# Protective effect of melatonin on adriamycin-induced cardiotoxicity in rats

## Sıçanlarda adriyamisinin oluşturduğu kardiyotoksisite üzerine melatoninin koruyucu etkisi

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## ABSTRACT

***Objectives:*** Adriamycin is one of the most widely used anticancer drugs. The major limiting factor of using this drug is the development of cardiotoxicity. However, melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous molecule as a good antioxidant that may protect the heart. We investigated whether or not pretreatment with melatonin can attenuate adriamycin-induced cardiotoxicity.

***Study design:*** All procedures and experiments were approved by the Animal Ethics Committee of Gazi Osman Paşa University (2012-HADYEK-022). Adult male Wistar-Albino rats were randomly divided into four groups, namely control (CON, n=7), melatonin (MEL, n=7), adriamycin (ADR, n=7), and adriamycin+melatonin (ADR+MEL, n=7) groups. Cardiotoxicity in rats was induced by adriamycin injection (cumulative dose: 18 mg/kg, intraperitoneal [i.p.]) at an interval of 24 hours (h) on the 5th, 6th and 7th days. Rats receiving melatonin treatment in the adriamycin group received melatonin (10 mg/kg/day, i.p.) for 7 days and were injected with adriamycin (18 mg/kg, i.p.) on 5th, 6th and 7th days. On the 8th day, gravimetric, electrocardiography (ECG) and biochemical parameters were assessed.

***Results:*** Adriamycin induction caused changes in the ECG pattern, including ST-segment elevation and decreased R-amplitude, increase in the serum levels of cardiac injury markers (creatine kinase [CK], CK-MB, aspartate transaminase, and lactate dehydrogenase), decrease in the antioxidant enzymes activity (superoxide dismutase, glutathione peroxidase), elevated lipid peroxidation (malondialdehyde), and altered lipid profile in the serum. Melatonin treatment prevented all the parameters of adriamycin-induced cardiotoxicity in rats.

***Conclusion:*** Melatonin has a protective effect on the heart against adriamycin-induced cardiotoxicity in rats.

## ÖZET

***Amaç:*** Adriyamisin yaygın olarak kullanılan anti kanser ilaçlardan birisidir. Bu ilacın kullanımını sınırlayan esas etmen kalpte toksisitenin oluşmasıdır. Melatonin (N-asetil-5-metoksitriptamin) ise kalbi koruyabilen iyi bir antioksidandır. Biz, melatonin tedavisinin adriyamisinin başlattığı kalp toksisitesini azaltıp, azaltmayacağını araştırdık.

***Çalışma planı:*** Bütün hayvan deneyleri Gazi Osman Paşa Üniversitesi Hayvan Etik Komitesi tarafından uygun bulundu (2012-HADYEK-022). Yetişkin erkek Wistar-Albino sıçanlar rastgele kontrol grubu (KON, n=7), melatonin (MEL, n=7), adriyamisin (ADR, n=7) ve adriyamisin+melatonin (ADR+MEL, n=7) ile tedavi edilmiş gruplar olmak üzere dört gruba ayrıldı. Sıçanlarda kalp toksisitesi, 24 saat aralıkla beşinci günde, altıncı günde ve yedinci günde adriyamisin enjeksiyonu (kümülatif doz 18 mg/kg, i.p.) ile başlatıldı. Melatonin ile tedavi edilmiş adriyamisin uygulanmış gruba yedi gün süre ile melatonin (10 mg/kg, i.p.) ve beşinci, altıncı ve yedinci günde adriyamisin (kümülatif doz 18 mg/kg, i.p.) enjekte edildi. Sekizinci günde tüm gruplarda ağırlık ölçümleri, elektrokardiyografi (EKG) ve biyokimyasal veriler incelendi.

***Bulgular:*** Adriyamisin uyarısı sonucu ST yükselmesi ve R dalgası genliğinde azalma içeren EKG değişiklikleri, serumdaki kalp hasarı belirleyicileri seviyesinde artma (kreatin kinaz, kreatin kinaz-MB, aspartat transaminaz ve laktat dehidrogenaz), antioksidan enzimlerinin aktivitesinde azalma (süperoksit dismutaz, glutatyon peroksidaz) ve lipit peroksidasyonda artma (malondialdehit) ve serum lipit profilinde değişiklikler oldu. Melatonin tedavisi sıçanlarda adriyamisinin başlattığı kalp toksisitesinin hemen hemen tüm parametrelerini düzeltti.

***Sonuç:*** Melatonin sıçanlarda adriyamisinin başlattığı kardiyotoksisiteye karşı önemli bir koruyucu etkiye sahiptir.

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Adriamycin (belonging to the class of anthracycline), an antitumor antibiotic, has been established as an effective agent against a wide range of malignant conditions. Adriamycin- induced cardiac toxicity includes a broad clinical spectrum, and both early and late effects are described. Early effects are those that can be observed after one dose of anthracycline and include a pericarditis-myocarditis syndrome, acute left ventricular dysfunction and arrhythmias. A late effect of the administration of adriamycin in some patients has been a dose-related cardiomyopathy, which may progress to overt congestive heart failure (CHF).[1] Acute toxicity is seen as transient changes on electrocardiograms (ECG), typically ST- and T-wave flattening, arrhythmias and sinus tachycardia.[2] Adriamycin-induced cardiotoxicity mechanisms include: the iron-mediated formation of reactive oxygen species (ROS) and promotion of myocardial oxidative stress; metabolism of adriamycin into more hydrophilic and cardiotoxic substances, which subsequently accumulate in cardiomyocytes; impaired expression of various important cardiac proteins; disruption of cellular and mitochondrial $Ca^{2+}$ homeostasis; induction of mitochondrial DNA lesions and disruption of mitochondrial bioenergetics; degradation of myofilamental and cytoskeletal proteins, including titin and dystrophin; and interference with various pro-survival kinases.[3]

Melatonin is the chief secretory product of the pineal gland of all mammals, and is known to influence a variety of biological processes including circadian rhythms, neuroendocrine, cardiovascular and immune functions, as well as thermoregulation.[4] Some investigations confirm the beneficial effects of melatonin on the physiology and morphology of the hypoxic/reoxygenated heart. Kaneko et al.[5] used isolated rat hearts that were subjected to 30 minutes (min) ischemia and 30 min perfusion. They used melatonin in the perfusion medium. The results showed that the duration of ventricular tachycardia and ventricular fibrillation were significantly reduced by melatonin relative to the durations of these arrhythmias in the control hearts.

One of the previous studies demonstrated the protective effect of melatonin against doxorubicin-induced cardiotoxicity on cardiac dysfunction, ultrastructural alterations and apoptosis in mouse hearts, especially on both left ventricular function and morphological study of the heart.[6]

The present study was designed to show the ability of melatonin to protect cardiac tissue from adriamycin-induced oxidative cardiac damage using enrichment antioxidant enzyme activities analysis, ECG, cardiac marker enzymes, and lipid profile.

**Abbreviations:**

| | |
|---|---|
| *AST* | *Aspartate aminotransferase* |
| *CK* | *Creatine kinase* |
| *cTnT* | *cardiac troponin T* |
| *ECG* | *Electrocardiograms* |
| *GSH-Px* | *Glutathione peroxidase* |
| *LDH* | *Lactate dehydrogenase* |
| *LDL* | *Low-density lipoprotein* |
| *MDA* | *Malondialdehyde* |
| *ROS* | *Reactive oxygen species* |
| *SOD* | *Superoxide dismutase* |
| *TBARS* | *Thiobarbituric acid reactive substance* |
| *VLDL* | *Very low-density lipoprotein* |

## MATERIALS AND METHODS

### Study design

This is a randomized controlled experimental study.

### Animals

Ten-week-old male Wistar-Albino rats (185±25 g) were housed in groups of two animals and maintained under standardized conditions (12-hour (h) light/dark cycle, 24±2ºC, 35-60% humidity). Rats were fed with standard laboratory chow with free access to water. All animal procedures and experiments described in present study were approved by the Animal Ethics Committee of Gazi Osman Paşa University (2012-HADYEK-022).

### Experimental procedures

The animals were randomly divided into the following groups consisting of 7 rats each:

Group 1 (CON): Animals received standard laboratory diet and drinking water ad libitum and served as the control group.

Group 2 (MEL): Animals received melatonin treatment (10 mg/kg/day, intraperitoneal (i.p.), Oxilife, 99.9% pure tablet soluble in pure water) for 7 days[4] and served as the melatonin group.

Group 3 (ADR): Animals were injected with adriamycin at a cumulative dose of 18 mg/kg i.p. at an interval of 24 h on the 5th, 6th and 7th days[7] and served as the adriamycin group.

Group 4 (ADR+MEL): Animals received melatonin treatment (10 mg/kg/day, i.p., Oxilife, 99.9% pure tablet soluble in pure water) for 7 days and were injected with adriamycin (cumulative dose: 18 mg/kg, i.p.) on the 5th, 6th and 7th days.

### Electrocardiography

At the end of the experimental period, the animals were anesthetized with ketamine (75 mg/kg, Pfizer, İstanbul, Turkey) plus xylazine (10 mg/kg, Bayer, İstanbul, Turkey). Needle electrodes were inserted under the skin of the animals in lead II position.[8] ECG recordings were made using computerized MP100 data acquisition system (BIOPAC, Santa Barbara, CA). Changes in ECG pattern (amplitude of ST-segment, R-amplitude, duration of P wave, QRS complex, QT interval, and R-R interval) were considered.

### Biochemical assays

For biochemical assays, approximately 5 mL of whole blood samples were taken from the abdominal aorta into Vacutainer serum-separated tubes (also called gold-topped tubes) shortly after the rats were anesthetized. After 30 min, the tubes were centrifuged at 1500 x g for 10 min. Then, the clear serum was used for all following biochemical assays.

### Measurements of cardiac enzymes

Creatine kinase (CK), creatine kinase-MB fraction (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and cardiac troponin T (cTnT) were estimated by kinetic determination using the commercial kits of Bechman by Bechman Coulter LX-2000 (Brea, CA, USA).

### Superoxide dismutase (SOD) assay

Total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al.[9] The principle of SOD activity determination method was based on the inhibition of nitroblue tetrazolium reduction by the xanthine-xanthine oxidase system as a superoxide radical generator. One unit of SOD was defined as the enzyme activity causing 50% inhibition in the nitroblue tetrazolium reduction rate. The SOD activity was expressed as units per milliliter (U/ml).

### Thiobarbituric acid reactive substance (TBARS) assay

TBARS level was determined using Wasowicz's method,[10] which was based on the reaction of malondialdehyde (MDA) with TBA (Sigma-Aldrich) at 95-100ºC. In the TBA test reaction, MDA or MDA-like substances and TBA react with the production of a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 90°C for 15 min. In order to precipitate proteins, a volume of the sample was mixed with two volumes of cold 10% (wt/vol) trichloroacetic acid. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (wt/vol) MDA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm (Ultraspec Plus; Pharmacia LKB Biocrom, Cambridge, England). The results were expressed as nanograms per milliliter (ng/ml) according to standard graphics, which were prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane.

### Glutathione peroxidase (GSH-Px) assay

GSH-Px activity was measured using the method described by Paglia and Valentine.[11] GSH-Px was measured by the enzymatic reaction, which was initiated by addition of $H_2O_2$ (Sigma-Aldrich) to the reaction mixture containing reduced glutathione, nicotinamide adenine dinucleotide phosphate (NADPH, Sigma-Aldrich), and glutathione reductase (Sigma-Aldrich). The change in the absorbance at 340 nm was monitored using a spectrophotometer and the enzymatic activity was given as nanomoles per milliliter (nmol/ml).

### Measurements of lipid profile

Serum total high-density lipoprotein (HDL)-cholesterol, triglycerides, and low-density lipoprotein (LDL)-cholesterol levels were analyzed with a Cobas C 6000 autoanalyzer (Roche Molecular Biochemicals, Mannheim, Germany). Very low-density lipoprotein (VLDL)-cholesterol was calculated by the following formula: VLDL = Triglycerides/5.

### Statistical analysis

All the values are expressed as mean±SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed Student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad). Differences were considered to be statistically significant at values of $p<0.05$.

## RESULTS

### Characteristics of experimental animals

There was no significant difference in the body weight between the groups, although adriamycin-treated ani-

mals showed a slight reduction in body weight, which was not significant when compared with the control group (data not shown).

## Electrocardiography

Electrocardiographic patterns (amplitude of ST-segment, R-amplitude, duration of both P wave and QRS complex, QT interval, R-R interval, and heart rate) of the control and experimental animals are shown in Figure 1 and Table 1. The control group showed a normal pattern on ECG,[12] whereas the adriamycin-treated group showed an elevation in ST-segment ($p<0.001$) and a decrease in R-amplitude ($p<0.05$) as compared to the control group. Moreover, an increase in the duration of P wave ($p<0.05$) and R–R interval ($p<0.001$) and a decrease in the duration of QRS complex ($p<0.01$) and heart rate ($p<0.001$) were observed in the adriamycin- treated group as compared to the control group. Adriamycin treatment did not show a significant effect on the QT interval when compared to the control group (Table 1). The melatonin-treated group did not show significant changes in any ECG parameters compared with the control group, but a decrease in amplitude of the ST-segment ($p<0.001$), increase in R-amplitude ($p<0.05$), decrease in duration of P wave ($p<0.05$), increase in duration of QRS complex ($p<0.001$), decrease in R-R interval ($p<0.001$), and an increase in heart rate ($p<0.001$) were observed as compared to the adriamycin-treated group. Melatonin pre-co-treatment in the adriamycin-treated

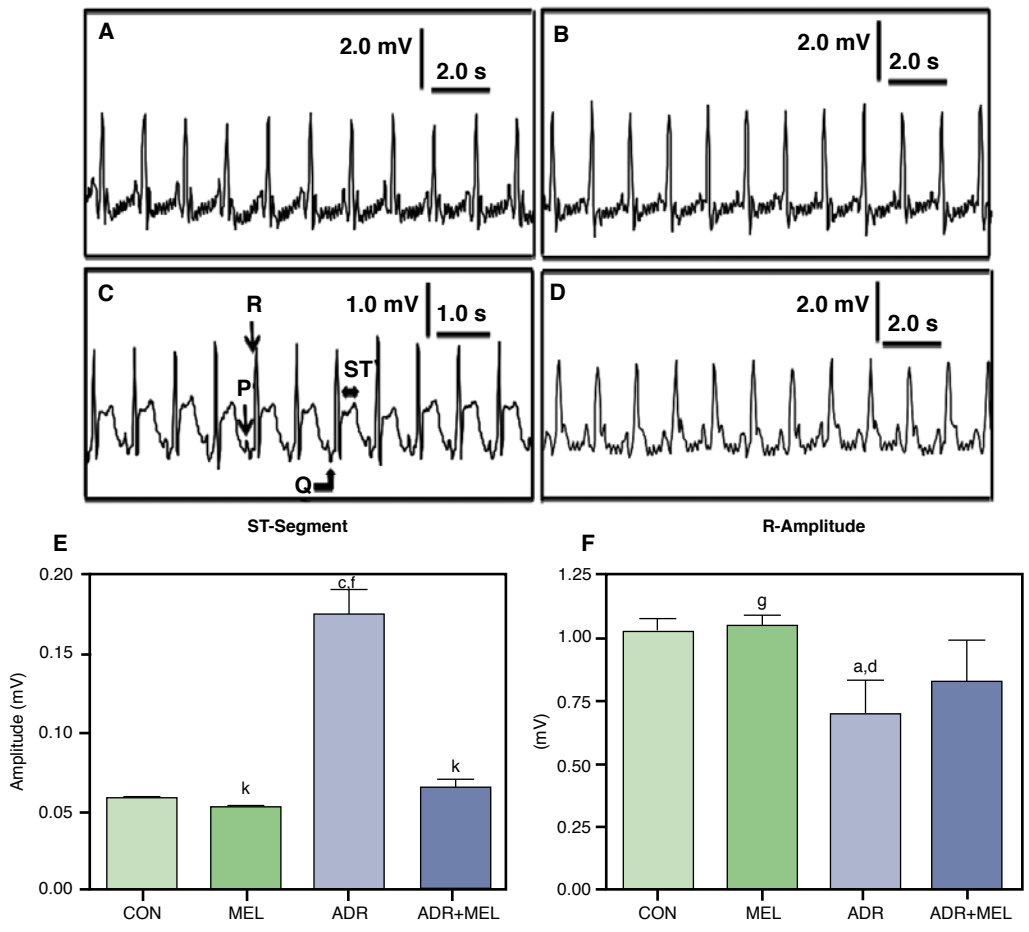


**Figure 1.** The effect of melatonin pre-treatment on the ECG pattern in the control group **(A)**, melatonin-treated group **(B)**, adriamycin administration group **(C)**, melatonin-treated adriamycin group **(D)**, amplitude of ST-segment **(E)**, and R-amplitude **(F)**. Data are presented as mean± SEM. One-way ANOVA and post-hoc Bonferroni multiple comparison test: a: $p<0.05$, b: $p<0.01$, c: $p<0.001$ vs CON; d: $p<0.05$, e: $p<0.01$, f: $p<0.001$ vs MEL; g: $p<0.05$, j: $p<0.01$, k: $p<0.001$ vs ADR. ADR: Adriamycin; CON: Control; MEL: Melatonin; ns: Non-significant.

**Table 1. Electrocardiographic parameters**

| | CON | MEL | ADR | ADR+MEL |
|---|---|---|---|---|
| P wave (duration, s) | 0.033±0.001 | 0.030±0.003$^{g}$ | 0.042±0.003$^{a,d}$ | 0.027±0.004$^{a,k}$ |
| QRS complex (duration, s) | 0.059±0.007 | 0.062±0.005$^{k}$ | 0.031±0.002$^{b,f}$ | 0.045±0.004$^{d,g}$ |
| QT interval (duration, s) | 0.065±0.005 | 0.063±0.004$^{ns}$ | 0.076±0.007$^{ns}$ | 0.068±0.005$^{ns}$ |
| R-R interval (duration, s) | 0.149±0.007 | 0.141±0.006$^{k}$ | 0.251±0.008$^{c,f}$ | 0.203±0.011$^{f,j}$ |
| Heart rate (bpm) | 333.3±16.9 | 345.4±12.2$^{k}$ | 230.6±9.6$^{c,f}$ | 289.7±18.2$^{d,g}$ |

Data are presented as mean±SEM.
One-way ANOVA with post-hoc Bonferroni multiple comparison test was used.
ap<0.05,bp<0.01, cp<0.001 as compared to CON; dp<0.05,ep<0.01, fp<0.001 as compared to MEL; gp<0.05, jp<0.01, kp<0.001 as compared to ADR.
ADR: Adriamycin; CON: Control; MEL: Melatonin; ns: Non-significant.

group showed a decrease in amplitude of ST-segment ($p<0.001$) and a slight increase in R-amplitude, which was not significant when compared to the adriamycin alone-treated group (Figure 1). Melatonin pre-co-treatment also resulted in a decrease in duration of P wave ($p<0.001$) and R-R interval ($p<0.05$), along with an increase both in the duration of the QRS complex ($p<0.05$) and heart rate ($p<0.05$) as compared to the adriamycin-intoxicated group (Table 1).

## Cardiac marker enzymes

Table 2 represents the effects of adriamycin and melatonin treatment on cardiac marker enzymes including CK, CK-MB, LDH, AST, and cTnT. The activities of CK ($p<0.05$), CK-MB ($p<0.05$), cTnT ($p<0.05$), LDH ($p<0.001$), and AST ($p<0.001$) were increased in the adriamycin- treated group as compared to the control group. The melatonin-treated group did not show any significant changes in cardiac marker enzymes when compared with the control group, but the activities of CK ($p<0.01$), CK-MB ($p<0.01$), LDH ($p<0.01$), AST ($p<0.001$), and cTnT ($p<0.05$) were decreased as compared to the adriamycin-treated group. The activities of CK ($p<0.05$), CK-MB ($p<0.05$), LDH ($p<0.001$), AST ($p<0.001$), and cTnT ($p<0.05$) were also decreased in melatonin pre-co-treatment in the adriamycin-treated group when compared to the adriamycin only-treated group.

## SOD, MDA and GSH-Px

The activities of the antioxidant enzymes SOD, GSH-Px and marker for oxidative stress MDA are shown as a graph in Figure 2. The adriamycin-treated group showed reduced levels of antiperoxidative enzyme (SOD, $p<0.05$) and glutathione-dependent enzyme (GSH-Px, $p<0.001$) as compared to the control group. The adriamycin treatment also resulted in an elevated level of MDA ($p<0.001$), end product of lipid peroxidation and marker of oxidative stress, as compared to the control group. The melatonin-treated group did not show any significant changes in levels of SOD, MDA and GSH-Px as compared to the control group,

**Table 2. Cardiac marker enzymes**

| | CON | MEL | ADR | ADR+MEL |
|---|---|---|---|---|
| CK (U/L) | 385.2±30.2 | 366.4±16.7$^{j}$ | 573.7±57.8$^{a,e}$ | 393.0±31.4$^{g}$ |
| CK-MB (U/L) | 471.5±54.5 | 432.3±37.6$^{j}$ | 794.2±83.2$^{a,e}$ | 523.8±89.5$^{g}$ |
| LDH (U/L) | 423.8±51.4 | 406.0±28.8$^{j}$ | 1089.6±65.7$^{c,e}$ | 671.8±77.8$^{a,d,k}$ |
| AST (U/L) | 110.6±7.3 | 100.9±4.7$^{k}$ | 254.1±14.6$^{c,f}$ | 163.8±17.8$^{a,e,k}$ |
| cTnT (ng/ml) | 1.2±0.2 | 1.1±0.2$^{g}$ | 2.4±0.3$^{a,d}$ | 1.3±0.3$^{g}$ |

Data are presented as mean±SEM.
One-way ANOVA with post-hoc Bonferroni multiple comparison test was used.
ap<0.05,bp<0.01, cp<0.001 as compared to CON; dp<0.05,ep<0.01, fp<0.001 as compared to MEL; gp<0.05, jp<0.01, kp<0.001 as compared to ADR.
ADR: Adriamycin; AST: Alanine aminotransferase; CK: Creatine kinase; CK-MB: Creatine kinase-MB fraction; CON: Control; cTnT: Cardiac troponin T; LDH: Lactate dehydrogenase; MEL: Melatonin.

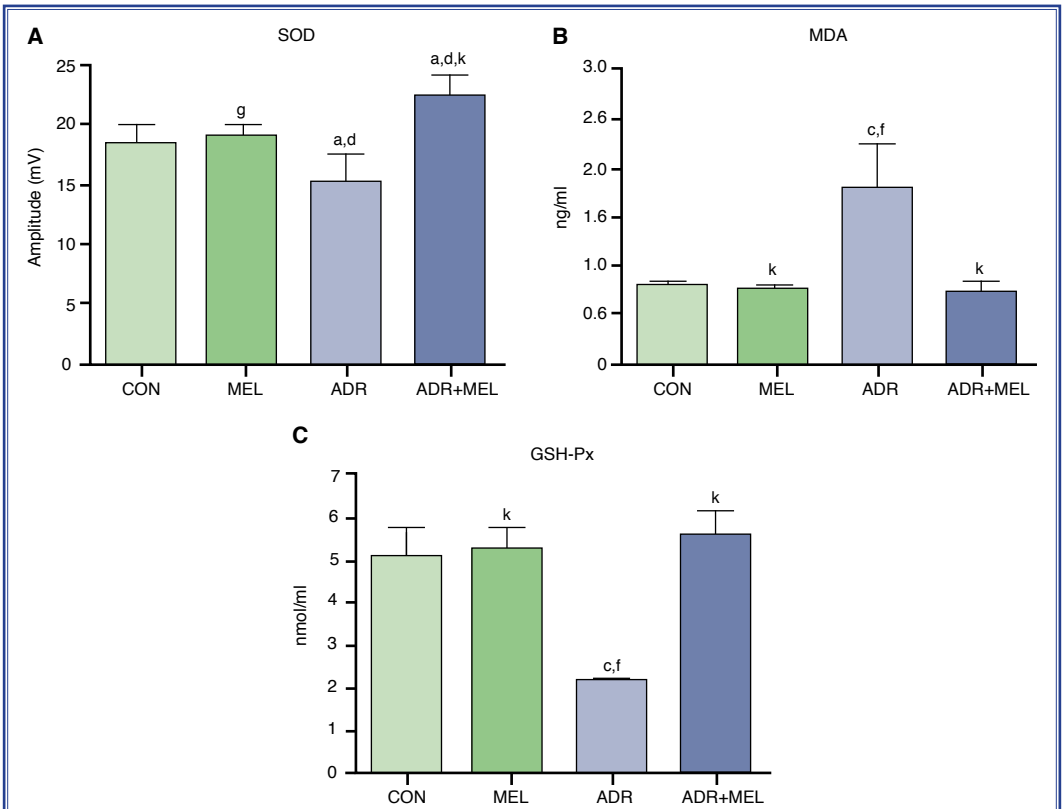


**Figure 2.** **(A)** Effect of melatonin pre-treatment on adriamycin-induced cardiotoxicity in superoxide dismutase (SOD), **(B)** malondialdehyde (MDA), and **(C)** glutathione peroxidase (GSH-Px) activities of rats. Data are presented as mean±SEM. One-way ANOVA and post-hoc Bonferroni multiple comparison test: a: p<0.05, b: p<0.01, c: p<0.001 vs CON; d: p<0.05, e: p<0.01, f: p<0.001 vs MEL; g: p<0.05, j: p<0.01, k: p<0.001 vs ADR. ADR: Adriamycin; CON: Control; MEL: Melatonin; ns: Non-significant.

but there were some increases both in the levels of SOD (p<0.05) and GSH-Px (p<0.001) and a decrease in the level of MDA (p<0.001) when compared with the adriamycin-treated group. Pre-co-treatment with melatonin in the adriamycin-intoxicated group increased the levels of SOD (p<0.001) and GSH-Px (p<0.001) as compared to the adriamycin alone-treated group. The prior administration of melatonin for 7 days along with adriamycin administration on the 5th, 6th and 7th days resulted in a decrease in MDA level (p<0.001) when compared with the adriamycin alone-treated group.

## Lipid profile

The effects of melatonin treatment on triglycerides, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol in the serum of control and adriamycin-treated groups are listed in Table 3. The adriamycin-treated group showed an increase in triglycerides (p<0.01), LDL-cholesterol (p<0.001) and VLDL-cholesterol (p<0.01) as compared to the control group. There was a decrease in the HDL-cholesterol level (p<0.05) in the adriamycin-intoxicated group as compared to the control group. The melatonin-treated group did not show any significant changes in lipid profile as compared with the control group, but there were some decreases in the levels of triglycerides (p<0.001), LDL-cholesterol (p<0.001) and VLDL-cholesterol (p<0.001) and an increase in the level of HDL-cholesterol (p<0.05) when compared with the adriamycin-treated group. Melatonin pre-co-treatment in the adriamycin-treated group showed a decrease in the levels of triglycerides (p<0.05), LDL-cholesterol (p<0.05) and VLDL-cholesterol (p<0.05) in comparison to the adriamycin alone-treated group. Furthermore, there was an increase in HDL-cholesterol level (p<0.01) in the melatonin pre-co-treatment in the adriamycin-intoxicated group as compared to the adriamycin alone-treated group.

**Table 3. Lipid profile**

| | CON | MEL | ADR | ADR+MEL |
|---|---|---|---|---|
| Triglycerides (mg/dl) | 39.0±3.5 | 35.1±3.7$^{k}$ | 63.7±5.7$^{b,f}$ | 47.7±2.9$^{d,g}$ |
| HDL-cholesterol (mg/dl) | 53.4±2.1 | 54.0±3.7$^{g}$ | 44.1±1.6$^{a,d}$ | 58.3±2.8$^{j}$ |
| LDL-cholesterol (mg/dl) | 7.6±0.5 | 6.5±0.4$^{k}$ | 27.6±2.9$^{c,f}$ | 18.5±2.0$^{b,f,g}$ |
| VLDL-cholesterol (mg/dl) | 110.4±7.3 | 100.3±8.6$^{k}$ | 254.1±14.4$^{b,f}$ | 163.4±17.3$^{a,e,g}$ |

Data are presented as mean±SEM.
One way ANOVA with post-hoc Bonferroni multiple comparison test was used.
ap<0.05,bp<0.01, cp<0.001 as compared to CON; dp<0.05,ep<0.01, fp<0.001 as compared to MEL; gp<0.05, jp<0.01, kp<0.001 as compared to ADR.
ADR: Adriamycin; CON: Control; HDL: High-density lipoproteins; LDL: Low- density lipoproteins; MEL: Melatonin; VLDL: Very low-density lipoproteins.

## DISCUSSION

The present study demonstrated some important effects of melatonin underlying the already-known alterations in both electrical and biochemical activity of the heart in the adriamycin-treated rat. First, all of the ECG parameters changed significantly in the adriamycin- treated group as compared to the control group, but melatonin restored the changes in ECG parameters in the adriamycin group to the pattern of the control group. Second, cardiac marker enzymes of the adriamycin-treated group increased significantly when compared with the control group, whereas melatonin treatment decreased the levels of cardiac marker enzymes as compared to the adriamycin group. Third, the lipid profile of the adriamycin-treated group was significantly changed as compared to the control group, but melatonin treatment restored the changes in lipid profile of the adriamycin group to the profile of the control group. These findings have important implications for our understanding of the role of melatonin as an important antioxidant in adriamycin-induced cardiotoxicity.

Intensive investigations of adriamycin-induced cardiotoxicity have continued for decades. The different lines of evidence have provided putative mechanisms, but the precise mechanism underlying adriamycin-induced cardiotoxicity is not completely elucidated. Most studies favor free radical-induced oxidative stress as playing a pivotal role, as it can be interpreted by the chemical structure of adriamycin possessing a tendency to generate ROS during drug metabolism.[13-15]

Holland et al.[16] demonstrated that adriamycin administration in rats resulted in a formation of pathological Q waves in ECG recordings. Previous studies have shown decreases in heart rate and contractility with repeated doxorubicin treatments, likely due to cumulative free radical cardiac damage.[17] It has been demonstrated that ECG analysis can reveal underlying cardiovascular disease as a risk factor in the heart's response to toxicant-induced injury in the rat and serve as a valuable tool to evaluate baseline vulnerability and assess cardiotoxicity.[18] Fisher et al.[19] demonstrated a strong correlation between ST-segment duration and adriamycin-induced cardiotoxicity. In the present study, adriamycin administration resulted in ST-segment elevation, decreased R-amplitude, increased duration of both P wave and R-R interval, and decreased duration of both QRS complex and heart rate. These changes could be due to the consecutive loss of cell membrane in the injured myocardium. ST-segment elevation, which is likely due to oxidant stress-induced repolarization changes, may be an indicator of more severe damage, and thus enhanced cardiac susceptibility to cardiotoxicants in animals with underlying disease. In the present study, melatonin pretreatment in the adriamycin-treated group prevented the pathological alterations in the ECG pattern including duration of both P wave and QRS complex, R-R interval, amplitude of ST-segment, R-amplitude, and also heart rate.

The degree of adriamycin-induced cardiotoxicity was assessed chemically by determining the levels CK, CK-MB, LDH, AST, and cTnT. In comparison to the control group, the adriamycin-treated group showed significant elevation in the levels of cardiac marker enzymes in the serum. This effect may be due to indication of adriamycin-induced necrotic damage of the myocardium and leakiness of the plasma membrane. Melatonin pre-co-treatment resulted in the lowered activity of the marker enzymes in the se-

rum. It demonstrated that melatonin could maintain membrane integrity, thereby restricting the leakage of these enzymes. In comparison to the adriamycin-treated group, only the melatonin-treated group showed a decrease in the levels of cardiac marker enzymes. Several investigators reported elevation in the levels of CK and LDH enzyme activities after adriamycin administration.[20-23]

In the present study, we demonstrated that an increase in lipid peroxidation (significant increase in the level of MDA and decrease in the levels of SOD and GSH-Px) following adriamycin administration was significantly prevented by melatonin. Narin et al.[24] showed that the level of MDA was elevated by doxorubicin. SOD converts superoxide radicals into hydrogen peroxide and molecular oxygen. Since hydrogen peroxide is still toxic to the cell, SOD works in conjunction with two other enzymes, catalase and GSH-Px, to convert hydrogen peroxide into water.[25,26] In our results, decrease in SOD and GSH-Px activities in the adriamycin-induced group may have been due to increased generation of ROS, such as superoxide and hydrogen peroxide, which in turn leads to inhibition of these enzymes. Melatonin treatment restored the decreased levels of SOD in the serum. This could be due to the direct free radical scavenging effect of melatonin, or to an indirect effect through its ability to protect antioxidant enzymes from oxidative damage. A prior study showed that GSH-Px, SOD and CAT enzyme activities were depressed by adriamycin.[27] However, Li et al.[24] showed that SOD and CAT enzyme activities were not changed, but GSH-Px activity was depressed by adriamycin.

Lipids play an important role in cardiovascular diseases due to the structure and stability of the cellular membranes.[8] The results of the present study showed a significant elevation in the level of triglycerides in the serum of the adriamycin-treated group. There were also some increases in LDL fraction and VLDL fraction, which implies that adriamycin toxicity is dependent upon the cumulative dose described by Iliskovic et al.[28] In contrast, we showed that there was a decrease in HDL-cholesterol level in the adriamycin administration group. With these results in the lipid profile, the lipid fraction may be diluted and delay their lipolysis and utilization. The pre-cotreatment with melatonin successfully restored the elevated triglycerides, LDL-cholesterol and VLDL-cholesterol levels in the serum and also restored the decreased HDL-cholesterol levels in the heart in the treatment group.

As a limitation of the study, the protective effect of melatonin on adriamycin-induced cardiotoxicity should have been confirmed by histopathological findings.

In conclusion, the present study demonstrated that intraperitoneal injections of adriamycin produced cardiac toxicity in rats as evidenced by the release of myocyte injury markers in the serum. In addition, the present study provided experimental evidence that melatonin maintained the antioxidant enzyme levels and improved cardiac performance following adriamycin administration. This finding might be of scientific support in understanding the beneficial effects of melatonin on cardioprotection against cardiac toxicity, in which oxidative stress has long been known to contribute to the pathogenesis.

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*Key words:* Adriamycin; antioxidants; cardiovascular diseases/prevention & control; coronary disease; disease models, animal; melatonin; rats, Wistar.

*Anahtar sözcükler:* Adriyamisin; antioksidanlar; kardiyovasküler hastalık/korunma ve kontrol; koroner hastalık; hastalık modeli, hayvan; melatonin; sıçanlar, Wistar.