safer from a cardiotoxicity standpoint than large bolus doses, and dose-fractionated weekly schedules can significantly reduce cardiac events without compromising effectiveness compared to the usual three-week regimen [119]. The mechanism of continuous DOX plasma formation in the heart is thought to increase the exposure of cardiomyocytes to DOX, thereby reducing the occurrence of DIC [120]. Administering DOX infusions over extended periods ranging from 48 to 96 hours reduced cardiotoxicity and displayed anticancer effectiveness. However, modifying the timing of administration did not produce comparable outcomes between adult patients and pediatric populations. While continuous infusions lasting 48 hours were reported to be safer for breast cancer patients, children receiving DOX treatment for acute lymphoblastic leukemia [ALL] did not benefit from cardiac protection [121- 123].

Vitamin E

Vitamin E, a fat-soluble compound, is formulated by combining four tocopherols and four tocotrienols [124,125]. Because vitamin E is an antioxidant, it shields cell membranes from reactive oxygen species. According to a study, vitamin E can prevent Dox-induced cardiotoxicity. Vitamin E is beneficial for Dox-induced acute cardiotoxicity. Compared to cardiotoxicity, other antioxidants such as vitamin C, the seven organic compounds PZ51, reduced glutathione, ambroxol, ursolic acid, and oleanolic acid only marginally improve cardiotoxicity [126,127].

Modified formulations

To lessen DOX's cardiotoxic effects, research teams have looked closely at modified formulations of the drug, such as liposomal encapsulation techniques [128-130]. Liposomal formulations can encapsulate hydrophilic drugs directly surrounded by the aqueous compartment or integrate nonpolar drugs into the lipid bilayer. In the market, non-PEG-related and PEGylated liposomal DOX formulations are obtainable, with the PEGylated versions being the predominant choice in the United States [131]. The PEGylated liposomal version of DOX, approved by the FDA, is called Doxil. Patients with multiple myeloma, ovarian cancer, and Kaposi's sarcoma who have had at least one prior chemotherapy treatment failure are prescribed it [132].

Because of their improved permeability retention effect, liposomal formulations of DOX accumulate preferentially at the tumor site and cause lower peak plasma concentrations of free DOX, which reduces their cardiotoxic effects [133]. In addition, PEGylation of liposomes prolongs their bloodstream circulation duration by preventing absorption by the reticuloendothelial system. This ensures sustained efficacy while enhancing safety by encapsulating free DOX [134]. Furthermore, Liposomal DOX formulations produced reduced cardiotoxicity while keeping breast cancer treatment effective, particularly when combined with other chemotherapy medications, according to an analysis of phase II and III clinical studies. This implies that liposomal versions of DOX may be a safer alternative to regular DOX in treating various malignancies [135]. Due to their versatility and efficacy, hybrid nanosystems are increasingly used in gene therapy, drug delivery, phototherapy, tissue regeneration, vaccines, antibacterials, biomolecule detection, imaging probes, and theranostics. These nanosystems can be classified based on their foundational components, accessory moieties, and hybridization architecture. The study of cardiac scaffolds focuses on material selection, surface engineering, processing methods, and electrical coupling between artificial scaffolds and native tissue. Nanofibrous hybrids and carbon and metal-based systems enable cardiac tissues, and functionalized CNTs have led to human-induced pluripotent stem cell-derived cardiomyocyte maturation [136]

Allicin

Allicin, a bioactive component of garlic, constitutes an organosulfur molecule. It demonstrates a range of pharmacological activities, such as antifungal, antioxidant, antibacterial, and anticarcinogenic qualities [137]. It functions as a cytoprotective drug to stop Dox-induced cardiotoxicity. In acute Dox intoxication, a study found that allicin effectively lowers myocardial oxidative stress, inflammation, and apoptosis. Following the deactivation of antioxidants, for example, superoxide dismutase [SOD], glutathione peroxidase, and catalase [CAT] by DOX, allicin works to replenish antioxidant levels. This process helps mitigate oxidative damage and reduce cardiac apoptosis [138].

Cardioprotective effects of 20[S]-ginsenoside Rh2 against

Ginseng has been a renowned traditional Chinese medicine for centuries. Its main active components, ginsenosides, offer a range of pharmacological benefits. These include enhancing cardiovascular health, boosting immune function, improving stress resistance, memory, and learning, and supporting the social and mental growth of chemotherapy patients and healthy persons. Ginsenoside Rh2 [139] is a well-known bioactive ginsenoside found in Panax ginseng Rh2 pretreatment reduces the cytotoxic effects of Dox-induced cardiomyocyte damage dose-dependently, increasing cardiac cell survival rates. Additionally, Rh2 therapy restores serum creatine kinase, lactate dehydrogenase, and lactate dehydrogenase, exhibiting cardioprotective effects [140]. Exposure to Dox diminishes antioxidants such as SOD, CAT, and glutathione while elevating malondialdehyde levels. In contrast, Rh2 administration rebalances this antioxidant imbalance [141]

Mediator as Hematopoietic Cytokines

Erythropoietin [EPO] is frequently used to treat anemia and is essential to hematopoiesis [142]. Erythropoietin [EPO] receptors are distributed across different organs and tissue types, such as the brain, heart, and skeletal muscle. EPO demonstrates efficacy both in vitro and in vivo in mice by stopping cardiomyocytes by programmed cell death, preventing heart atrophy, and mitigating left ventricular dysfunction. A study examining the cardioprotective properties of EPO in male Wistar rats unveiled a reduction in oxidative stress and cardiomyocyte apoptosis [143] SIRT1 to EPO-mediated cardio defense alongside DIC through mitochondrial dysfunction and toxicity, highlighting the importance of SIRT1 in cellular health [144]. Similarly, TPO [thrombopoietin] demonstrated cardio-protective effects in H9c2 cells, primary myocytes from newborn rats, and animal models [145]. Studies conducted on rat models subjected to severe and prolonged DOX management revealed that thrombopoietin [TPO] reinstated heart function, proposing its mechanisms of action through the Akt and ERK pathways [146,147]

Probucol

Probucol is an antihyperlipidemic medication that effectively reduces lipid levels and acts as an antioxidant. It demonstrates a cardioprotective effect by lowering heart failure and cardiac muscle damage without interfering with Dox's antineoplastic effects [148].

MiR-181c shields cardiomyocytes from injury by averting cell apoptosis through modulation of the PI3K/Akt signaling pathway.

Heart failure, which raises the risk of death from cardiovascular diseases, is caused by apoptosis in the cardiac muscle. After receiving Dox, the microRNA miR181c—which promotes muscle cell survival through the PI3K/Akt signaling pathway—is downregulated. Although MiR-181c therapy reduces apoptosis in Dox-treated H9C2 cells, downregulation of MiR-181c promotes cardiotoxicity by activating toxic markers like TNF-α, Fas, and IL-6 [149].

Nanocarrier-based delivery systems, such as liposomal formulations, have demonstrated reduced cardiotoxicity by selectively targeting tumor tissues. Antioxidants like Vitamin E and novel agents like allicin have shown promise in mitigating oxidative stress, though their clinical translation remains limited by inconsistent results [150]

DNA gene editing using CRISPR/Cas9

Classical CRISPR-Cas9 technology has been used to engineer isogenic lines of human induced pluripotent stem cells (hiPSCs) to investigate the association of genotype to disease phenotype in a precise, robust, and reproducible way. This technology has allowed the in vitro and in vivo study of many monogenic diseases of high or incomplete penetrance. For example, patient-specific hiPSC-derived cardiomyocytes and microtissues harboring a mutation in PKP2 display impaired desmosomal stability compared to isogenic controls generated using CRISPR-Cas9.

The researchers improved cardiac function in the mutant mice by performing CRISPR-Cas9-mediated in vivo genome editing combined with adeno-associated virus (AAV)9 delivery to inactivate the mutant allele. This study provided the first preclinical evidence that CRISPR-mediated disruption of the PLN R14del allele can improve cardiac function in a humanized mouse model and could be valuable for treating the arrhythmogenic phenotype in human carriers. CRISPR-Cas9 editing has successfully generated cell lines and mouse models to study genetic cardiomyopathies [151].

RNA-based therapeutics

RNA-based therapeutics, including small interfering RNA (siRNA), antisense oligonucleotides (ASOs), and messenger RNA (mRNA), have the potential to revolutionize heart failure management. These therapies target specific genetic and molecular pathways involved in heart failure pathogenesis. However, challenges include delivery methods, stability of RNA molecules, and off-target effects. RNA-based therapeutics can promote cardiac regeneration and repair by promoting the expression of factors that enhance tissue regeneration, such as growth factors or stem cell differentiation factors. Combining RNA-based therapies with existing treatment modalities, such as pharmacological agents or medical devices, may offer synergistic effects and improved clinical outcomes. For example, combining RNA-based therapies with beta-blockers or angiotensin-converting enzyme inhibitors may have additive or synergistic effects on cardiac remodeling and function[152]

MANAGEMENT FOR CONGESTIVE HEART FAILURE

Congestive heart failure [CHF] can be treated by reducing heart pressure and minimizing its causing factors. Treatment methods include ACE inhibitors or angiotensin II receptor antagonists, beta-blockers, and anti-mineralcorticoids like spironolactone. These medications help reduce high blood pressure by reducing fluid retention and reducing the need for oxygen. Additionally, they protect the heart from stress hormones, allowing it to beat steadier and require less oxygen. Beta-blockers like carvedilol, ACE inhibitors such as enalapril, and mineralocorticoid receptor antagonists like spironolactone have been effective in managing heart failure associated with DOX. These drugs, administered at optimized dosages, reduce cardiac workload and mitigate remodeling [153]

Erythropoietin

The hormone erythropoietin is produced in tiny amounts by healthy kidneys and the liver. A glycoprotein cytokine, erythropoietin, is primarily released by the kidney during periods of cellular hypoxia [154]. A few anti-anemic medications also exhibit cardioprotection against the cardiotoxic effects of Dox. An anti-anemic medication called erythropoietin is used to treat anemia by chemotherapy. When given prophylactically, erythropoietin reduces apoptosis and cardiomyopathy to protect against Dox-induced cardiotoxicity [155].

SUMMARY

This review looks at the effect of DOX on the Heart. DOX is a chemotherapy medication that has been employed to treat cancer for more than 30 years. DOX has the potential to be fatally toxic to major organs, especially cardiotoxicity. Its biological activities include intercalating with DNA base pairs and binding

to enzymes connected to DNA. It can also target several different molecular targets, which might damage DNA and change gene expression—a typical kind of cardiotoxicity. DOX activates various molecular signals, leading to a pro-apoptotic protein expression is up while anti-apoptotic protein expression is down. This occurs through activating AMPK, influencing programmed cell death, and inducing the Bcl-2/Bax apoptosis mechanism. Consequently, the Bcl-2/Bax ratio is disrupted, triggering the breakdown and stimulation of caspase 3 and 9. Additionally, DOX prompts can cause apoptosis and permanent cell death in healthy tissues, leading to injuriousness in vital organs such as the liver, brain, renal tubules, and heart. This impedes cardiac healing mechanisms and destroys local bone marrow stem cells, rendering patients susceptible to cardiotoxicity. Increased expression of NFKB results in inflammation and necrosis of cardiac tissue, leading to cardiomyopathy. DOX is a medication used to treat cancer, and several studies have looked into drug delivery methods for this medication. The ability of liposomes to enter intracerebral malignancies without harming healthy brain tissue has demonstrated encouraging outcomes. Cells communicate through extracellular molecules like nucleotides, lipids, miRNA, or proteins, which cells can release in microvesicles and bind to receptors on other cells, causing intracellular signaling and modifying the physiological state of recipient cells. Exosomes in various biological fluids contain proteins, lipids, and genetic material like mRNA and miRNA. The discovery and use of exosomes as clinical biomarkers of cardiovascular disease risk is potentially aid in the development of new therapeutics. Recent hydrogel systems and nanoparticles have been altered to improve efficacy. If DOX's chemistry, transport, and toxicity are improved, its clinical use is anticipated to continue for a more extended period. Fulfilling these three objectives is necessary for these enhancements to succeed.

CONCLUSION

Owing to its wide range of effects, DOX is a commonly utilized cancer treatment medication. Its dosage, meanwhile, needs to be carefully addressed because it can have negative effects on the Heart and several organs. Although DOX's exact processes are unknown, it inserts into DNA, is responsible for inhibiting topoisomerase II enzymes, damages the mitochondrial wall, and raises the production of free radicals and oxidative damage. Developing efficient delivery methods, creating DOX mimics, and giving antioxidant or anti-apoptotic medications are some strategies to lessen negative effects. Nevertheless, specific approaches have not been able to reduce anthracycline toxicity in clinical trials. More efficient approaches ought to be investigated to minimize toxicity and maintain or improve the therapeutic effects of DOX. While animal models have provided significant insights into the molecular mechanisms of DOX cardiotoxicity, translating these findings to human clinical outcomes remains challenging due to differences in drug metabolism, genetic factors, and comorbidities.

FUTURE DIRECTIONS

Patients treated with DOX, a medication used to treat cancer, may experience cardiotoxicity, which could be fatal and harm other organs. 'ROS and iron' is the most widely accepted theory. Researchers should investigate how iron buildup and ROS levels are related to toxicity and how DOX raises ROS levels to prevent toxicity. Adverse effects include immune system activation, altered gene expression, and compromised heart repair mechanisms. Research should concentrate on target-specific DOX delivery mechanisms to avoid impeding the regeneration and mobilization of bone marrow cells. AI is expected to significantly enhance personalized medicine, even in simple disease treatments. While physicians have intuition, AI can link data in a way not typically achievable by humans. With accurate data, AI can diagnose cases that clinicians wouldn't, helping patients fight diseases earlier. AI may also revolutionize medication and drug design, allowing for more efficient disease treatment and early detection.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Md. Amaan, Dr. Radha Goel, Surovi Paul, and Iqbal Danish equally participated in the article preparation. They made substantial contributions to the conception of the article, to drafting it, and to revising it critically for important intellectual content. All authors have approved the final article, which is the version to be submitted.

REFERENCES

[1] Thorn CF, Oshiro C, Marsh S, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. *Pharmacogenet*

Genomics.,**21,** 440-446(2011) https://doi.org/10.1097/FPC.0b013e32833ffb56

[2] Basser RL, Green MD. Strategies for prevention of anthracycline cardiotoxicity. *Cancer Treat. Rev*., **19,** 57-77 (1993) https://doi.org/10.1016/0305-7372(93)90027-o

[3] Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. *Curr. Med. Chem*., **16**, 3267-85 (2009) https://doi.org/10.2174/092986709788803312

[4] Afrin H, Salazar CJ, Kazi M, Ahamad SR, Alharbi M, Nurunnabi M. Methods of screening, monitoring and management of cardiac toxicity induced by chemotherapeutics. *Chin. Chem. Lett.*, **33 ,** 2773-82 (2022) https://doi.org/10.1016/j.cclet.2022.01.011.

[5] Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. *Nat. Rev. Cancer*., **9,** 338-50. https://doi.org/10.1038/nrc2607

[6] Subapriya R, Kumaraguruparan R, Ramachandran CR, Nagini S. Oxidant-antioxidant status in patients with oral squamous cell carcinomas at different intraoral sites. *Clin Biochem.*, **35,** 489-93 (2002) https://doi.org/10.1016/s0009-9120(02)00340-5

[7] Quiles JL, Huertas JR, Battino M, Mataix J, Ramírez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. *Toxicol.,* **180,** 79-95 (2002) https://doi.org/10.1016/s0300-483x(02)00383-9

[8] Xiao B, Hong L, Cai X, Mei S, Zhang P, Shao L. The true colors of autophagy in doxorubicin-induced cardiotoxicity. *Oncol. Letters.*, **18,** 2165-72 (2019) https://doi.org/10.3892/ol.2019.10576

[9] Di Meo S, Reed TT, Venditti P, Victor VM. Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxid. Med. Cell. Longev*., 2016,1245049 (2016) https://doi.org/10.1155/2016/1245049

[10] Vitale R, Marzocco S, Popolo A. Role of oxidative stress and inflammation in doxorubicin-induced cardiotoxicity: A brief account. *Int. J. Mol. Sci.*, **25(13),** 7477(2022) https://doi.org/10.3390/ijms25137477

[11] Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. *Int. J. Cardiol. Heart Vasc*., **10,** 17-24 (2016) https://doi.org/10.1016/j.ijcha.2015.11.004

[12] Kamińska K, Cudnoch-Jędrzejewska A. A Review on the Neurotoxic Effects of Doxorubicin. *Neurotox. Res*., **41,** 383-397 (2023) https://doi.org/10.1007/s12640-023-00652-5

[13] Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology.*, **115,** 155-62 (2010) https://doi.org/10.1159/000265166

[14] Fabiani I, Aimo A, Grigoratos C, Castiglione V, Gentile F, Saccaro LF, Arzilli C, Cardinale D, Passino C, Emdin M. Oxidative stress and inflammation: determinants of anthracycline cardiotoxicity and possible therapeutic targets. *Heart Fail. Rev.*, **26**, 881-90 (2021) https://doi.org/10.1007/s10741-020-10063-9

[15] Sakata Y, Dong JW, Vallejo JG, Huang CH, Baker JS, Tracey KJ, Tacheuchi O, Akira S, Mann DL. Toll-like receptor 2 modulates left ventricular function following ischemia-reperfusion injury. *Am. J. Physiol. Heart. Circ. Physiol*., **292**, H503-9 (2007) https://doi.org/10.1152/ajpheart.00642.2006

[16] Zhu H, Sarkar S, Scott L, et al. Doxorubicin Redox Biology: Redox Cycling, Topoisomerase Inhibition, and Oxidative Stress. *React. Oxyg. Species (Apex)*., 1, 189-98 (2016) https://doi.org/10.20455/ros.2016.835

[17] Shi Y, Moon M, Dawood S, McManus B, Liu PP. Mechanisms and management of doxorubicin cardiotoxicity. *Herz*., **36,** 296-305 (2011) https://doi.org/10.1007/s00059-011-3470-3

[18] Cardoso S, Santos RX, Carvalho C, Correia S, Pereira GC, Pereira SS, Oliveira PJ, Santos MS, Proença T, Moreira PI. Doxorubicin increases the susceptibility of brain mitochondria to Ca^{2+}-induced permeability transition and oxidative damage. *Free Radic. Biol. Med.*, **45,**1395-1402 (2008) https://doi.org/10.1016/j.freeradbiomed.2008.08.008

[19] Tsang WP, Chau SP, Kong SK, Fung KP, Kwok TT. Reactive oxygen species mediate doxorubicin induced p53-independent apoptosis. *Life Sci*., **73,**2047-58 (2003) https://doi.org/10.1016/s0024-3205(03)00566-6

[20] Ashikawa K, Shishodia S, Fokt I, Priebe W, Aggarwal BB. Evidence that activation of nuclear factor-κB is essential for the cytotoxic effects of doxorubicin and its analogues. *Biochem. Pharmacol.*, **67,** 353-64 (2004) https://doi.org/10.1016/j.bcp.2003.08.039

[21] Bien S, Ritter CA, Gratz M, Sperker B, Sonnemann J, Beck JF, Kroemer HK. Nuclear factor-κB mediates up-regulation of cathepsin B by doxorubicin in tumor cells. *Mol. Pharmacol.*, **65,** 1092-1102 (2004) https://doi.org/10.1124/mol.65.5.1092

[22] Lefrak EA, Piťha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity, *Cancer.*, **32,** 302-14 (1973) https://doi.org/10.1002/1097-0142(197308)32:2<302::aid-cncr2820320205>3.0.co;2-2

[23] Herman E, Mhatre R, Lee IP, Vick J, Waravdekar VS. A comparison of the cardiovascular actions of daunomycin, adriamycin and N-acetyldaunomycin in hamsters and monkeys. *Pharmacol.*, **6,** 230-41 (1971) https://doi.org/10.1159/000136248

[24] van Acker SA, Kramer K, Voest EE, Grimbergen JA, Zhang J, van der Vijgh WJ, Bast A, van Acker SA. Doxorubicin-induced cardiotoxicity monitored by ECG in freely moving mice A new model to test potential protectors: A new model to test potential protectors. *Cancer Chemother. Pharmacol.*, **38**, 95-101 (1996) https://doi.org/10.1007/s002800050453

[25] Von Hoff DD, Layard MW, Basa P, DAVIS Jr HL, Von Hoff AL, Rozencweig M, Muggia FM. Risk factors for doxorubicin-lnduced congestive heart failure. *Ann. Intern. Med.* **, 91,** 710-17 (1979) https://doi.org/10.7326/0003-4819-91-5-710

[26] Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. *N. Engl. J. Med*.**, 339,** 900-5 (1998) https://doi.org/10.1056/NEJM199809243391307

[27] Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer.*, 97, 2869-79 (2003) https://doi.org/10.1002/cncr.11407

[28] Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer*., 97, 2869-79 (2003) https://doi.org/10.1002/cncr.11407

[29] Tu VC, Bahl JJ, Chen QM. Signals of oxidant-induced cardiomyocyte hypertrophy: key activation of p70 S6 kinase-1 and phosphoinositide 3-kinase. *J. Pharmacol. Exp. Ther*. , **300,** 1101-10, (2002) https://doi.org/10.1124/jpet.300.3.1101

[30] Merten KE, Jiang Y, Feng W, Kang YJ. Calcineurin activation is not necessary for Doxorubicin-induced hypertrophy in H9c2 embryonic rat cardiac cells: involvement of the phosphoinositide 3-kinase-Akt pathway. *J. Pharmacol. Exp. Ther.*, **319,** 934-40 (2006) https://doi.org/10.1124/jpet.106.108845

[31] Sardão VA, Oliveira PJ, Holy J, Oliveira CR, Wallace KB. Morphological alterations induced by doxorubicin on H9c2 myoblasts: nuclear, mitochondrial, and cytoskeletal targets. *Cell. Bio. Toxicol.*, **25**, 227-43 (2009) https://doi.org/10.1007/s10565-008-9070-1

[32] Zordoky BN, El-Kadi AO. Induction of several cytochrome P450 genes by doxorubicin in H9c2 cells. *Vascul. Pharmacol.*, **49,** 166-72 (2008) https://doi.org/10.1016/j.vph.2008.07.004

[33] El-Kadi AO, Zordoky BN. Modulation of cardiac and hepatic cytochrome P450 enzymes during heart failure. *Curr. Drug Metabol.*, **9,** 122-8 (2008) https://doi.org/10.2174/138920008783571792

[34] Goormaghtigh E, Huart P, Praet M, Brasseur R, Ruysschaert JM. Structure of the adriamycin-cardiolipin complex: role in mitochondrial toxicity. *Biophys. Chem*., **35,** 247-57 (1990) https://doi.org/10.1016/0301-4622(90)80012-v

[35] Berthiaume JM, Wallace KB. Adriamycin-induced oxidative mitochondrial cardiotoxicity. *Cell. Biol. Toxicol*., **23,** 15-25 (2007) https://doi.org/10.1007/s10565-006-0140-y

[36] Odom AL, Hatwig CA, Stanley JS, Benson AM. Biochemical determinants of adriamycin® toxicity in mouse liver, heart and intestine. *Biochem. Pharmacol.*, **43,** 831-36 (1992) https://doi.org/10.1016/0006-2952(92)90250-m

[37] Okoye CN, Koren SA, Wojtovich AP. Mitochondrial complex I ROS production and redox signaling in hypoxia. *Redox Biol.*, **67**, 102926 (2023) https://doi.org/10.1016/j.redox.2023.102926.

[38] Larsson NG, Holme E, Kristiansson B, Oldfors A, Tulinius M. Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayre syndrome. *Pediatr. Res*., **28,** 131-36, 1990 https://doi.org/10.1203/00006450-199008000-00011

[39] Lebrecht D, Setzer B, Ketelsen UP, Haberstroh J, Walker UA. Time-dependent and tissue-specific accumulation of mtDNA and respiratory chain defects in chronic doxorubicin cardiomyopathy. *Circulation.*, **108,** 2423-29 (2003) https://doi.org/10.1161/01.CIR.0000093196.59829.DF

[40] Tokarska-Schlattner M, Zaugg M, Zuppinger C, Wallimann T, Schlattner U. New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics. *J. Mol. Cell. Cardiol*., **41,**389-405 (2006) https://doi.org/10.1016/j.yjmcc.2006.06.009

[41] Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. *Prog. Cardiovasc. Dis.*, **49,** 330-352 (2007) https://doi.org/10.1016/j.pcad.2006.10.002

[42] Renu K, Abilash VG, PB TP, Arunachalam S. Molecular mechanism of doxorubicin-induced cardiomyopathy–An update. *Eur. J. Pharmacol*., **818**, 241-253 (2018) https://doi.org/10.1016/j.ejphar.2017.10.043

[43] Goormaghtigh E, Huart P, Brasseur R, Ruysschaert JM. Mechanism of inhibition of mitochondrial enzymatic complex I–III by adriamycin derivatives. *Biochim. Biophys. Acta*., **861,** 83-94 (1996) https://doi.org/10.1016/0005-2736(86)90374-3

[44] Cheung KG, Cole LK, Xiang B, Chen K, Ma X, Myal Y, Hatch GM, Tong Q, Dolinsky VW. Sirtuin-3 (SIRT3) protein attenuates doxorubicin-induced oxidative stress and improves mitochondrial respiration in H9c2 cardiomyocytes. *J. Biol. Chem.*, **290,** 10981–93 (2015) https://doi.org/10.1074/jbc.M114.607960

[45] Schlame M, Rua D, Greenberg ML. The biosynthesis and functional role of cardiolipin. *Prog. Lipid Res*., **39,** 257-88(2000) https://doi.org/10.1016/s0163-7827(00)00005-9

[46] Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev*.**, 56,**185-229 (2004) https://doi.org/10.1124/pr.56.2.6

[47] Goormaghtigh E, Pollakis G, Ruysschaert JM. Mitochondrial membrane modifications induced by adriamycin-mediated electron transport. *Biochem. Pharmacol*., **32**, 889-93 (1983) https://doi.org/10.1016/0006-2952(83)90593-2

[48] Marcillat O, Zhang Y, Davies KJ. Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochem. J.*, **259,** 181-89 (1991) https://doi.org/10.1042/bj2590181

[49] Delemasure S, Vergely C, Zeller M, Cottin Y, Rochette L. Preventing the cardiotoxic effects of anthracyclins. From basic concepts to clinical data. *In Annales de cardiologie et d'angeiologie* , **5,** 104-12 (2006) https://doi.org/10.1016/j.ancard.2006.02.005

[50] Wang Y, Lilienfeldt N, Hekimi S. Understanding coenzyme Q. *Physiol Rev*. **104,** 1533-1610 (2024) https://doi.org/10.1152/physrev.00040.2023

[51] Turrens JF. Mitochondrial formation of reactive oxygen species. *J. Physiol*., **552,** 335-44 (2003) https://doi.org/10.1113/jphysiol.2003.049478

[52] Priya LB, Baskaran R, Huang CY, Padma VV. Neferine ameliorates cardiomyoblast apoptosis induced by doxorubicin: possible role in modulating NADPH oxidase/ROS-mediated NFκB redox signaling cascade. *Sci Rep*., **7,** 12283 (2017) https://doi.org/10.1038/s41598-017-12060-9

[53] Mizutani H, Tada-Oikawa S, Hiraku Y, Kojima M, Kawanishi S. Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. *Life Sci*., **76**, 1439-53 (2005) https://doi.org/10.1016/j.lfs.2004.05.040

[54] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. *Free Radic. Biol. Med*. 2009, **47,** 333-43. https://doi.org/10.1016/j.freeradbiomed.2009.05.004

[55] Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am. J. Physiol. Heart Circ. Physiol*., **309,** H1453-67 (2015) https://doi.org/10.1152/ajpheart.00554.2015

[56] Podyacheva E, Toropova Y. SIRT1 activation and its effect on intercalated disc proteins as a way to reduce doxorubicin cardiotoxicity. *Front Pharmacol*., **13,** 1035387 (2022) https://doi.org/10.3389/fphar.2022.1035387

[57] Yu H, Rong W, Yang J, et al. Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL): A Novel Biomarker for Prognostic Assessment and Risk Stratification of Acute Pulmonary Embolism. *J. Clin. Med*., **11**, 3908 (2022) https://doi.org/10.3390/jcm11133908

[58] Floros KV, Thomadaki H, Florou D, Talieri M, Scorilas A. Alterations in mRNA expression of apoptosis-related genes BCL2, BAX, FAS, caspase-3, and the novel member BCL2L12 after treatment of human leukemic cell line HL60 with the antineoplastic agent etoposide. *Ann. N. Y. Acad. Sci*.,1090, 89-97 (2006) https://doi.org/10.1196/annals.1378.009

[59] Liu TJ, Yeh YC, Ting CT, Lee WL, Wang LC, Lee HW, Wang KY, Lai HC, Lai HC. Ginkgo biloba extract 761 reduces doxorubicin-induced apoptotic damage in rat hearts and neonatal cardiomyocytes. *Cardiovasc. Res*., **80 ,** 227-35 (2008) https://doi.org/10.1093/cvr/cvn192

[60] Li Y, Yan J, Yang P. The mechanism and therapeutic strategies in doxorubicin-induced cardiotoxicity: Role of programmed cell death. *Cell Stress Chaperones*., **29,** 666-80 (2024) https://doi.org/10.1016/j.cstres.2024.09.001

[61] Liu J, Mao W, Ding B, Liang CS. ERKs/p53 signal transduction pathway is involved in doxorubicin-induced apoptosis in H9c2 cells and cardiomyocytes. *Am. J. Physiol. Heart. Circ. Physiol*., **295 ,** H1956-1965 (2008) https://doi.org/10.1152/ajpheart.00407.2008

[62] Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. *Sci. Rep.*,**7** ,44735 (2017) https://doi.org/10.1038/srep44735

[63] Moreira AC, Branco AF, Sampaio SF, Cunha-Oliveira T, Martins TR, Holy J, Oliveira PJ, Sardão VA. Mitochondrial apoptosis-inducing factor is involved in doxorubicin-induced toxicity on H9c2 cardiomyoblasts. *Biochim. Biophys. Acta.*, **1842,** 2468-2478 (2014) https://doi.org/10.1016/j.bbadis.2014.09.015

[64] Songbo M, Lang H, Xinyong C, Bin X, Ping Z, Liang S. Oxidative stress injury in doxorubicin-induced cardiotoxicity. *Toxicol Lett*., 307:41-48 (2019) https://doi.org/10.1016/j.toxlet.2019.02.013

[65] Wenningmann N, Knapp M, Ande A, Vaidya TR, Ait-Oudhia S. Insights into Doxorubicin-induced Cardiotoxicity: Molecular Mechanisms, Preventive Strategies, and Early Monitoring. *Mol. Pharmacol*. , **96**, 219-32 (2019) https://doi.org/10.1124/mol.119.115725

[66] Mancilla TR, Iskra B, Aune GJ. Doxorubicin-Induced Cardiomyopathy in Children. *Compr. Physiol*., **9,** 905-31(2019) https://doi.org/10.1002/cphy.c180017

[67] Zhao Y, He R, Oerther S, Zhou W, Vosough M, Hassan M. Cardiovascular Complications in Hematopoietic Stem Cell Transplanted Patients. *J. Pers. Med*., **12,** 1797 (2022) https://doi.org/10.3390/jpm12111797

[68] Wallace KB. Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis. *Cardiovasc. Toxicol.*, **7,** 101-7 (2007) https://doi.org/10.1007/s12012-007-0008-2

[69] Nicolay K, Fok JJ, Voorhout W, Post JA, de Kruijff B. Cytofluorescence detection of adriamycin-mitochondria interactions in isolated, perfused rat heart *Biochim. Biophys. Acta*., **887,**35-41 (1986) https://doi.org/10.1016/0167-4889(86)90119-9

[70] Fernandez-Chas M, Curtis MJ, Niederer SA. Mechanism of doxorubicin cardiotoxicity evaluated by integrating multiple molecular effects into a biophysical model. *Br. J. Pharmacol*., **175**, 763-81 (2018) https://doi.org/10.1111/bph.14104

[71] Arai M, Tomaru K, Takizawa T, Sekiguchi K, Yokoyama T, Suzuki T, Nagai R. Sarcoplasmic reticulum genes are selectively down-regulated in cardiomyopathy produced by doxorubicin in rabbits. *Mol. Cell Cardiol*., **30,** 243-54 (1998) https://doi.org/10.1006/jmcc.1997.0588

[72] Kalivendi SV, Konorev EA, Cunningham S, Vanamala SK, Kaji EH, Joseph J, Kalyanaraman B. Doxorubicin activates nuclear factor of activated T-lymphocytes and Fas ligand transcription: role of mitochondrial reactive oxygen species and calcium. *Biochem. J*., **389,** 527-39 (2005) https://doi.org/10.1042/BJ20050285

[73] Little GH, Saw A, Bai Y, Dow J, Marjoram P, Simkhovich B, Leeka J, Kedes L, Kloner RA, Poizat C. Critical role of nuclear calcium/calmodulin-dependent protein kinase IIδB in cardiomyocyte survival in cardiomyopathy. *J. Biol. Chem*., **5284,** 24857-68 (2009) https://doi.org/10.1074/jbc.M109.003186

[74] Sag CM, Köhler AC, Anderson ME, Backs J, Maier LS. CaMKII-dependent SR Ca leak contributes to doxorubicin-induced impaired Ca handling in isolated cardiac myocytes. *J. Mol. Cell Cardiol*., **51**, 749-59 (2011) https://doi.org/10.1016/j.yjmcc.2011.07.016

[75] Takaseya T, Ishimatsu M, Tayama E, Nishi A, Akasu T, Aoyagi S. Mechanical unloading improves intracellular Ca2+ regulation in rats with doxorubicin-induced cardiomyopathy. *J .Am. Coll. Cardiol*., **44,** 2239-46 (2004) https://doi.org/10.1016/j.jacc.2004.08.057

[76] Lebrecht D, Kirschner J, Geist A, Haberstroh J, Walker UA. Respiratory chain deficiency precedes the disrupted calcium homeostasis in chronic doxorubicin cardiomyopathy. *Cardiovasc. Pathol*. , **19,** e167-74 (2010) https://doi.org/10.1016/j.carpath.2009.06.006

[77] Agustini FD, Arozal W, Louisa M, Siswanto S, Soetikno V, Nafrialdi N, Suyatna F. Cardioprotection mechanism of mangiferin on doxorubicin-induced rats: Focus on intracellular calcium regulation. *Pharm .Biol*., **54,**1289-97 (2016) https://doi.org/10.3109/13880209.2015.1073750

[78] Gao J, Chen T, Zhao D, Zheng J, Liu Z. Ginkgolide B exerts cardioprotective properties against doxorubicin-induced cardiotoxicity by regulating reactive oxygen species, Akt and calcium signaling pathways in vitro and in vivo. *PloS One*, **11,** e0168219 (2016) https://doi.org/10.1371/journal.pone.0168219

[79] Böhm F, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovasc. Res.*, **76,** 8-18 (2007) https://doi.org/10.1016/j.cardiores.2007.06.004

[80] Bien S, Riad A, Ritter CA, Gratz M, Olshausen F, Westermann D, Grube M, Krieg T, Ciecholewski S, Felix SB, Staudt A. The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. *Cancer Res.*, **67**, 10428-435 (2007) https://doi.org/10.1158/0008-5472.CAN-07-1344

[81] Picard P, Smith PJ, Monge JC, Rouleau JL, Nguyen QT, Calderone A, Stewart DJ. Coordinated upregulation of the cardiac endothelin system in a rat model of heart failure. *J. Cardiovasc. Pharmacol.*, **31**, S294-297 (1998) https://doi.org/10.1097/00005344-199800001-0008

[82] Sayed-Ahmed MM, Khattab MM, Gad MZ, Osman AM. Increased plasma endothelin-1 and cardiac nitric oxide during doxorubicin-induced cardiomyopathy. *Pharmacol. Toxicol.* **, 89,** 140-44 (2001) https://doi.org/10.1034/j.1600-0773.2001.d01-148.x

[83] Suzuki T, Miyauchi T. A novel pharmacological action of ET-1 to prevent the cytotoxicity of doxorubicin in cardiomyocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **280,** R1399-1406 (2001) https://doi.org/10.1152/ajpregu.2001.280.5.R1399

[84] Schwebe M, Ameling S, Hammer E, Monzel JV, Bonitz K, Budde S, Schult K, Oswald S, Scheuch E, Grube M, Poesch A. Protective effects of endothelin receptor A and B inhibitors against doxorubicin-induced cardiomyopathy. *Biochem. Pharmacol*., **94,** 109-29 (2015) https://doi.org/10.1016/j.bcp.2015.01.014

[85] Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science.*, **226,** 466-8 (1984) https://doi.org/10.1126/science.6093249

[86] Pendleton M, Lindsey RH Jr, Felix CA, Grimwade D, Osheroff N. Topoisomerase II and leukemia. *Ann. N. Y. Acad. Sci*., 1310, 98-110 (2014) https://doi.org/10.1111/nyas.12358

[87] Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, Yeh ET. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat. Med.*, **18 ,**1639-42 (2012) https://doi.org/10.1038/nm.2919

[88] van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. *Cochrane Database Syst. Rev*.. **6,** 23-28, (2011) https://doi.org/10.1002/14651858.CD003917.pub3

[89] Deng S, Yan T, Jendrny C, Nemecek A, Vincetic M, Gödtel-Armbrust U, Wojnowski L. Dexrazoxane may prevent doxorubicin-induced DNA damage via depleting both topoisomerase II isoforms. *BMC Cancer.*, 14,842 (2014) https://doi.org/10.1186/1471-2407-14-842

[90] Yan T, Deng S, Metzger A, Gödtel-Armbrust U, Porter AC, Wojnowski L. Topoisomerase IIα-dependent and-independent apoptotic effects of dexrazoxane and doxorubicin. *Mol. Cancer Ther*., **8**, 1075-85 (2009) https://doi.org/10.1158/1535-7163.MCT-09-0139

[91] de Oliveira BL, Niederer S. A biophysical systems approach to identifying the pathways of acute and chronic doxorubicin mitochondrial cardiotoxicity. *PLoS Comput. Biol*., **12,** e1005214 (2016) https://doi.org/10.1371/journal.pcbi.1005214

[92] Pointon AV, Walker TM, Phillips KM, Luo J, Riley J, Zhang SD, Parry JD, Lyon JJ, Marczylo EL, Gant TW. Doxorubicin in vivo rapidly alters expression and translation of myocardial electron transport chain genes, leads to ATP loss and caspase 3 activation. *PLoS One***, 5,** e12733 (2010) https://doi.org/10.1371/journal.pone.0012733

[93] Bahat A, Gross A. Mitochondrial plasticity in cell fate regulation. *J Biol Chem*., **294**, 13852-63 (2019) https://doi.org/10.1074/jbc.REV118.000828

[94] Nakao M. Epigenetics: interaction of DNA methylation and chromatin. *Gene*. , **278,** 25-31 (2001) https://doi.org/10.1016/s0378-1119(01)00721-1

[95] Jain D. Cardiotoxicity of doxorubicin and other anthracycline derivatives. *J. Nucl. Cardiol*., **7,**53-62 (2000) https://doi.org/10.1067/mnc.2000.103324

[96] Lu P. Monitoring cardiac function in patients receiving doxorubicin. *Semin. Nucl. Med*., **35,**197-201 (2005) https://doi.org/10.1053/j.semnuclmed.2005.02.005

[97] Totzeck M, Aide N, Bauersachs J, Bucerius J, Georgoulias P, Herrmann K, Hyafil F, Kunikowska J, Lubberink M, Nappi C, Rassaf T. Nuclear medicine in the assessment and prevention of cancer therapy-related cardiotoxicity: prospects and proposal of use by the European Association of Nuclear Medicine (EANM). *Eur. J. Nucl. Med. Mol. Imaging*., **50**, 792-812 (2023) https://doi.org/10.1007/s00259-022-05991-7

[98] Hiroe M, Ohta Y, Fujita N, Nagata M, Toyozaki T, Kusakabe K, Sekiguchi M, Marumo F. Myocardial uptake of 111In monoclonal antimyosin Fab in detecting doxorubicin cardiotoxicity in rats. Morphological and hemodynamic findings. *Circulation,* **86,** 1965-72 (1992) https://doi.org/10.1161/01.cir.86.6.1965

[99] Lekakis J, Prassopoulos V, Athanassiadis P, Kostamis P, Moulopoulos S. Doxorubicin-induced cardiac neurotoxicity: study with iodine 123-labeled metaiodobenzylguanidine scintigraphy. *J. Nucl. Cardiol*., **3,** 37-41 (1996) https://doi.org/10.1016/s1071-3581(96)90022-7

[100] Min PK, Lim S, Kang SJ, et al. Targeted ultrasound imaging of apoptosis with annexin a5 microbubbles in acute Doxorubicin-induced cardiotoxicity. *J Cardiovasc. Ultrasound*., **18**,91-97 https://doi.org/10.4250/jcu.2010.18.3.91

[101] Goldstein DS. Sympathetic neuroimaging. *Handb. Clin. Neurol*., **117,**365-70 (2013) https://doi.org/10.1016/B978-0-444-53491-0.00029-8

[102] Laursen AH, Thune JJ, Hutchings M, et al. ^{123} I-MIBG imaging for detection of anthracycline-induced cardiomyopathy. *Clin. Physiol. Funct. Imaging*.,**38,** 176-85 (2018) https://doi.org/10.1111/cpf.12419

[103] Camilli M, Cipolla CM, Dent S, Minotti G, Cardinale DM. Anthracycline Cardiotoxicity in Adult Cancer Patients: *JACC: CardioOncology* State-of-the-Art Review. *JACC CardioOncol*., **6,** 655-77 (2024) https://doi.org/10.1016/j.jaccao.2024.07.016

[104] Unverferth BJ, Magorien RD, Balcerzak SP, Leier CV, Unverferth DV. Early changes in human myocardial nuclei after doxorubicin. *Cancer* , **52**, 215-21 (1983) https://doi.org/10.1002/1097-0142(19830715)52:2<215::aid-cncr2820520206>3.0.co;2-f

[105] Lipshultz SE, Lipsitz SR, Mone SM, Goorin AM, Sallan SE, Sanders SP, Orav EJ, Gelber RD, Colan SD. Female sex and higher drug dose as risk factors for late cardiotoxic effects of doxorubicin therapy for childhood cancer. *N. Engl. J. Med*.,**332,** 1738-44 (1995) https://doi.org/10.1056/NEJM199506293322602

[106] Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N. Engl. J. Med*., **324**, 808-15 (1991) https://doi.org/10.1056/NEJM199103213241205

[107] Elmore S. Apoptosis: a review of programmed cell death. *Toxico pathol*, ***35***, 495–516. https://doi.org/10.1080/01926230701320337 Panjrath GS, Patel V, Valdiviezo CI, Narula N, Narula J, Jain D. Potentiation of doxorubicin cardiotoxicity by iron loading in a rodent model. *J. Am. Coll. Cardiol*., **49,** 2457-64 (2007) https://doi.org/10.1016/j.jacc.2007.02.060

[108] Darzynkiewicz Z, Galkowski D, Zhao H. Analysis of apoptosis by cytometry using TUNEL assay. *Methods*, **44,** 250-54 (2008) https://doi.org/10.1016/j.ymeth.2007.11.008

[109] Atas E, Kismet E, Kesik V, Karaoglu B, Aydemir G, Korkmazer N, Demirkaya E, Karslioglu Y, Yurttutan N, Unay B, Koseoglu V. Cardiac troponin-I, brain natriuretic peptide and endothelin-1 levels in a rat model of doxorubicin-induced cardiac injury. *J. Cancer Res. Ther*., **11,** 882-86 (2015) https://doi.org/10.4103/0973-1482.144636

[110] Adamcova M, Skarkova V, Seifertova J, Rudolf E. Cardiac Troponins are Among Targets of Doxorubicin-Induced Cardiotoxicity in hiPCS-CMs. *Int. J. Mol. Sci*., **20,** 2638 (2019) https://doi.org/10.3390/ijms20112638

[111] Kısmet E, Varan A, Ayabakan C, Alehan D, Portakal O, Büyükpamukçu M. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. *Pediatr Blood Cancer*., **42,** 220-24 (2004) https://doi.org/10.1002/pbc.10368

[112] Koh E, Nakamura T, Takahashi H. Troponin-T and brain natriuretic peptide as predictors for adriamycin-induced cardiomyopathy in rats. *Circ. J*., **68**, 163-67 (2004) https://doi.org/10.1253/circj.68.163

[113] Clark SJ, Pippon M, Hemsworth S, Newland P, Pizer B. Cardiac troponin T following anthracycline chemotherapy in children and adolescents. *J. Chemother.*, **19,** 332-34 (2007) https://doi.org/10.1179/joc.2007.19.3.332

[114] Advani P, Hoyne J, Moreno-Aspita A, Dubin M, Brock S, Harlow C, Chumsri S, Suter T, Blackshear JL. High-sensitivity troponin T and NT-proBNP kinetics in breast cancer chemotherapy. *Chemotherapy*, **62**, 334-38 (2017) https://doi.org/10.1159/000477797

[115] Wang S, Wang Y, Zhang Z, Liu Q, Gu J. Cardioprotective effects of fibroblast growth factor 21 against doxorubicin-induced toxicity via the SIRT1/LKB1/AMPK pathway. *Cell Death Dis.*, **8,** e3018 (2017) https://doi.org/10.1038/cddis.2017.410

[116] Nousiainen T, Vanninen E, Jantunen E, Puustinen J, Remes J, Rantala A, Vuolteenaho O, Hartikainen J. Natriuretic peptides

during the development of doxorubicin-induced left ventricular diastolic dysfunction. *J. Intern. Med*., **251,** 228-34 (2002) https://doi.org/10.1046/j.1365-2796.2002.00951.x

[117] Hayakawa Y, Takeda K, Yagita H, Van Kaer L, Saiki I, Okumura K. Differential regulation of Th1 and Th2 functions of NKT cells by CD28 a nd CD40 costimulatory pathways. *J. Immunol*., **166,** 6012-18 (2001) https://doi.org/10.4049/jimmunol.166.10.6012

[118] Daugaard G, Lassen U, Bie P, Pedersen EB, Jensen KT, Abildgaard U, Hesse B, Kjaer A. Natriuretic peptides in the monitoring of anthracycline induced reduction in left ventricular ejection fraction. *Eur. J. Heart Fail*., **7,** 87-93 (2005) https://doi.org/10.1016/j.ejheart.2004.03.009

[119] Lum BL, Svec JM, Torti FM. Doxorubicin: alteration of dose scheduling as a means of reducing cardiotoxicity. *Drug Intell. Clin. Pharm*., **19,** 259-64 (1985) https://doi.org/10.1177/106002808501900403

[120] Tabuchi M, To H, Sakaguchi H, et al. Therapeutic index by combination of adriamycin and docetaxel depends on dosing time in mice. *Cancer Res*., **65**, 8448-54 (2005) https://doi.org/10.1158/0008-5472.CAN-05-1161

[121] Legha SS, Benjamin RS, Mackay B, Yap HY, Wallace S, Ewer M, Blumenschein GR, Freireich EJ. Adriamycin therapy by continuous intravenous infusion in patients with metastatic breast cancer. *Cancer*, **49,** 1762-66 (1982) https://doi.org/10.1002/1097-0142(19820501)49:9<1762::aid-cncr2820490905>3.0.co;2-q

[122] Hortobagyi GN, Frye D, Buzdar AU, Ewer MS, Fraschini G, Hug V, Ames F, Montague E, Carrasco CH, Mackay B, Benjamin RS. Decreased cardiac toxicity of doxorubicin administered by continuous intravenous infusion in combination chemotherapy for metastatic breast carcinoma. *Cancer*, **63(1**), 37-45 (1989) https://doi.org/10.1002/1097-0142(19890101)63:1<37::aid-cncr2820630106>3.0.co;2-z

[123] Moghrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. *Blood*., **109,** 896-904 (2007) https://doi.org/10.1182/blood-2006-06-027714

[124] López-Martínez MI, Miguel M, Garcés-Rimón M. Protein and Sport: Alternative Sources and Strategies for Bioactive and Sustainable Sports Nutrition. *Front Nutr*., **9,** 926043 (2022) https://doi.org/10.3389/fnut.2022.926043

[125] Blumberg JB, Frei BB, Fulgoni Iii VL, Weaver CM, Zeisel SH. Impact of frequency of multi-vitamin/multi-mineral supplement intake on nutritional adequacy and nutrient deficiencies in US adults. *Nutrients*, **9(8),** 849 (2017) https://doi.org/10.3390/nu9080849

[126] Choksey A, Timm KN. Cancer Therapy-Induced Cardiotoxicity-A Metabolic Perspective on Pathogenesis, Diagnosis and Therapy. *Int. J. Mol. Sci*., **23,** 441 (2021)https://doi.org/10.3390/ijms23010441

[127] El-Demerdash E, Ali AA, Sayed-Ahmed MM, Osman AM. New aspects in probucol cardioprotection against doxorubicin-induced cardiotoxicity. *Cancer Chemother. Pharmacol*., **52,** 411-16 (2003) https://doi.org/10.1007/s00280-003-0676-y

[128] Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J. Pharm. Pharmacol*., **65,** 157-70 (2013) https://doi.org/10.1111/j.2042-7158.2012.01567.x

[129] Tahover E, Patil YP, Gabizon AA. Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index: focus on liposomes. *Anti-cancer Drugs* .,**26,**241-58 (2015) https://doi.org/10.1097/CAD.0000000000000182

[130] van den Hurk C, Breed W, Dercksen W. Nonpegylated liposomal doxorubicin: reduction in cardiotoxicity, although still severe alopecia. *Anti-Cancer Drugs.*, **26,** 687 (2015) https://doi.org/10.1097/CAD.0000000000000239

[131] Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.* , **65,** 36-48 (2013) https://doi.org/10.1016/j.addr.2012.09.037

[132] Barenholz YC. Doxil®—The first FDA-approved nano-drug: Lessons learned. *J. Control Release*., **160,** 117-34 (2012) https://doi.org/10.1016/j.jconrel.2012.03.020

[133] Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin. Pharmacokinet*., **42,** 419-36 (2003) https://doi.org/10.2165/00003088-200342050-00002

[134] Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat. Rev. Drug Discov*., **4,** 145-60 (2005) https://doi.org/10.1038/nrd1632

[135] Franco YL, Vaidya TR, Ait-Oudhia S. Anticancer and cardio-protective effects of liposomal doxorubicin in the treatment of breast cancer. *Breast Cancer: Targets and Therapy*, **11,** 131-41 (2018) https://doi.org/10.2147/BCTT.S170239

[136] Seaberg J, Montazerian H, Hossen MN, Bhattacharya R, Khademhosseini A, Mukherjee P. Hybrid Nanosystems for Biomedical Applications. *ACS Nano*., 15, 2099-2142 (2021) https://doi.org/10.1021/acsnano.0c09382

[137] Reyes BA, Dufourt EC, Ross J, Warner MJ, Tanquilut NC, Leung AB. Selected phyto and marine bioactive compounds: Alternatives for the treatment of type 2 diabetes. *Studies Nat Products Chem.*, **55,** 111-43 (2018) https://doi.org/10.1016/B978-0-444-64068-0.00004-8.

[138] Abdel-Daim MM, Kilany OE, Khalifa HA, Ahmed AA. Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. *Cancer Chemother. Pharmacol.,* **80,** 745-53, 2018 https://doi.org/10.1007/s00280-017-3413-7

[139] Li X, Chu S, Lin M, Gao Y, Liu Y, Yang S, Zhou X, Zhang Y, Hu Y, Wang H, Chen N. Anticancer property of ginsenoside Rh2

from ginseng. *Eur. J. Med. Chem*.**, 203,**112627 (2020) https://doi.org/10.1016/j.ejmech.2020.112627

[140] Rajadurai M, Prince PS. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. *Toxicology*, **230,**178-88 (2007) https://doi.org/10.1016/j.tox.2006.11.053

[141] Wang H, Yu P, Gou H, Zhang J, Zhu M, Wang ZH, Tian JW, Jiang YT, Fu FH. Cardioprotective effects of 20 -ginsenoside Rh2 against doxorubicin-induced cardiotoxicity in vitro and in vivo. *Evid. Based Complement. Alternat. Med*., **2012,** 506214 (2012) https://doi.org/10.1155/2012/506214

[142] Perreault AA, Venters BJ. Integrative view on how erythropoietin signaling controls transcription patterns in erythroid cells. *Curr. Opin. Hematol*., **25,** 189-95 (2018) https://doi.org/10.1097/MOH.0000000000000415

[143] Li L, Takemura G, Li Y, Miyata S, Esaki M, Okada H, Kanamori H, Khai NC, Maruyama R, Ogino A, Minatoguchi S. Preventive effect of erythropoietin on cardiac dysfunction in doxorubicin-induced cardiomyopathy. *Circulation,* **113,** 535-43 (2006) https://doi.org/10.1161/CIRCULATIONAHA.105.568402

[144] Ammar HI, Saba S, Ammar RI, Elsayed LA, Ghaly WB, Dhingra S. Erythropoietin protects against doxorubicin-induced heart failure. Int. Cardiovasc. Physiol. Pathophysiol., **301(6),** H2413-21 (2011) https://doi.org/10.1152/ajpheart.01096.2010

[145] Cui L, Guo J, Zhang Q, Yin J, Li J, Zhou W, Zhang T, Yuan H, Zhao J, Zhang L, Carmichael PL. Erythropoietin activates SIRT1 to protect human cardiomyocytes against doxorubicin-induced mitochondrial dysfunction and toxicity. *Toxicol. Lett.*, **275,** 28-38 (2017) https://doi.org/10.1016/j.toxlet.2017.04.018

[146] Li K, Sung RY, Huang WZ, Yang M, Pong NH, Lee SM, Chan WY, Zhao H, To MY, Fok TF, Li CK. Thrombopoietin protects against in vitro and in vivo cardiotoxicity induced by doxorubicin. *Circulation,* **113,** 2211-20 (2006) https://doi.org/10.1161/CIRCULATIONAHA.105.560250

[147] Chan KY, Xiang P, Zhou L, Li K, Ng PC, Wang CC, Zhang L, Deng HY, Pong NH, Zhao H, Chan WY. Thrombopoietin protects against doxorubicin-induced cardiomyopathy, improves cardiac function, and reversely alters specific signalling networks. *Eur. J. Heart Fail*., **13**, 366-76 (2011) https://doi.org/10.1093/eurjhf/hfr001

[148] Siveski-Iliskovic N, Hill M, Chow DA, Singal PK. Probucol protects against adriamycin cardiomyopathy without interfering with its antitumor effect. *Circulation* **91,** 10-15 (1995) https://doi.org/10.1161/01.cir.91.1.10

[149] Li X, Zhong J, Zeng Z, Wang H, Li J, Liu X, Yang X. MiR-181c protects cardiomyocyte injury by preventing cell apoptosis through PI3K/Akt signaling pathway. *Cardiovasc. Diagn. Ther*., **10,** 849-58 (2020) https://doi.org/10.21037/cdt-20-490

[150] Han HS, Koo SY, Choi KY. Emerging nanoformulation strategies for phytocompounds and applications from drug delivery to phototherapy to imaging. *Bioact. Mater*., **14,** 182-205 (2022) https://doi.org/10.1016/j.bioactmat.2021.11.027

[151] Liu N, Olson EN. CRISPR Modeling and Correction of Cardiovascular Disease. *Circ Res*., **130,** 1827-50 (2022) https://doi.org/10.1161/CIRCRESAHA.122.320496

[152] Aderinto N, Abdulbasit MO, Olatunji G, Edun M, Aboderin G. The promise of RNA-based therapeutics in revolutionizing heart failure management - a narrative review of current evidence. *Ann. Med. Surg. (Lond)*., 85, 4442-53 (2023) https://doi.org/10.1097/MS9.0000000000001118

[153] Souma T, Suzuki N, Yamamoto M. Renal erythropoietin-producing cells in health and disease. *Front. Physiol*., **6,** 167 (2015) https://doi.org/10.3389/fphys.2015.00167

[154] Ramond A, Sartorius E, Mousseau M, Ribuot C, Joyeux-Faure M. Erythropoietin pretreatment protects against acute chemotherapy toxicity in isolated rat hearts. *Exp. Biol. Med.*, **233,** 76-83 (2008) https://doi.org/10.3181/0706-RM-152

[155] Elmorsy EA, Saber S, Hamad RS, Abdel-Reheim MA, El-Kott AF, AlShehri MA, Morsy K, Negm S, Youssef ME. Mechanistic Insights into Carvedilol's Potential Protection Against Doxorubicin-Induced Cardiotoxicity. *Eur. J. Pharm. Sci*.,**200**, 106849 (2024) https://doi.org/10.1016/j.ejps.2024.106849