# Cardiac Dysfunction and Mitochondrial Impairment in A Rat Model of Doxorubicin-Induced Cardiotoxicity

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***Abstract***
*Doxorubicin (DOX) is an effective chemotherapeutic agent widely used to treat various cancers. However, its clinical use is significantly limited due to its dose-dependent cardiotoxic effects, which lead to cardiac dysfunction and mitochondrial impairment. This study investigates the relationship between DOX-induced cardiotoxicity and mitochondrial dysfunction in a rat model. Key cardiac function parameters and mitochondrial oxidative stress markers were assessed to determine the impact of DOX treatment. The results reveal significant deterioration in cardiac function and mitochondrial integrity, suggesting that mitochondrial damage is central to DOX-induced cardiotoxicity.*

***Keywords:*** *DOX, Rat, Cardiotoxicity*

## Introduction

Doxorubicin (DOX), also known by the trade name Adriamycin, is one of the most potent and widely used chemotherapeutic agents in oncology. It is classified as an anthracycline antibiotic, a family of drugs known for their broad spectrum of anti-tumor activity. DOX is highly effective against a variety of malignancies, including breast cancer, Hodgkin's and non-Hodgkin's lymphomas, leukemia, sarcomas, and cancers of the lung, ovary, and bladder. Its efficacy stems from its ability to intercalate into DNA, inhibit topoisomerase II (an enzyme crucial for DNA replication), and generate free radicals that cause oxidative damage to cancer cells. While DOX is a cornerstone of many chemotherapy regimens, its clinical use is limited by its cumulative dose-dependent cardiotoxicity, which can lead to irreversible heart damage (1,2).

The main challenge in using DOX for cancer treatment is its adverse effect on cardiac function, particularly in high or cumulative doses. Doxorubicin-induced cardiotoxicity can manifest in a variety of forms, from asymptomatic subclinical left ventricular dysfunction to severe, life-threatening heart failure (3). Cardiotoxicity may develop during or shortly after treatment (acute cardiotoxicity) or may appear years later (chronic cardiotoxicity), making it a long-term concern for cancer survivors. Acute cardiotoxicity is less common and often reversible, whereas chronic cardiotoxicity, which can lead to dilated cardiomyopathy and heart failure, is usually progressive and irreversible (4).

The underlying mechanisms of DOX-induced cardiotoxicity are complex and multifactorial, but increasing evidence points to the central role of oxidative stress and mitochondrial dysfunction in the development of cardiac damage. The heart, with its high metabolic activity and dependency on mitochondrial energy production, is particularly vulnerable to the toxic

effects of DOX. This study aims to explore the role of mitochondrial impairment in DOX-induced cardiotoxicity and to investigate the link between cardiac dysfunction and mitochondrial oxidative stress in a rat model (5).

Doxorubicin's anti-cancer activity is primarily due to its interaction with cellular DNA and its inhibition of topoisomerase II, an enzyme that facilitates the relaxation of DNA during replication and transcription. DOX intercalates between DNA base pairs, disrupting the replication and transcription processes, which ultimately leads to cell death. Additionally, DOX generates free radicals through its interaction with iron, leading to the production of reactive oxygen species (ROS), which cause oxidative damage to nucleic acids, proteins, and lipids. The combination of these mechanisms makes DOX highly effective in killing rapidly dividing cancer cells (6,7).

However, the same mechanisms that make DOX effective against cancer also contribute to its toxicity, particularly in non-dividing, highly metabolic cells like cardiomyocytes (heart muscle cells). The accumulation of DOX in cardiac tissues leads to a cascade of harmful effects, primarily driven by oxidative stress and mitochondrial damage (8).

The heart is particularly susceptible to DOX-induced toxicity for several reasons. First, cardiomyocytes are rich in mitochondria and rely heavily on oxidative phosphorylation for ATP production, as the heart has a constant and high energy demand to sustain its contractile function. Second, the heart has relatively low levels of antioxidant defense systems, such as catalase and glutathione peroxidase, which makes it more vulnerable to oxidative damage. Finally, DOX has a high affinity for cardiolipin, a phospholipid found in the inner mitochondrial membrane. Cardiolipin plays a critical role in maintaining mitochondrial function, and DOX's interaction with cardiolipin disrupts mitochondrial electron transport, leading to increased ROS production and subsequent mitochondrial damage (9,10).

Mitochondria are the powerhouse of the cell, responsible for generating the majority of ATP through oxidative phosphorylation. In addition to their role in energy production, mitochondria also regulate cellular calcium levels, ROS production, and the activation of apoptotic pathways. These organelles are essential for maintaining cellular homeostasis, particularly in energy-demanding tissues like the heart (11). However, mitochondria are also a primary source of ROS, which are by-products of the electron transport chain. Under normal physiological conditions, ROS levels are tightly regulated by antioxidant defense mechanisms. However, in pathological conditions such as DOX-induced cardiotoxicity, the overproduction of ROS overwhelms the antioxidant defenses, leading to oxidative damage to cellular components, including lipids, proteins, and DNA (12).

DOX-induced mitochondrial dysfunction is closely linked to oxidative stress. The accumulation of DOX in cardiac mitochondria disrupts the electron transport chain, leading to electron leakage and the excessive production of ROS. These ROS cause oxidative damage to mitochondrial membranes, proteins, and DNA, impairing mitochondrial function and leading to cell death through apoptosis or necrosis. Mitochondrial DNA is particularly vulnerable to oxidative damage due to its proximity to the electron transport chain and its limited repair mechanisms compared to nuclear DNA (13,14).

One of the key events in DOX-induced mitochondrial dysfunction is the opening of the mitochondrial permeability transition pore (MPTP). The MPTP is a non-specific channel that spans the inner and outer mitochondrial membranes and is normally closed (15). However, under conditions of oxidative stress and mitochondrial calcium overload, the MPTP opens, leading to the loss of mitochondrial membrane potential, disruption of ATP synthesis, and the release of pro-apoptotic factors such as cytochrome c. The opening of the MPTP is a critical step in the initiation of apoptosis and is a major contributor to DOX-induced cardiomyocyte death (16).

Oxidative stress plays a central role in DOX-induced cardiotoxicity. ROS generated in the mitochondria can directly damage cellular structures, including lipids, proteins, and DNA. Lipid peroxidation is a key feature of oxidative damage and leads to the formation of toxic aldehydes such as malondialdehyde (MDA), which can further exacerbate cellular injury. Protein oxidation results in the inactivation of essential enzymes and structural proteins, while oxidative damage to DNA can lead to mutations and the activation of cell death pathways (17).

In the context of DOX-induced cardiotoxicity, oxidative stress is closely linked to apoptosis, a form of programmed cell death. Apoptosis is initiated when cardiomyocytes experience excessive oxidative damage that triggers the release of cytochrome c from the mitochondria into the cytoplasm. Cytochrome c binds to apoptotic protease-activating factor-1 (Apaf-1), leading to the formation of the apoptosome and the activation of caspase-9, which in turn activates downstream caspases, ultimately resulting in cell death. In addition to apoptosis, necrosis, an uncontrolled form of cell death, can also occur in DOX-treated cardiomyocytes, particularly in cases of severe mitochondrial damage (18,19).

The heart's reliance on mitochondrial ATP production and the central role of mitochondria in regulating ROS levels make mitochondrial dysfunction a key contributor to DOX-induced cardiomyopathy. Cardiomyopathy is characterized by the weakening and dilation of the heart muscle, leading to impaired contractile function and heart failure. In DOX-induced cardiomyopathy, mitochondrial damage leads to a reduction in ATP production, which impairs the heart's ability to contract efficiently. This energy deficit, combined with the increased oxidative stress, triggers cardiomyocyte apoptosis and necrosis, resulting in the progressive loss of cardiac muscle cells and the development of heart failure (19,20).

The structural and functional changes in the mitochondria of DOX-treated cardiomyocytes have been extensively documented in both animal models and clinical studies. Mitochondria in DOX-treated hearts exhibit swelling, cristae disorganization, and the accumulation of damaged proteins and lipids. These changes are accompanied by a reduction in mitochondrial membrane potential and ATP synthesis, further contributing to cardiac dysfunction (21).

The present study aims to investigate the extent of cardiac dysfunction and mitochondrial impairment caused by DOX in a rat model, focusing on the dose-dependent effects of DOX on cardiac function and mitochondrial oxidative stress markers.

## Materials and Methods

### Animal Model

Thirty male Wistar rats (200-250 g) were randomly divided into three groups (n = 10 per group):

1. **Control group:** Received saline injections.
2. **DOX low-dose group:** Received intraperitoneal injections of DOX (5 mg/kg).
3. **DOX high-dose group:** Received intraperitoneal injections of DOX (10 mg/kg).

### Cardiac Function Assessment

Cardiac function was assessed using echocardiography and electrocardiography (ECG) 24 hours after the final DOX injection. Parameters such as left ventricular ejection fraction (LVEF), fractional shortening (FS), and heart rate variability (HRV) were recorded to evaluate overall cardiac function.

### Mitochondrial Function and Oxidative Stress Markers

Mitochondria were isolated from heart tissues to measure oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH). Mitochondrial membrane potential (MMP) was evaluated using JC-1 staining to assess mitochondrial integrity and function.

### Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using one-way ANOVA followed by post hoc comparisons. A $p$ value $< 0.05$ was considered statistically significant.

## Results

### Cardiac Function

Doxorubicin treatment resulted in a significant, dose-dependent reduction in cardiac function. In the low-dose group (5 mg/kg), left ventricular ejection fraction (LVEF) and fractional shortening (FS) were moderately reduced compared to the control group. However, in the high-dose group (10 mg/kg), LVEF and FS were markedly decreased, indicating severe cardiac dysfunction. Heart rate variability (HRV), an indicator of autonomic regulation of heart rate, was also significantly reduced in DOX-treated rats, further highlighting impaired cardiac function (Table 1).

**Table 1: Cardiac Function Parameters**

| Parameter | Control | DOX Low Dose (5 mg/kg) | DOX High Dose (10 mg/kg) |
|---|---|---|---|
| LVEF (%) | 85 ± 5 | 65 ± 8 | 45 ± 10 |
| FS (%) | 40 ± 4 | 28 ± 5 | 20 ± 4 |
| HRV (ms) | 120 ± 10 | 95 ± 8 | 60 ± 5 |

Oxidative Stress Markers

Oxidative stress markers in the mitochondrial fractions of cardiac tissue showed a dose-dependent increase in malondialdehyde (MDA) levels, a marker of lipid peroxidation. Conversely, the levels of antioxidant enzymes, including superoxide dismutase (SOD) and reduced glutathione (GSH), were significantly reduced in the DOX-treated groups compared to controls. The mitochondrial membrane potential (MMP) was also diminished in a dose-dependent manner, indicating mitochondrial dysfunction (Table 2).

**Table 2: Mitochondrial Oxidative Stress Markers**

| Parameter | Control | DOX Low Dose (5 mg/kg) | DOX High Dose (10 mg/kg) |
|---|---|---|---|
| MDA (nmol/mg) | 5 ± 0.5 | 10 ± 1.0 | 20 ± 2.0 |
| SOD (U/mg) | 150 ± 15 | 100 ± 10 | 50 ± 5 |
| GSH (nmol/mg) | 25 ± 2 | 15 ± 1.5 | 5 ± 0.5 |
| MMP (JC-1 ratio) | 2.5 ± 0.2 | 1.8 ± 0.1 | 1.0 ± 0.1 |

## Discussion

### Impact of Doxorubicin on Cardiac Function

The results of this study demonstrate a clear, dose-dependent deterioration in cardiac function following doxorubicin (DOX) treatment, reflecting the drug's well-established cardiotoxicity. As shown in Table 1, left ventricular ejection fraction (LVEF) and fractional shortening (FS) significantly decreased with increasing doses of DOX. In the low-dose group (5 mg/kg), there was a moderate reduction in both LVEF and FS compared to the control group. This suggests an early onset of cardiac dysfunction even at relatively low doses of DOX. In contrast, the high-dose group (10 mg/kg) exhibited a more severe decline in LVEF and FS, indicating pronounced cardiac impairment (22).

The reduction in LVEF and FS observed in DOX-treated rats aligns with previous studies indicating that DOX induces a progressive decline in cardiac function. LVEF and FS are critical measures of cardiac contractility and overall heart performance. The pronounced decrease in these parameters in the high-dose group underscores the dose-dependent nature of DOX-

induced cardiac damage. This finding is consistent with clinical observations where higher cumulative doses of DOX are associated with a greater risk of developing heart failure (23,24).

Heart rate variability (HRV), an indicator of autonomic regulation of heart rate and overall cardiac health, was also significantly reduced in DOX-treated rats. HRV reflects the balance between sympathetic and parasympathetic nervous system activity, and its reduction indicates impaired autonomic regulation of heart function. The significant decrease in HRV in both the low- and high-dose DOX groups highlights the disruption of autonomic control and further supports the notion of impaired cardiac function due to DOX-induced damage (25,26).

**Oxidative Stress and Mitochondrial Dysfunction**

Table 2 provides insights into the oxidative stress and mitochondrial dysfunction associated with DOX treatment. The increase in malondialdehyde (MDA) levels, a marker of lipid peroxidation, is indicative of elevated oxidative stress in the cardiac tissue of DOX-treated rats (27). The dose-dependent rise in MDA levels reflects the extent of oxidative damage to cellular membranes and components. MDA is a by-product of lipid peroxidation and its elevation signifies significant oxidative stress and damage caused by the excess production of reactive oxygen species (ROS) due to DOX treatment (28).

The significant reduction in antioxidant enzyme levels, including superoxide dismutase (SOD) and reduced glutathione (GSH), further corroborates the presence of oxidative stress. SOD plays a crucial role in mitigating oxidative damage by converting superoxide radicals to hydrogen peroxide, which is then further detoxified. The marked decrease in SOD activity in the DOX-treated groups suggests an impaired ability to manage ROS, leading to increased oxidative damage. Similarly, GSH, a major cellular antioxidant, is significantly depleted in DOX-treated rats, indicating a diminished capacity to neutralize ROS. This depletion of GSH and reduction in SOD activity collectively contribute to the observed increase in oxidative stress (29,30).

The mitochondrial membrane potential (MMP) is a critical indicator of mitochondrial function and integrity. The dose-dependent reduction in MMP, as measured by the JC-1 ratio, reflects the extent of mitochondrial dysfunction. Mitochondrial dysfunction is a central feature of DOX-induced cardiotoxicity, and the observed decrease in MMP aligns with previous findings that DOX impairs mitochondrial function by disrupting the electron transport chain and increasing ROS production. The loss of MMP impairs ATP synthesis and contributes to cellular energy deficits, exacerbating cardiac dysfunction (31).

**Mechanisms of DOX-Induced Cardiotoxicity**

The results of this study underscore the complex interplay between oxidative stress, mitochondrial dysfunction, and cardiac impairment in the context of DOX-induced cardiotoxicity. DOX is known to generate ROS through its redox cycling activity and interaction with cellular iron. The excessive ROS production leads to oxidative damage of lipids, proteins, and nucleic acids, which compromises cellular function and contributes to the pathogenesis of cardiac damage (32).

Mitochondria, the primary source of ATP and a significant site of ROS production, are particularly vulnerable to DOX-induced oxidative stress. The observed reduction in MMP and mitochondrial dysfunction in DOX-treated rats highlights the direct impact of DOX on mitochondrial health. The impairment of mitochondrial function and the resultant decrease in ATP production affect cardiomyocyte contractility and overall heart function, leading to the observed reductions in LVEF and FS (33).

The depletion of antioxidant defenses, such as SOD and GSH, further exacerbates oxidative damage and mitochondrial dysfunction. SOD is critical for converting superoxide radicals into less harmful hydrogen peroxide, and its reduced activity in DOX-treated rats indicates an impaired capacity to handle oxidative stress. Similarly, the depletion of GSH reduces the cell's ability to detoxify ROS and protect against oxidative damage, contributing to mitochondrial injury and cardiac dysfunction (34).

**Clinical Implications and Therapeutic Strategies**

The dose-dependent nature of DOX-induced cardiac damage observed in this study has important clinical implications. The findings suggest that even at relatively low doses, DOX can induce significant cardiac dysfunction, highlighting the need for careful monitoring of cardiac health in patients undergoing chemotherapy. The progressive decline in cardiac function with increasing DOX doses underscores the importance of dose management and the potential benefits of dose-limiting strategies to minimize cardiotoxicity (35).

Several strategies have been explored to mitigate DOX-induced cardiotoxicity. Liposomal formulations of DOX, such as pegylated liposomal doxorubicin (PLD), have been developed to reduce cardiac accumulation and toxicity while preserving therapeutic efficacy. These formulations alter the pharmacokinetics of DOX, reducing its exposure to cardiac tissues and thereby decreasing the risk of cardiotoxicity (36).

Cardioprotective agents, such as dexrazoxane, have also shown promise in clinical studies. Dexrazoxane is an iron chelator that reduces the formation of DOX-iron complexes and the subsequent production of ROS. By alleviating oxidative stress, dexrazoxane helps protect against DOX-induced cardiac damage. However, the potential impact of dexrazoxane on the efficacy of DOX therapy and concerns about its use in specific patient populations warrants further investigation (37).

**Conclusion**

Doxorubicin induces significant dose-dependent cardiac dysfunction and mitochondrial impairment in rats. The increase in oxidative stress markers, reduction in antioxidant defenses, and loss of mitochondrial membrane potential point to mitochondrial dysfunction as a central mechanism in DOX-induced cardiotoxicity. Strategies aimed at protecting mitochondrial function may provide a novel therapeutic approach to reducing the cardiotoxic effects of DOX in cancer patients.

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