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Isosorbide dinitrate improves doxorubicin-induced cardiotoxicity via diminishing proinflammatory mediators, oxidative stress, and apoptosis

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ABSTRACT

Cardiotoxicity is the presence of cardiac dysfunction resulting from electrical or muscle injury, which results in heart toxicity. The heart weakens and becomes less efficient in pumping blood. Cardiotoxicity is one of chemotherapy most serious side effects, with significantly increased morbidity and mortality. Isosorbide dinitrate is an antianginal agent used to treat chest pain in people with a certain heart condition (coronary artery disease). This medication belongs to the nitrate drug class. It works by relaxing and widening blood vessels, allowing more blood to flow to the heart. The role of isosorbide dinitrate in doxorubicin-induced cardiotoxicity reduction or prevention is briefly discussed in this work. The 28 male rats were randomly split into four groups (7 rats in each group). The control group of rats was provided with natural food and drink. For two weeks, rats in the normal saline group were fed 0.9% normal saline. For those in the doxorubicin-induced group, 2.5 mg/kg was administered thrice weekly to the rats for two weeks.

ISDN group (treated with ISDN): ISDN was administered orally (10 mg/kg/d) for two weeks. Heart damage was a result of doxorubicin treatment. Cardiac tissues of doxorubicin-treated rats showed elevated tumor necrosis factor-alpha, interleukin-1beta, malondialdehyde, and caspase-3 levels ($p<0.05$), while total antioxidant capacity and Bcl-2 levels were considerably decreased ($P<0.05$). Inflammatory markers (TNF-α and IL-1β) are reduced after ISDN treatment, providing strong evidence that ISDN considerably mitigates doxorubicin-induced cardiotoxicity ($P<0.05$). In addition, total antioxidant capacity was considerably increased in the ISDN group compared to the doxorubicin-only group ($P<0.05$), whereas the oxidative marker malondialdehyde in cardiac tissue was decreased ($P<0.05$). ISDN dramatically mitigated doxorubicin-induced cardiotoxicity in rats by modulating oxidative stress, the inflammatory response, and the apoptotic pathway. This research aimed to see if ISDN may prevent doxorubicin-induced cardiotoxicity by limiting the effects of the medication on inflammation pathways, oxidative pathways, and apoptotic pathways.

Keywords: *isosorbide dinitrate, doxorubicin-induced cardiotoxicity, inflammation, oxidative stress, and apoptosis*

INTRODUCTION

Isosorbide dinitrate is a longer-acting medicine organic nitrate class. It has been primarily used to prevent and treat myocardial ischemia in individuals with coronary artery disease and is extremely successful in alleviating acute bouts of angina pectoris. It also has a therapeutic role in the management of heart failure. It was discovered in 1939 (Wang et al., 2005). For their actions, organic nitrates are rapidly denigrated enzymatically in smooth muscle cells, resulting in the release of nitric oxide (NO), which activates cytosolic soluble guanylyl cyclase, increasing cGMP and causing dephosphorylation of myosin light chain kinase (MLCK) via a cGMP dependent protein kinase. The reduced availability of phosphorylated (active) MLCK interferes with myosin activation, causing it to fail to connect with actin to cause contraction. As a result, relaxation occurs. Increased intracellular cGMP may also inhibit Ca2+ entry, contributing to relaxation. Veins express more mitochondrial aldehyde dehydrogenase that generates NO from organic nitrate than arteries. This may account for the predominant vasodilator action. Lastly, The NO produced by nitrates also stimulates cGMP synthesis in platelets, resulting in a moderate antiaggregatory action. This action may be beneficial in cases with unstable angina (Thadani & Opie, 2012). Nitrates cause venodilution (increased volume capacitance and reduction volume return lead to decrease end-diastolic volume resulting in cardiac work leading to decrease oxygen demand) and artery dilation (decreased systemic vascular resistance leads to decrease afterload resulting in decreased cardiac work and decrease oxygen demand and also cause coronary dilation to lead to increased oxygen supply) (Banerjee et al., 2021). Chronic heart failure and coronary artery disease patients who take isosorbide dinitrate have a lower risk of cardiac complications and mortality from cardiovascular disease(Napoli et al., 2021). Consequently, this drug is a promising candidate for counteracting cardiotoxicity.

Antineoplastic drugs often cause cardiotoxicity, a serious adverse effect that can significantly affect a patient's prognosis and quality of life (Nardin et al., 2020). Cardiotoxicity is a broad term that refers to "toxicity that affects the heart" since it is a condition in which the heart muscle is damaged. Cardiotoxicity may impair the heart's ability to pump blood throughout the body. Included in this idea are the direct effects of chemotherapy on the cardiovascular system as a whole and the indirect effects brought on by a thrombogenic state or a shift in hemodynamic flow (Mancilla et al., 2019). Direct cellular toxicity causes myocardial injury that worsens over time, leading to diastolic and systolic dysfunction; coagulation system effects cause ischemic events; arrhythmogenic effects; and myocardial and pericardial inflammation causes myocardial dysfunction or pericardial sequelae (Abdul-Rahman et al., 2023). Certain cancer treatments may cause heart problems, either temporarily or permanently. Doxorubicin, a drug used to treat solid malignancies such as leukemia, lymphoma, and breast cancer, has been related to adverse heart effects in certain patients (Renu et al., 2022).

Doxorubicin hydrochloride is a water-soluble crystalline orange-red powder that is an anticancer anthracycline antibiotic. It is a hydroxylated version of daunorubicin. It was created using the bacteria Streptomyces peacetime var. caesius (Gonçalves, 2018). Doxorubicin therapeutic activity is performed by intercalating between DNA bases as HCL and stops protein synthesis and DNA replication. Increased enzyme complex and DNA stability during DNA replication is another benefit of doxorubicin inhibition of topoisomerase-2. The actions of the medication on DNA inhibit the re-joining of broken strands of nucleotides following a double-strand break (Norouzi et al., 2020). Doxorubicin exacerbates the toxicity of anthracycline antibiotics and has cardiac and cutaneous vascular effects because it oxidizes the lipids in cell membranes (Songbo et al., 2019). Currently, no single drug reduces doxorubicin toxicity, has no side effects, makes cancer more sensitive to treatment, or raises the incidence of cancer. Hence, we tested the effectiveness of isosorbide dinitrate in cardiotoxicity by observing its effects on the hearts of male rats. This experiment aims to see if isosorbide

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dinitrate can protect rats against the cardiotoxicity caused by doxorubicin.

MATERIAL AND METHODS

Animal preparation

The Faculty of Science at the University of Kufa provided 28 male Sprague Dawley rats ranging in weight from 150 g to 240 g and in age from 10 to 12 weeks. The rats were kept in the animal house of the University of Kufa Faculty of Science. They were given their specialized group caging setup, where the temperature and humidity were kept at a constant 24±2 C°. The animals were fed fresh water and their standard diet at regular intervals, with the amount of food given to each group determined by how much they ate. The animals were given medication and isolated for two weeks to adjust to their new environment.

Study design

For this experiment, 28 male rats (3 months old, 150-240 G in weight) were employed and randomly split into four groups (7 rats in each group). Rats in the control group were fed and watered as usual during the research. For the "normal saline" (N/S) group: Two weeks of administration of 0.9% normal saline, a vehicle of isosorbide dinitrate, to rats at a dose of 10 ml/kg/day orally; Group Doxorubicin (Induction of Cardiotoxicity): 2.5 mg/kg IP three times weekly for two weeks (total dose 15 mg/kg) (Moutabian et al., 2022). Isosorbide dinitrate (ISDN) group (ISDN treated group): ISDN given by oral route at a dose of 10 mg/kg/day over two weeks (Plotkine et al., 1992), and doxorubicin was administered similarly to that of the doxorubicin group.

Collection of Samples

Blood sample collection

Each animal body weight was recorded 48 hours following the previous anticancer medicine treatment. We administered 10 mg/kg of xylazine and 100 mg/kg of ketamine intravenously to anesthetize the animals. The animals were put to sleep, a thoracoabdominal incision was made, and blood was drawn via heart perforation from the left ventricle. Clot activator gel was then added to the tubes containing the blood samples. After centrifuging the blood at 4000 rpm for 10 minutes, the serum was separated. A commercial ELISA assay was performed on the serum to detect TNF-α and LI-1β.

Tissue sample preparation

Hearts were removed, cleansed, and weighed before being divided into apical and basal halves and split into two halves; the basal side of the heart was washed with ice-cold saline to remove any red blood cells or clots, then frozen at -80 degrees Celsius, weighed after thawing, and homogenized in a 0.1 M phosphate buffer saline (pH7.4) containing 1% Triton-100 and a protease using a high-intensity ultrasonic liquid processor. (Quagliariello et al., 2021).The supernatants were collected, homogenized, and centrifuged at 14000 rpm at 4 degrees Celsius for 10 minutes before being used with readily available ELISA kits to measure caspase-3, MDA, TAC, Bcl2 by using colorimetric assay kits according to the manufacturer's instruction (PARS Bio chem, china).

Tissue Sampling for Histopathology

The apical portion was kept, fixed in 10% neutral formalin, embedded in a paraffin block, and cut into 5 M thick pieces for histological examinations using a microtome. Tissue sections were viewed under a microscope stained with hematoxylin and eosin(Yay et al., 2020).

Statistical Analysis

GraphPad Prism 8 was used for analysis. Data were presented as mean + standard error of the mean (SEM). For comparisons between all groups, we utilized one-way ANOVA. One-way ANOVA was used to examine the histopathological variations across the groups, with post hoc testing performed using the Bonferroni multiple comparison test. The threshold for statistical significance was set at $P<0.001$ for all analyses.

RESULTS

Doxorubicin at a dose of 2.5 mg/kg caused cardiotoxicity. Compared to the N/S group, TAC and Bcl-2 levels were much lower in the doxorubicin group cardiac tissues, while TNF-α, IL-1β, MDA, and caspase-3 levels increased dramatically. Isosorbide dinitrate effectively reduced doxorubicin-induced cardiotoxicity, as evidenced by lower levels of the inflammatory mediators TNF-α and IL-1β (Figure 1 and Figure 2). In cardiac tissue (Figure 3), the oxidative marker MDA was much lower in the isosorbide dinitrate group compared to the doxorubicin-only group, whereas TAC was significantly higher in the isosorbide dinitrate group (Figure 4). Also, cardiac caspase-3 levels were lower in the isosorbide dinitrate group than in the doxorubicin-only group (Figure 5), and Bcl-2 levels were significantly higher in the isosorbide dinitrate group than in the doxorubicin-only group (Figure 6). Also, isosorbide dinitrate dramatically improved scores for cardiomyopathy histological lesions compared to the doxorubicin group.

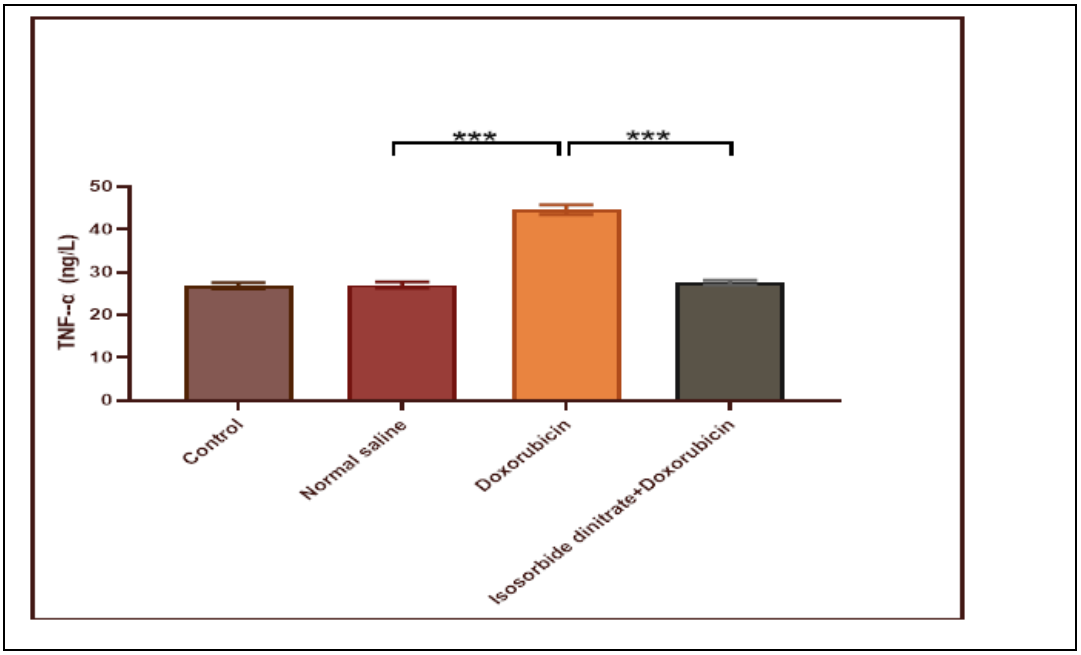

FIGURE 1: Serum TNF-α level of experimental groups.

Untreated (control) and treated rats received either a saline solution (N/S), doxorubicin (2.5 mg/kg), or doxorubicin (2.5 mg/kg) plus isosorbide dinitrate (10mg/kg). Serum TNF-α levels were determined using an ELISA kit. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni multiple comparison test. ***P<0.001

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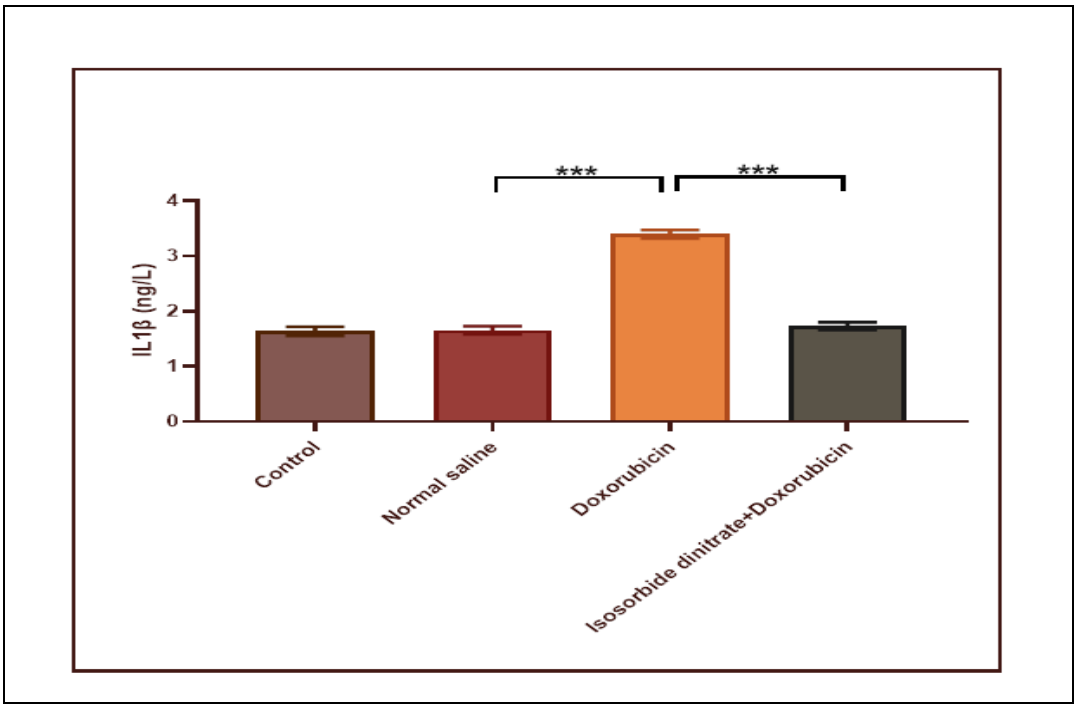

FIGURE 2: Serum interleukin-1β level of experimental groups.

Doxorubicin (2.5 mg/kg), doxorubicin (2.5 mg/kg) + isosorbide dinitrate (10 mg/kg), or vehicle (N/S) was administered to rats. The concentration of IL-1α in the blood was determined with the help of an ELISA kit. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni's multiple comparison tests. ***P<0.001

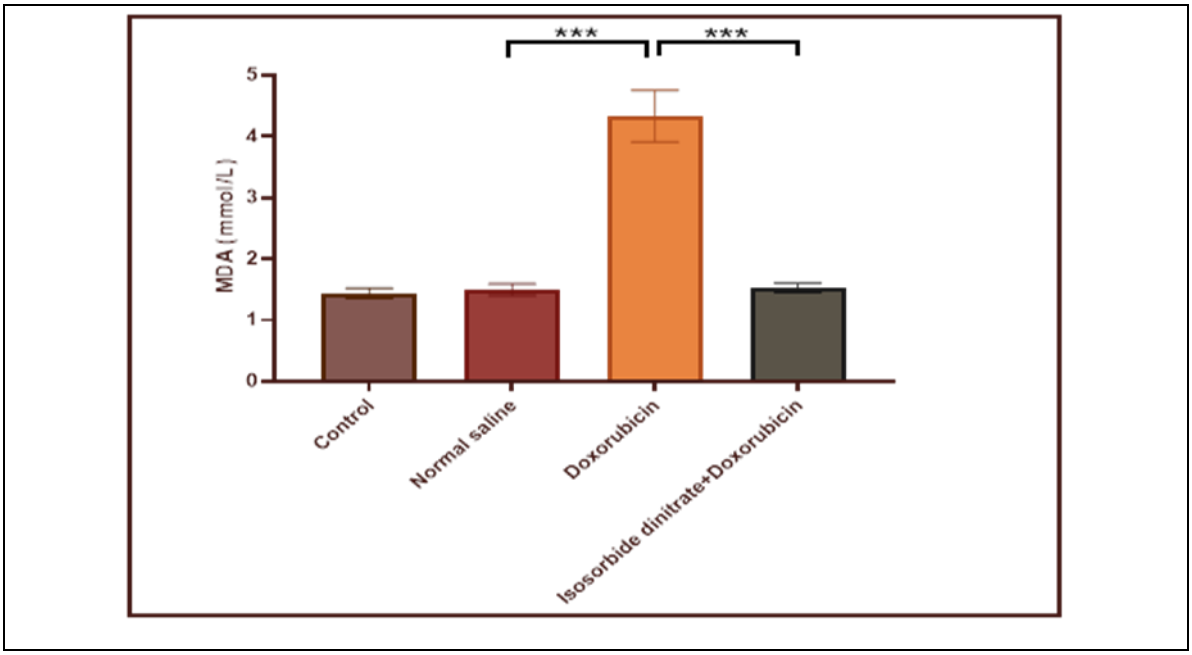

FIGURE 3: Cardiac MDA level of experimental groups.

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Doxorubicin (2.5 mg/kg), doxorubicin (2.5 mg/kg) + isosorbide dinitrate (10 mg/kg), or vehicle (N/S) was administered. MDA ELISA kit was used to measure MDA levels in the heart. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni's multiple comparison tests. ***P<0.001

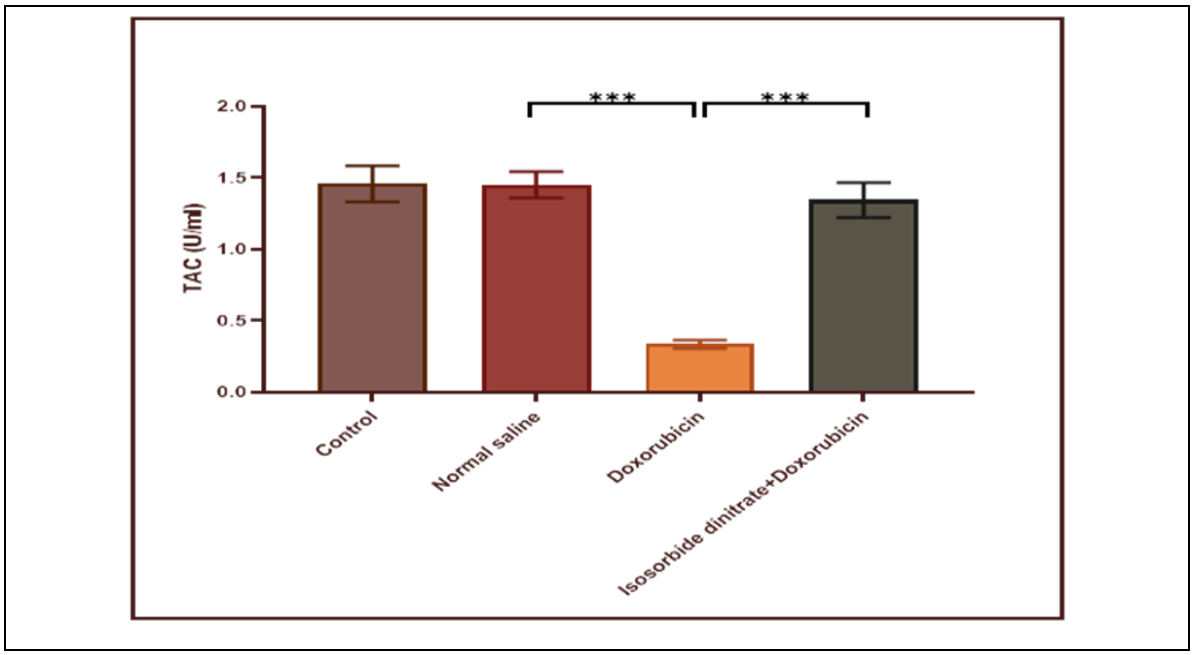

FIGURE 4: Cardiac TAC level of experimental groups.

Doxorubicin (2.5 mg/kg), doxorubicin (2.5 mg/kg) + isosorbide dinitrate (10 mg/kg), or vehicle (N/S) was administered to rats. The TAC ELISA kit was used to measure the TAC levels in the heart. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni's multiple comparison tests. ***P<0.001

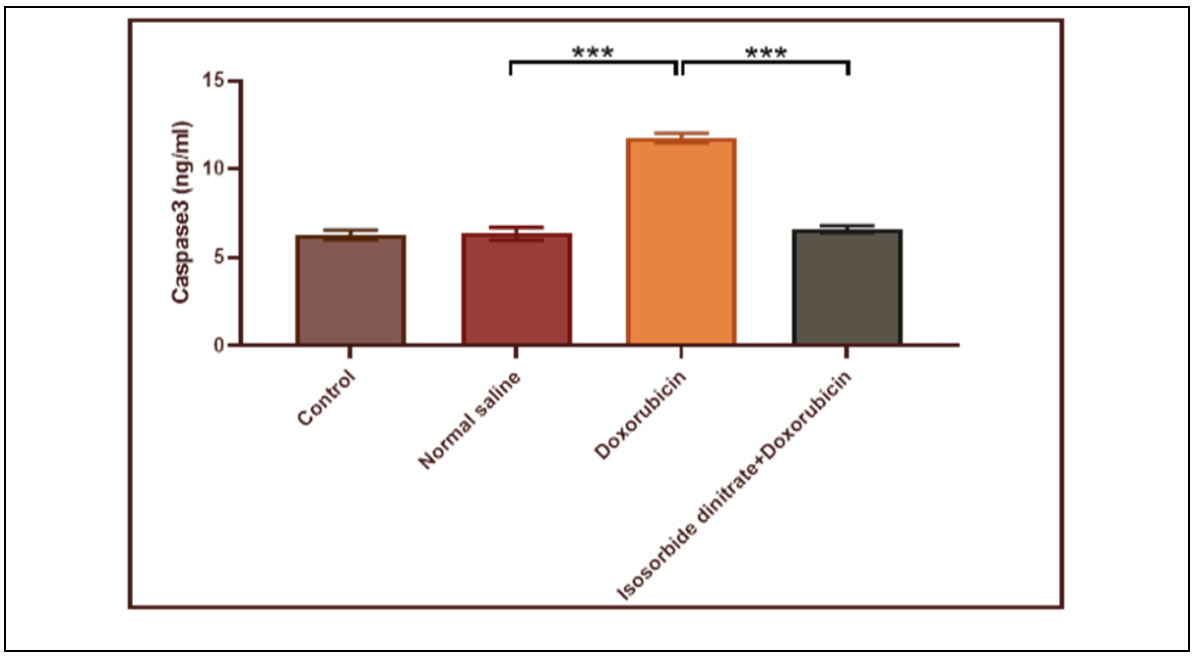

FIGURE 5: Cardiac caspase-3 level of experimental groups.

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Doxorubicin (2.5 mg/kg), doxorubicin (2.5 mg/kg) + isosorbide dinitrate (10 mg/kg), or vehicle (N/S) was administered to rats. Using an ELISA kit specific for caspase-3, the amount of caspase-3 in the heart was measured. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni's multiple comparison tests. ***P<0.001

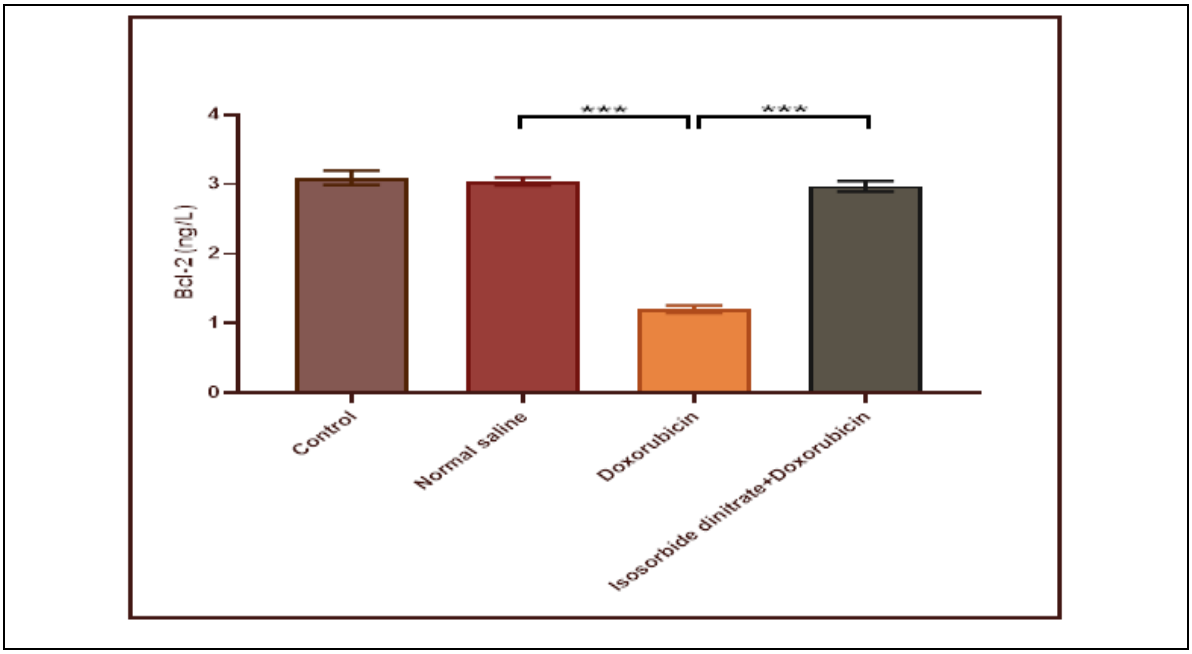

FIGURE 6: Cardiac Bcl2 level of experimental groups.

Doxorubicin (2.5 mg/kg), doxorubicin (2.5 mg/kg) + isosorbide dinitrate (10 mg/kg), or vehicle (N/S) was administered to rats. A BCL-2 ELISA kit was used to measure BCL-2 levels in cardiac tissue. The data are shown as a mean±SEM using one-way ANOVA with Bonferroni's multiple comparison tests. ***P<0.001

TABLE 1: Analyzing and contrasting the experimental groups based on the mean histopathological score.

Group	SEM±Mean	Comparison	P-Value
Control	0 ± 0	Normal saline versus control	>0.9999
Normal saline	0 ± 0	Isosorbide dinitrate vs. Saline	0.0579
Doxorubicin	3.76 ± 0.18	Doxorubicin-Saline Comparison	<0.0001
Isosorbide dinitrate	1.2 ± 0.18	Doxorubicin vs. Isosorbide dinitrate	0.7144

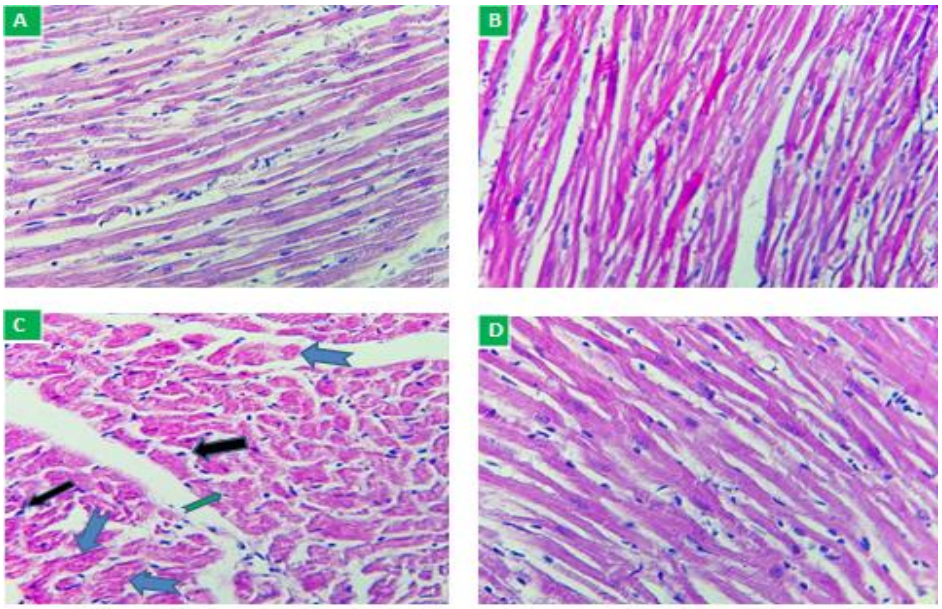

FIGURE 7: The histology of the myocardium in the control group is normal, and the appearance is unblemished. H&E. The myocardial of those given a placebo (normal saline) was B. There is no evidence of injury, indicating normal histology. H&E. C. A. the appearance of the myocardium of doxorubicin (2.5 mg/kg) plus nebivolol (4 mg/kg) treated rats is free of damage, demonstrating normal histology, organized myocardial fibrils and staining H&E. B. the appearance of the myocardium of doxorubicin (2.5 mg/kg) plus nebivolol (4 mg/kg) treated rats is free of damage.

DISCUSSION

Doxorubicin is a potent cytotoxic antitumor agent used alone or combined to eradicate cancer (Pérez-Herrero & Fernández-Medarde, 2015). New ways have been tested to reduce or minimize doxorubicin-induced cardiotoxicity while keeping the drug's capacity to kill cancer cells. However, no clinically useful preventative medicine has yet to be created (Wenningmann et al., 2019).

Inflammatory cytokines such as IL-1β and TNF-α have been related to the development and progression of various cardiac diseases, causing negative inotropic effects and unfavorable effects on left ventricular remodeling (Mann, 2015). Doxorubicin causes a cascade of inflammatory events in the myocardium by activating NF-κB and boosting the synthesis of numerous proinflammatory cytokines, including TNF-α (Nunes et al., 2021). A progressive increase in proinflammatory cytokines in heart tissue was discovered to be the etiology of doxorubicin-induced cardiomyopathy (Meeran et al., 2019). Similarly, the current study findings validate the inflammatory process associated with doxorubicin-induced cardiotoxicity, as seen by a significant rise in cardiac IL-1β and TNF-α in the doxorubicin group over the control group. These findings are consistent with other investigations (Alyasiry et al., 2022; Aziz et al., 2020; Eid et al., 2021; Rusul et al., 2022). Isosorbide dinitrate markedly decreased the elevated proinflammatory markers IL-1β and TNF-α in rats compared to the induced untreated doxorubicin group. Isosorbide dinitrate's potential protective role against doxorubicin-induced cardiac damage has never before been studied. Isosorbide dinitrate, on the other hand, dramatically lowered TNF-α and IL-1β levels in rats with cysteamine-induced chronic duodenal ulcers in a prior study (Fouad et al., 2017). Furthermore, NO donors have been demonstrated to boost cyclo-oxygenase activity while creating protective prostaglandin-E2 (Calatayud et al., 2001). Noguchi and colleagues revealed that isosorbide dinitrate dramatically reduces plasma TNF-α and IL-1β levels in a mouse model of lung fibrosis compared to an induced untreated bleomycin group (Noguchi et al., 2014).

There are various and intricate molecular pathways underpinning doxorubicin-induced carcinogenicity (Okpara et al., 2022). Despite decades of study, the precise mechanism underlying doxorubicin-induced cardiotoxicity remains unknown, making it an important area of attention in cardio-oncology (Koutsoukis et al., 2018). Heart failure brought on by doxorubicin has been linked to oxidative stress. (Xu et al., 2020). Our findings demonstrated a significant increase in lipid peroxidation as evidenced by a rise in aldehydic lipid peroxide products, MDA, and a significant decrease in the total antioxidant capacity of cardiac tissue in doxorubicin-treated rats compared to the control group. These findings are consistent with the findings of several investigations (Sakr et al., 2016; Singla et al., 2014). Apoptosis has been connected to the pathophysiology of doxorubicin-induced cardiotoxicity. Doxorubicin-induced oxidative stress activates several signaling pathways, including caspase-3, resulting in myocyte death (Kong et al., 2022; Zhang et al., 2019). A set of proteins regulates apoptosis. Members of the Bcl2 protein family are key apoptosis regulators (Singh et al., 2019). Caspase-3 is required for apoptosis. Doxorubicin directly or indirectly activates the caspase-3 pathway (Abd El-Hamid et al., 2019). Apoptotic cell death is a critical component of doxorubicin-induced cardiotoxicity, but the exact triggers/mechanisms are unknown (Vo et al., 2021). Compared to the control group, this study demonstrated a significant increase in caspase-3 activity and decreased Bcl-2 activity in cardiac tissue. This is comparable to a recent study that found that rats treated with doxorubicin had enhanced caspase-3 activation (Ma et al., 2019). The doxorubicin redox cycle initially generates hydrogen peroxides, which results in oxidative DNA damage; this oxidative DNA damage then indirectly generates more peroxides by activating NADPH oxidase, which causes disruption of the mitochondrial membrane potential and an increase in membrane permeability, as well as the release of cytochrome c from the mitochondria to the cytosol and subsequent activation of caspase-3, which will start the doxorubicin (Mikhed et al., 2015). Cell death was inhibited by isosorbide dinitrate, as evidenced by a decrease in caspase-3 levels and an increase in Bcl-2 levels in cardiac tissue compared to the DOX group. This study is the first to examine the role of isosorbide dinitrate in DOX-induced cardiotoxicity and apoptosis. Previous research, however, showed that after the anticancer drug cisplatin-induced melanoma in a mouse model, nitric oxide or ISDN reduced caspase-3. (Perrotta et al., 2007). Acute myocardial infarction raised caspase-3, but treatment with isosorbide dinitrate dramatically decreased the rise in the early-stage apoptotic ratio caused by AMI in cardiomyocytes (reduction in caspase-3 and increased in Bcl2) (Mo et al., 2011).

The myocardium in the control and isosorbide dinitrate groups had a normal morphological appearance, with normal-sized cells with centrally positioned nuclei, regular-ordered cells with obvious cross striations, and normal arteries, according to the histologic study of heart tissue. Significant histological abnormalities, such as cardiac enlargement, cytoplasmic vacuolization, perinuclear vacuolization, myocardial fiber disarray, and myofibrillar loss, were seen in the doxorubicin-treated rats. These histological modifications align with earlier research on doxorubicin-induced cardiomyopathy in rats (Baniahmad et al., 2020; Kitakata et al., 2022; Sheta et al., 2016).

Several molecular mechanisms, including an increase in ROS production, promotion of lipid peroxidation, and consequent oxidative stress, cause doxorubicin-induced cardiotoxicity. Damage to the cell membranes of the mitochondria, myocytes, and cellular macromolecules (Kong et al., 2022; Rawat et al., 2021). The release of proinflammatory mediators like TNF-α and IL-1β demonstrates a second, heightened inflammatory response in the myocardium. The fourth process, called cardiomyocyte apoptosis, also involves programmed cell death brought on by mitochondrial malfunction and cellular degeneration (Sun et al., 2016). The current work adds to the expanding body of proof showing apoptosis, oxidative stress, and inflammation all play significant roles in the pathophysiology of doxorubicin-induced heart injury.

Isosorbide dinitrate reduced doxorubicin-induced heart tissue damage at the histology level. According to Fouad and colleagues, Isosorbide dinitrate demonstrated anti-ulcerative, antioxidant, and anti-inflammatory properties against a cysteamine-induced duodenal ulcer in rats (Fouad et al., 2017). Results showed that isosorbide dinitrate blocked the oxidative pathway, as seen by reduced lipid peroxidation, and maintained cardiac antioxidant status. Also, it reduced myocardial inflammation (as measured by cytokines such as TNF-α and IL-1β) and blocked the terminal phase of apoptosis (as measured by Bcl-2) in the heart. These results suggest that isosorbide dinitrate's cardioprotective effect in DOX-induced cardiomyopathy may be explained mechanistically. Research into the preventive effect of isosorbide dinitrate against a different kind of anticancer agent, as well as other nitrates, is advised.

To conclude, ISDN significantly reduced doxorubicin-induced cardiotoxicity in rats, most likely through interfering with TNF-α, IL-1β, MDA, TAC, Bcl2, and caspase-3.

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