# The protection of the myocardium by amifostine against mitoxantrone-induced acute cardiotoxicity in rats

*Sıçanlarda mitoksantronun yol açtığı akut kardiyotoksisteye karşı amifostin ile miyokardın korunması*

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## Abstract

**Objective:** Amifostine (AMI) has been used for the prevention of doxorubicin-induced cardiotoxicity in several experimental and a few clinical studies. The aim of this study was to investigate the effects of AMI on lipid peroxidation, protective enzymes, and mitoxantrone (MITO)-induced acute cardiotoxicity in the rat heart using biochemical tests and histopathological examinations.

**Materials and Methods:** Thirty-six rats were divided into six groups (n=6 in each). Control rats were given intraperitoneal (i.p.) serum saline and AMI group rats were given 200 mg/kg AMI i.p. Rats received MITO-2.5 and 5 mg/kg i.p. in the MITO-2.5 and MITO-5 groups. AMI 200 mg/kg i.p. was administered 30 min. before the same doses of MITO in the MITO-2.5+AMI and MITO-5+AMI groups.

**Results:** The levels of cardiac enzymes such as creatinine phosphokinase-myocardial band and cardiac troponin T did not change. Malondialdehyde (MDA) levels increased in MITO groups compared to controls. Catalase and glutathione (GSH) levels in the MITO and MITO+AMI groups were higher than in controls. Superoxide dismutase and glutathione peroxidase levels were not different between MITO groups and controls. There was no difference in MDA levels between MITO+AMI groups and controls. Calcium deposition was not detected. The scores of fibrosis, apoptosis, inflammation, and degeneration in MITO groups were higher than in controls. The scores of fibrosis, degeneration and inflammation in MITO+AMI groups were lower.

**Conclusion:** MITO caused lipid peroxidation and myocardial damage, and the myocardium increased catalase and GSH levels to prevent this damage. AMI can protect against MITO-induced acute cardiotoxicity, decreasing myocardial damage and lipid peroxidation. *(Turk J Hematol 2010; 27: 62-9)*

**Key words:** Amifostine, acute cardiotoxicity, mitoxantrone, lipid peroxidation

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## Özet

**Amaç:** Amifostin (AMI) doksorubisinin yol açtığı kardiyotoksisiteden korunmada çeşitli deneysel ve bir kaç klinik çalışmada kullanılmıştır. Bu çalışmanın amacı Sıçan kalbindeki lipid peroksidasyonu, koruyucu enzimler ve mitoksantronun (MITO) yol açtığı akut kardiyotoksisite üzerinde AMI'nin etkilerini biyokimyasal ve histopatolojik incelemeler ile araştırmaktı.
**Yöntem ve Gereçler:** Her bir grupta 6 sıçan olmak üzere 36 sıçan 6 gruba bölündü. İntraperitoneal (ip) olarak kontrol grubuna serum fizyolojik ve AMI grubuna 200 mg/kg AMI verildi. MITO 2.5 ve 5 gruplarındaki sıçanlar ip MITO 2.5 ve 5 mg/kg aldı. MITO 2.5+AMI ve MITO 5+AMI gruplarında aynı dozlarda MITO'dan 30 dk önce 200 mg/kg AMI uygulandı.
**Bulgular:** Kretainin fosfokinaz-miyokardial bant ve kardiyak troponin T gibi kardiyak enzimlerin düzeyi değişiklik göstermedi. MITO gruplarındaki malondialdehid (MDA) düzeyleri kontrollere kıyasla yüksekti. MITO ve MITO+AMI gruplarındaki katalaz ve glutatyon düzeyleri kontrollerden yüksekti. MITO+AMI ve kontroller arasında süperoksit dismutaz ve glutatyon peroksidaz düzeyleri bakımından fark yoktu. Kalsiyum birikimi saptanmadı. Fibrozis, dejenerasyon ve inflamasyon skorları MITO+AMI gruplarında daha düşüktü.
**Sonuç:** MITO lipid peroksidasyonu ve miyokardiyal zaralanmaya neden olurken miyokardiyum bu zaralanmadan korunmak için katalaz ve GSH düzeylerini arttırmaktadır. AMI miyokardiyal zararlanma ve lipid peroksidasyonu azaltarak MITO'nun yol açtığı akut kardiyotoksisiteye karşı koruyucu olabilmektedir. *(Turk J Hematol 2010; 27: 62-9)*
**Anahtar kelimeler:** Amifostin, akut kardiyotoksisite, mitoksantron, lipid peroksidasyonu

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## Introduction

Anthracyclines are antineoplastic drugs used in the treatment of hematological malignancies and solid tumors [1]. The mechanisms of the antitumor effect of these drugs are the inhibition of topoisomerase II, the intercalation between DNA base pairs, inhibiting synthesis of macromolecules, and the generation of free oxygen radicals, causing DNA damage and lipid peroxidation. The most important toxic effects of anthracyclines are myelosuppression and cardiotoxicity. These toxicities limit both their doses and effectiveness [1,2].

Anthracycline-induced chronic cardiotoxicity is dose-dependent and cumulative. The cumulative cardiotoxicity doses are 500-550 mg/m$^2$ for doxorubicin, 150 mg/m$^2$ for idarubicin, and 40-100 mg/m$^2$ for mitoxantrone (MITO), respectively. In these patients, left ventricular dysfunction and congestive heart failure occurred because of dilated cardiomyopathy [2-5].

Although myocardial adrenergic dysfunction including down-regulation of myocardial β-adrenergic receptors, intracellular calcium overload, induction of apoptosis, and release of cardiotoxic cytokines such as tumor necrosis factor-alpha and interleukin-2 may play a role in the pathogenesis of anthracycline-induced cardiotoxicity, focus in recent years has been on free oxygen radicals and lipid peroxidation [2,3,6-10].

One electron addition to quinine moiety in the tetracyclic ring of anthracyclines results in the formation of semiquinone and free oxygen radicals such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH^\bullet$), by NADPH (nicotinamide-adenine dinucleotide phosphate)-cytochrome c-reductase. $O_2^{-\bullet}$ converts to $H_2O_2$ by superoxide dismutase (SOD), and then $H_2O_2$ is metabolized to $H_2O$ by catalase. Ferrous ($Fe^{2+}$) ions catalyze the conversion of $H_2O_2$ to $OH^\bullet$. The $OH^\bullet$ is capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. As a result of the interaction of the anthracycline-iron ($Fe^{3+}$) complex, these radicals can cause both extensive tissue and DNA damage and decrease in peptides containing sulfhydryl, reacting with macromolecules, such as membrane lipids, proteins and nucleic acids in myocardial tissue. Moreover, free oxygen radicals can decrease the levels of glutathione (GSH) and protective enzymes such as catalase, SOD, glutathione peroxidase (Gpx), and glutathione reductase [2,6,8,10-12].

Amifostine (AMI) is a cytoprotective prodrug that is dephosphorylated by alkaline phosphatase in tissues to active free thiol metabolite [11,12]. AMI has been used for the prevention of doxorubicin-induced cardiotoxicity in several experimental and a few clinical studies [9,11-16]. AMI protects the myocardium, inhibiting lipid peroxidation, apoptosis and the production of free oxygen radicals [9,11,12,15,17].

Mitoxantrone (MITO) is an anthracenedione antineoplastic drug, but its cardiotoxicity is less than that of doxorubicin. The mechanism of MITO-induced cardiotoxicity is probably similar to that of other anthracyclines [18-20]. There are limited experimental studies on MITO-induced cardiotoxicity [6,7,19,21,22], but we did not find any experimental study in the literature on the cardioprotective effects of AMI against MITO-induced acute cardiotoxicity.

We thus investigated both the effects of AMI on lipid peroxidation and protective enzymes and the cardioprotective effects of AMI against MITO-induced acute cardiotoxicity in the rat heart using biochemical tests and histopathological examinations.

## Materials and Methods

### *Chemicals*

MITO (Mitoxantron Ebewe 20 mg/10 ml, Liba/Turkey), AMI (Ethyol 500 mg/10 ml, Er-Kim/Turkey), and serum saline were used as drugs in rats.

### *Animals and Treatment Protocols*

Thirty-six male Wistar rats, 4 months old, weighing 156±30 g (88-242 g) were obtained from the Experimental Research Center of Ege University. Rats were kept in ventilated rooms at

23±2°C with 10 hour (h) darkness/14 h light cycle and relative humidity of 60-75%. They were fed on standard feed (Best Feed-Turkey) and water *ad libitum*.

Rats were divided into six groups of six rats each. There was no difference between groups with respect to weight ($p>0.05$). In the control group, rats received 1 ml/100 g serum saline intraperitoneally (i.p.). In the AMI group, 200 mg/kg AMI i.p. was administered. MITO-2.5 mg/kg and 5 mg/kg i.p. were injected in rats in the MITO-2.5 and MITO-5 groups, respectively. AMI 200 mg/kg i.p. was administered to rats 30 minutes before the same doses of MITO in the MITO-2.5+AMI and MITO-5+AMI groups, respectively.

In previous experimental studies on MITO-induced chronic cardiotoxicity, 0.2-0.6 mg/kg MITO for 12-13 weeks was used. The cumulative doses were 2.4-7.2 mg/kg [20-23]. However, there is no study with the exception of cell culture on MITO-induced acute cardiotoxicity in rats. We thus performed this study with a single dose of 2.5 or 5 mg/kg MITO. Moreover, acute cardiotoxicity was evaluated 7 days after MITO administration, because anthracycline-induced cardiotoxicity is usually detected on the $7^{th}$ day of administration [18,24].

Blood samples were obtained from the tail vein for cardiac enzymes before administration, one day after drug administration, and before sacrifice. Sera were separated and stored until analysis. The rats were sacrificed 7 days after administration by cervical dislocation and their hearts were removed. The hearts were washed with cold serum saline. Half of the heart was stored at -80°C for biochemical tests until analysis. The other half was fixed in 4% formaldehyde solution, and then embedded into paraffin for histopathological evaluations. An expert pathologist examined 4 µm-thick tissue sections, hematoxylin and eosin-stained, without prior knowledge of the drugs given to the rats.

The Institutional Animal Care and Use Committee and National Institutes of Health Guidelines for Animal Care were followed throughout the study [25]. The Local Animal Ethical Committee of the Veterinary Faculty of Adnan Menderes University approved our study. The Project Fund of Adnan Menderes University supported this study financially (Project Number: 6022).

### *Biochemical Assays*

The serum levels of cardiac enzymes, such as creatinine phosphokinase-myocardial band (CK-MB) and cardiac troponin T (cTnT) were measured by electrochemiluminescence immunoassay method (Elecsys 2010 instruments, cTnT STAT and CK-MB STAT kits, Roche Diagnostics, Indianapolis, Indiana, USA).

The heart tissues were thawed and homogenized with appropriate buffer solutions. All analyses including malondialdehyde (MDA), catalase, SOD, total GSH, and Gpx in the heart tissues were performed with Oxis Research products (Foster City, California, USA) using spectrophotometric/colorimetric methods.

**MDA**, a lipid peroxidation product, was measured with a method using the commercial MDA-586 kit. This method is based on the reaction of a chromogenic reagent, N-methyl-2-phenylidone (NMPI) at 45°C [26]. **Catalase** was analyzed using the commercial catalase-520 kit. When incubated with $H_2O_2$, the tissue containing catalase is quenched with sodium azide. The amount of remaining $H_2O_2$ is determined by oxidative coupling reaction of 4-aminophenazone and 3,5-dichloro-2-hydroxybenzenesulfonic acid and then catalyzed by horseradish peroxidase (HRP). The resulting quinone imine dye is measured at 520 nm [27]. **SOD** was measured with the method of Nebot et al. [28] using the commercial SOD-525 kit. This method is based on the SOD-mediated increase in the rate of autoxidation of 5,6,6,6 a,11b tetrahydro - 3,9,10 - trihydroxybenzo [c] fluorine (TTF) in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. **Total GSH** was assayed with the method using the commercial GSH-420 kit. This method is based on the formation of a chromophoric thione [29]. **Gpx** was measured using the commercial Gpx-340 kit. Gpx catalyzes the reduction of $H_2O_2$ to $H_2O$ and organic peroxides to alcohols using GSH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm for indirect measuring of Gpx activity [30].

### *Histopathological Evaluation*

Myocardial fibrosis, degeneration, apoptosis, inflammation, and calcium deposition were evaluated using grading described previously [31,32]. The grading for *fibrosis* was 1: minimal fibrosis in ventricles, septum or papillary muscles, 2: small foci of fibrosis involving small foci at multiple locations, 3: multiple foci of fibrosis involving more than one area, and 4: large diffuse fibrosis area involving ventricular septum and left ventricular papillary muscles. The grading for *inflammation* was 1: few scattered inflammatory cells, 2: minimal inflammatory infiltrates, 3: small localized multiple foci of inflammatory cells involving more than one area, and 4: diffuse severe inflammatory infiltrates. The grading for *apoptosis* was 1: single myocytes randomly distributed in ventricles, septum or papillary muscles, 2: single foci consisting of a few myocytes involving more than previously described locations, and 3: small localized, multiple foci of myocytic apoptosis involving more than one area. Billingham's score for *degeneration* was 1: <5%, 1.5: 5-15%, 2: 16-25%, 2.5: 26-35%, and 3: >35%. The grading for *calcium deposition* was 1: occasional calcium deposits in ventricles, septum or papillary muscles, 2: apparent calcium deposits in previously described area, 3: apparent calcium deposits involving more than one area, and 4: large diffuse area of calcium deposition involving ventricular septum and left ventricular papillary muscles.

### *Statistical Analysis*

All values were given as mean ± standard deviation. One-way ANOVA, Tukey, and Bonferroni tests of post-hoc analysis were used for the comparison of multiple groups. Cardiac enzymes were compared with two-paired t test. SPSS 13.0 for Windows was used for all tests. P value <0.05 was as considered to be significant.

## Results

### *Rats*

In the MITO-5 group, one rat died on the 7th day of the study. The heart was removed and washed with cold serum saline. The same procedures were done until analysis.

### *Cardiac Enzymes*

The levels of cTnT and CK-MB were not different between the six groups and treatment days in all groups (p>0.05). The results of cardiac enzymes are shown in Table 1.

### *Lipid Peroxidation, GSH and Protective Enzymes*

***MDA levels*** in MITO groups were significantly higher than in control and AMI groups (p<0.05 for MITO-2.5 group and p<0.001 for MITO-5 group). There was no difference in MDA levels between MITO+AMI, control, and AMI groups (p>0.05). MDA levels of MITO-5+AMI group were significantly lower than of the MITO-5 group (p<0.005).

***Total GSH levels*** in MITO-2.5 (p<0.001 for both groups) and MITO-5 groups (p<0.001 and p<0.01) were higher than control and AMI groups. Moreover, these levels in MITO-2.5+AMI (p<0.001) and MITO-5+AMI groups (p<0.001 and p<0.005) were still higher than in controls.

***Catalase levels*** in the MITO-2.5 (p<0.001 and p<0.005), MITO-5 (p<0.001), and MITO+AMI groups (p<0.001) were higher than in control and AMI groups.

***SOD levels*** of the MITO-5+AMI group were higher than in controls (p<0.005), but there was no difference between the other groups (p>0.05).

***Gpx levels*** of the MITO-5+AMI group were lower than in the MITO-2.5 group (p<0.05), but these levels were not different between the other groups (p>0.05). The results of all biochemical analyses are shown in Table 1.

### *Histopathological Changes*

***Fibrosis score*** in the MITO-2.5 group was higher than in the control (p<0.005), AMI (p<0.05), and MITO-5 groups (p<0.005). Fibrosis decreased in the MITO-5+AMI group compared to the MITO-2.5 group (p<0.05). There was no difference between control and MITO-5+AMI groups (p>0.05).

***Inflammation scores*** in the AMI and MITO-2.5 (p<0.001 for both groups) and MITO- 5 groups (p<0.05) were higher than in the control group. Inflammation decreased in the MITO+AMI groups compared to the MITO-2.5 group (p<0.05 and p<0.001). There was no difference between control and MITO+AMI groups (p>0.05).

***Apoptosis score*** in the MITO-2.5 group was higher than in control, AMI (p<0.001 for both groups), and MITO-5 groups (p<0.05). While the score in the MITO-2.5+AMI group was higher than in controls (p<0.005), there was no difference between control and MITO-5+AMI groups (p>0.05).

***Billingham's scores*** in the AMI, MITO-2.5, MITO-2.5+AMI (p<0.001 for all), and MITO-5 groups (p<0.01) were higher than in the control group. Degeneration in MITO-5+AMI group was decreased when compared to the MITO-2.5 group (p<0.005). There was no difference between controls and the MITO-5+AMI groups (p>0.05). There was no *calcium deposition* in any rat.

Histopathological changes in all groups are given in Table 2. The figures of Grade 3 fibrosis, inflammation, apoptosis, and degeneration are seen in Figures 1-4, respectively.

**Table 1. The results of biochemical analysis in the groups**

| | Control (n=6) | Amifostine (n=6) | Mitoxantrone-2.5 (n=6) | Mitoxantrone-5 (n=6) | Mitoxantrone-2.5 + Amifostine (n=6) | Mitoxantrone-5 + Amifostine (n=6) |
|---|---|---|---|---|---|---|
| Weight (g) | 163±18 | 150±29 | 149±25 | 144±39 | 150±32 | 165±14 |
| MDA (nmol/g tissue) | 45.9±10.1 | 43±12.2 | 69.2±10.1[a,b] | 82.7±14.8[c,d] | 63.5±7.2 | 52.3±12.8[e] |
| Gpx (mU/g protein) | 14.2±7 | 20.7±10.5 | 23±1.8 | 18±7.3 | 15.1±5.3 | 6±2.3[g] |
| Catalase (U/g protein) | 1.15±0.15 | 1.3±0.25 | 2.61±0.43[d,f] | 3.32±1.2[c,d] | 3±0.47[c,d] | 3.27±0.55[c,d] |
| SOD (U/g protein) | 19.1±2.7 | 27.8±18.3 | 29.1±9.9 | 26.6±13.5 | 29.4±7.8 | 45.2±21[f] |
| GSH (µmol/g protein) | 85±27.4 | 120.3±30.3 | 258±60.5[c,d] | 196.4±29.5[c,h] | 215.6±36[c,d] | 198.1±30.8[c,i] |
| Troponin-T (ng/ml) | | | | | | |
| Day 0 | 0.01±0 | 0.01±0 | 0.01±0 | 0.01±0 | 0.01±0 | 0.03±0.04 |
| Day 1 | 0.01±0 | 0.01±0 | 0.01±0 | 0.01±0 | 0.01±0 | 0.01±0 |
| Day 8 | 0.01±0 | 0.1±0.15 | 0.23±0.33 | 0.09±0.1 | 0.04±0.05 | 0.83±1.8 |
| CK-MB (ng/ml) | | | | | | |
| Day 0 | 0.12±0.02 | 0.15±0.05 | 0.19±0.05 | 0.2±0.07 | 0.18±0.07 | 0.14±0.06 |
| Day 1 | 0.1±0 | 0.13±0.07 | 0.1±0 | 0.18±0.18 | 0.12±0.04 | 0.01±0.05 |
| Day 8 | 0.1±0 | 0.1±0 | 0.1±0 | 0.1±0 | 0.1±0 | 0.17±0.12 |

[a]:p < 0.05 as compared to AMI group, [b]:p < 0.05 as compared to control group, [c]:p < 0.001 as compared to control group, [d]:p < 0.001 as compared to AMI group, [e]:p < 0.005 as compared to MITO-5 group, [f]:p < 0.005 as compared to control group, [g]:p< 0.05 as compared to MITO-2.5 group, [h]:p < 0.01 as compared to AMI group, [i]:p < 0.005 as compared to AMI group

**Table 2. The results of histopathological evaluation in the groups**

| | Control (n=6) | Amifostine (n=6) | Mitoxantrone-2.5 (n=6) | Mitoxantrone-5 (n=6) | Mitoxantrone-2.5 + Amifostine (n=6) | Mitoxantrone-5 + Amifostine (n=6) |
|---|---|---|---|---|---|---|
| Inflammation | 1±0 | 2.3±0.5[a] | 2.8±0.4[a] | 2±0.6[b] | 1.8±0.4[c] | 1.5±0.5[d] |
| Degeneration | 1±0 | 2.3±0.5[a] | 2.8±0.4[a] | 2±0.6[e] | 2.3±0.3[a] | 1.7±0.4[f] |
| Fibrosis | 1.2±0.4 | 1.3±0.5[c] | 2.3±0.5[g] | 1.2±0.4[f] | 1.7±0.5 | 1.3±0.5[c] |
| Calcium deposition | 1±0 | 1±0 | 1±0 | 1±0 | 1±0 | 1±0 |
| Apoptosis | 1±0 | 1.3±0.5 | 2.7±0.5[a,h] | 1.7±0.5[c] | 2.2±0.4[g] | 1.8±0.8 |

[a]:p < 0.001 as compared to control group, [b]:p < 0.05 as compared to control group, [c]:p < 0.05 as compared to MITO-2.5 group, [d]:p < 0.001 as compared to MITO-2.5 group, [e]:p < 0.01 as compared to control group, [f]:p < 0.005 as compared to MITO-2.5 group, [g]:p < 0.005 as compared to control group, [h]:p < 0.001 as compared to AMI group

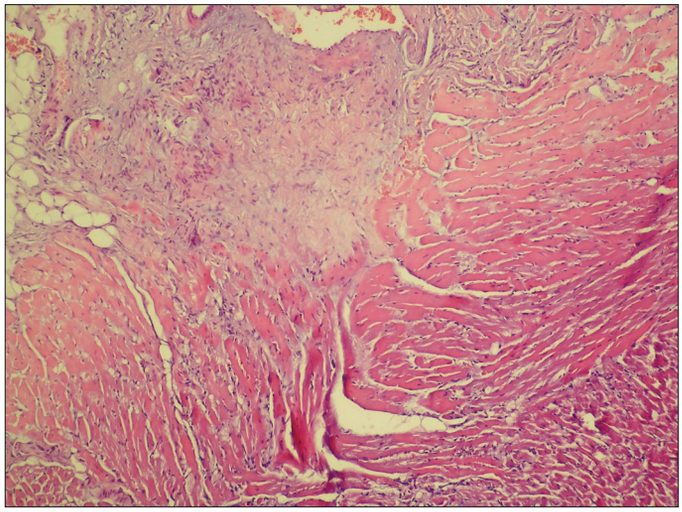

**Figure 1.** Grade 3 fibrosis in heart tissue
(multiple foci of fibrosis involving more than one area; 4 µm thick paraffin section, hematoxylin and eosin, original magnification x200)

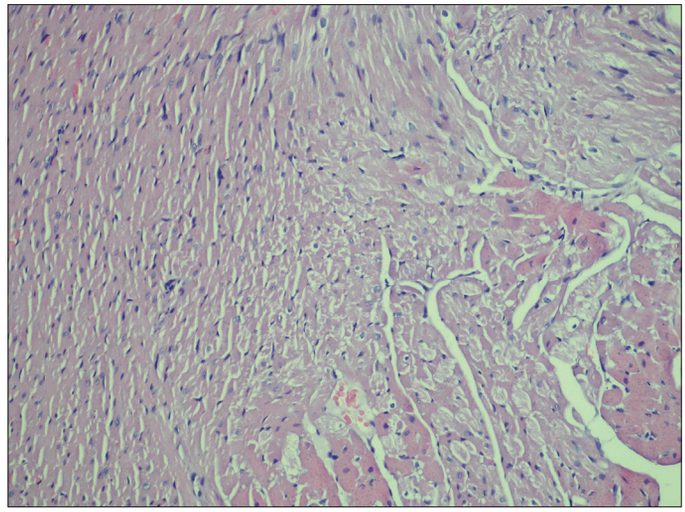

**Figure 3.** Grade 3 apoptosis in heart tissue
(small localized, multiple foci of myocytic apoptosis involving more than one area; 4 µm thick paraffin section, hematoxylin and eosin, original magnification x200)

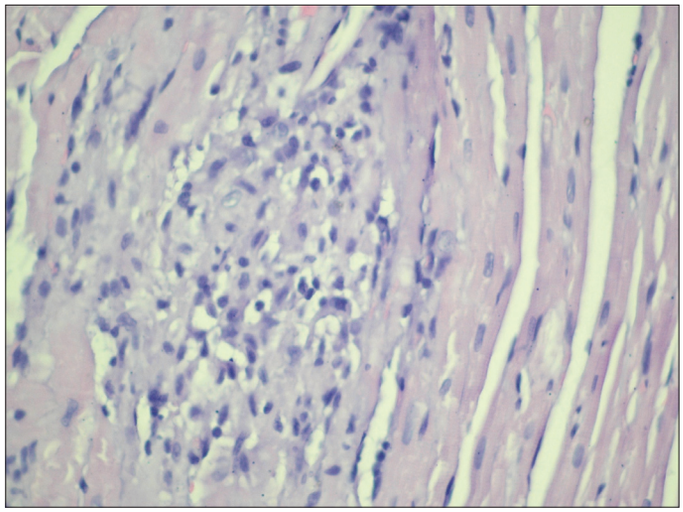

**Figure 2.** Grade 3 inflammation in heart tissue
(small localized multiple foci of inflammatory cells involving more than one area; 4 µm thick paraffin section, hematoxylin and eosin, original magnification x400)

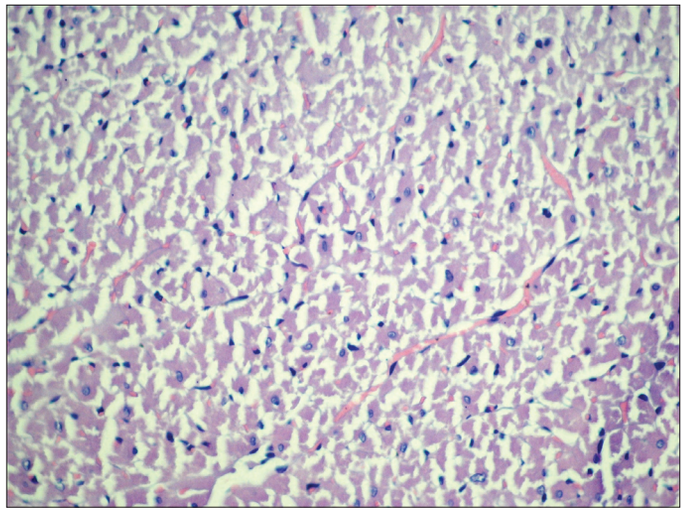

**Figure 4.** Grade 3 degeneration in heart tissue
(more than 35% of degeneration; 4 µm thick paraffin section, hematoxylin and eosin, original magnification x200)

## Discussion

In this study, MITO caused inflammation, degeneration, fibrosis, and apoptosis in heart tissue. Moreover MITO induced lipid peroxidation and increased catalase and total GSH levels.

It was shown in previous experimental studies that doxorubicin causes the generation of free oxygen radicals and lipid peroxidation. The effects of doxorubicin on GSH and protective enzymes such as catalase, Gpx and SOD are controversial

[9,11,12,33,34]. Although MITO induced lipid peroxidation and the generation of free oxygen radicals in liver microsomes [3,8,18], there are only a few experimental studies in which these effects in the heart are demonstrated [22,23]. In these studies, 0.5-0.6 mg/kg MITO for 12-13 weeks was administrated to investigate MITO-induced chronic cardiotoxicity. Moreover, histopathological changes for chronic cardiotoxicity due to the cumulative effects of MITO's repeated administration were detected. In our study, we investigated the acute cardiotoxicity of single-dose MITO. Lipid peroxidation played a role in MITO-induced acute cardiotoxicity in our study. Lipid peroxidation was especially more evident in the MITO-5 group.

Catalase, which dismutases $H_2O_2$ to $H_2O$ and $O_2^{-\bullet}$, is an antioxidant enzyme. While catalase activities are highest in liver and erythrocytes, they are lowest in the heart and brain. Catalase activity in the heart is about 2% of that in the liver of humans, mice, and rats [33]. SOD, which catalyzes the dismutation of $O_2^{-\bullet}$ to $H_2O_2$ and $O_2$, is a metalloenzyme. Fe-SOD in prokaryotes, Cu/Zn-SOD in cytosol and nuclei of eukaryotes and Mn-SOD in mitochondrial matrix of prokaryotes and eukaryotes are found [35]. Gpx catalyzes the conversion of $H_2O_2$ and reduced-GSH to $H_20$ and oxidized GSH [36]. Catalase, SOD and Gpx play critical roles in protecting the myocardium from lipid peroxidation and free oxygen radicals under oxidative damage.

It was detected in some experimental studies that doxorubicin and idarubicin decreased GSH, catalase, Gpx, and SOD levels in the heart tissue [9,11,37,38]. However, over-expression of catalase and Mn-SOD activities were detected after doxorubicin administration. Expression of catalase activity 60 to 100-fold higher than normal can exhibit protection from doxorubicin-induced lipid peroxidation in the heart of transgenic mice. But more than 200-fold increase in catalase activity can not provide this protection [2,33,39-41].

In some studies, no increase in the levels of GSH and these protective enzymes was found after MITO administration [20,39,41]. In our study, while Gpx and SOD levels did not change, GSH and catalase levels increased. Although it was not previously reported, this condition may be related to myocardial protection from MITO-induced cardiotoxicity.

Doxorubicin can cause edema, cytoplasmic vacuolization, degeneration, myofibrillar loss, inflammation, apoptosis, and fibrosis in cardiomyocytes [13,14,21,22]. Billingham's score was higher than in controls in the evaluation of chronic doxorubicin cardiotoxicity in two studies [14,21]. MITO may reduce cell viability acutely and accumulate in cardiomyocytes more than other anthracyclines such as epirubicin, idarubicin and carminomycin [6]. Moreover, MITO can induce apoptosis in the rat heart [2,42]. MITO-induced chronic cardiotoxicity was compared with doxorubicin in two studies. Koutinos et al. [22] administered 2 mg/kg doxorubicin and 0.5 mg/kg MITO once a week for 12 weeks. At the end of 12 weeks, Grade 1 and 2 degenerations were observed in 33% and 50% of rats treated with doxorubicin and in 83.3% and 16.6% of rats treated with MITO, respectively. Herman et al. [21] gave 1 mg/kg doxorubicin and 0.5 mg/kg MITO weekly for 12 weeks. Degeneration ≥ Grade 2 was detected in 100% of the doxorubicin group and 91% of the MITO group. In both studies, histopathological findings in the doxorubicin group were more abundant than those of the MITO group. In our study, the scores of inflammation, degeneration, fibrosis, and apoptosis were higher in the MITO groups. Billingham's score was more than Grade 1.5 in the MITO groups. Interestingly, histopathological changes were more apparent in the MITO-2.5 group.

In spite of histopathological and biochemical changes in heart tissue, we did not detect a significant increase in serum cardiac enzymes in our study. cTnT and CK-MB have been used for the detection of anthracycline-induced cardiotoxicity in experimental studies. These enzymes increased in both acute and chronic doxorubicin cardiotoxicity [21,40,43]. However, Nazeyrollas et al. [13] found these enzyme levels as normal. cTnT levels were high in rats with chronic MITO-induced cardiotoxicity in only one study [21].

AMI decreased MITO-induced lipid peroxidation in our study. This decrease was especially evident in the MITO-5+AMI group. While catalase and total GSH levels in all MITO+AMI groups, and SOD levels in only the MITO-5+AMI group increased, Gpx levels decreased in the MITO-5+AMI group compared to controls. However, AMI did not have any favorable effect on these parameters compared to the MITO groups. AMI did not change cardiac enzymes. Although AMI inhibits lipid peroxidation and the production of free oxygen radicals, and it increases the protective enzymes such as SOD, catalase, and GSH against doxorubicin-induced cardiotoxicity in some experimental studies [10,13-15]. we did not find any study in which the effects of AMI on MITO-induced cardiotoxicity were investigated. Thus, we could not compare our results with other studies.

In our study, AMI caused degeneration and inflammation when compared to controls. AMI administration together with MITO decreased degeneration, apoptosis, inflammation, and fibrosis especially in the MITO-5+AMI group. Alone, AMI administration resulted in degeneration and inflammation in a few experimental studies, although these effects were less than those of doxorubicin [13,14,21]. AMI can decrease the histopathological findings of doxorubicin-induced cardiotoxicity including edema, vacuolization, myofibrillar loss, necrosis, and degeneration [11,13,14,44]. Dragojevic-Simic et al. [14] and Herman et al. [45] detected that AMI significantly decreased these cardiac damage scores.

Dexrazoxane reduces the formation of free oxygen radicals induced by the doxorubicin-iron complex and the chronic cardiotoxic effects of doxorubicin in experimental and clinical studies [11,45]. Bjelogrlic et al. [44] and Herman et al. [45] compared the protective effects of dexrazoxane with AMI on doxorubicin-induced cardiotoxicity in two studies. Dexrazoxane was more cardioprotective than AMI. In one study, dexrazoxane attenuated the histopathological and biochemical changes of both doxorubicin- and MITO-induced cardiotoxicity [21].

In conclusion, MITO induced lipid peroxidation and caused myocardial damage. The myocardium increased catalase and GSH to prevent MITO-induced acute cardiotoxicity. AMI decreased both myocardial damage and MITO-induced lipid peroxidation. Thus, AMI can protect against MITO-induced acute cardiotoxicity.

**Conflict of interest**

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

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