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RESEARCH ARTICLE

Determination of acute and chronic effects of cadmium on the cardiovascular system of rats

Ikbal M. Ozturk[1], Belgin Buyukakilli[1], Ebru Balli[2], Burak Cimen[3], Sevgi Gunes[1], and Semra Erdogan[4]

[1]Department of Biophysics, [2]Department of Histology and Embryology, [3]Department of Biochemistry, and [4]Department of Biostatistics, Faculty of Medical, University of Mersin, Mersin, Turkey

Abstract

In this study, the systemic hemodynamics induced by acute and chronic cadmium (Cd^{+2}) intoxication in the cardiovascular system of rats using thoracic electrical bioimpedance were examined and the acute and chronic effects of Cd^{+2} intoxication on the activities of antioxidant enzymes and malondialdehyde (MDA) were compared. Also, in this study, ultrastructural changes in the heart and aorta of rats were evaluated. Thirty-eight male Wistar albino rats were randomly divided into control, acute, and chronic groups. Chronic group was administered by oral gavage an aqueous solution of $CdCl_2$ for 60 days, at dose of 15 mg Cd^{+2}/kg/day. Acute group was administered by oral gavage an aqueous solution of $CdCl_2$ with a single dose of 15 mg Cd^{+2}/kg. Cadmium increased the stroke volume and cardiac output of rats in the chronic group, but did not change the heart rate significantly. Antioxidant enzymes activities and MDA level significantly increased in the chronic group. In ultrastructural examination, there were widespread degenerative changes in heart muscle cells of the chronic group but endothelial cells and smooth muscle cells in the aorta tissue samples had normal morphological features in all groups. All of the findings indicate that Cd^{+2} toxication can cause deformation in heart muscle cells due to an increase in free radicals and lipid peroxidation. Also, this study has confirmed that a long-term-Cd^{+2} exposure increased stroke volume (SV) and cardiac output (CO), but did not change the heart rate (HR).

Keywords: *Cadmium; cardiac output; hemodynamics; rat; thoracic electrical bioimpedance*

Introduction

Cadmium (Cd^{+2}) is a potentially toxic element being ubiquitously present in environments to which humans and animals are exposed via a variety of routes, including industrial contamination, food sources, and tobacco smoke (Nation et al. 1987; Hudecova and Ginter 1992). Inhibition of bioamine uptake, Na-K ATPase, and voltage-dependent Ca^{+2} channels are among the numerous effects of Cd^{+2} poisoning.

When Cd^{+2} enters the body, it reaches the liver within the first 6 h and binds to metallothionein, which is a protein with a low molecular weight (6000–10,000 Da), and rich in cystein (Cherian and Goyer 1978; Chan and Cherian 1993). The cadmium–metallothionein complex generated in the liver was reported to be mainly distributed to the kidney and other tissues, and hence it causes damage in these tissues (Foulkes 1990; Liu et al. 1996).

Free radical levels are important for the growth and development of all cells. Free radical species affect all important components of cells such as lipids, proteins, carbohydrates, and nucleic acids (Sarkar et al. 1997). As a result of free radical attack, lipids are oxidized, and hence membranes are damaged (Sarkar et al. 1998). It has been reported that administration of Cd^{+2} via different routes causes increased lipid peroxidation in membranes of erythrocytes and tissues such as the liver, kidney, brain, and testes where malondialdehyde (MDA) is used as an indicator of oxidative damage (Gutteridge 1995; Stohs et al. 2001). Intake of Cd^{+2} results in consumption of gluthatione and protein-binding sulfydryl groups, and subsequently the levels of free radicals such as hydrogen peroxide, hydroxide, and superoxide are increased. Increased lipid peroxidation results in changes in intracellular stability, DNA damage, and apoptosis (Stohs et al. 2001). Bagchi et al. (1996) reported that the levels of

Address for Correspondence: Associate Professor Dr (PhD) Belgin Buyukakilli, Mersin Üniversitesi, Tıp Fakültesi, Yenişehir Kampüsü, Biyofizik Anabilim Dalı, 33161 Mersin, Turkey. Tel: 0 90 324 341 28 18. Fax: 0 90 324 341 24 00. Email: bbuyukakilli@mersin.edu.tr

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glutathione peroxidase (GSHPx) were increased, whereas a reduction was observed in the activity of glutathione reductase in experimental cadmium-induced toxicity. Moreover, free radicals are increased due to the inhibition of protective antioxidant mechanisms related to Cd^{+2} toxicity (Gulati et al. 1986; Sidhu et al. 1993).

Notably, like lead, Cd^{+2} has also been shown to increase atherosclerosis in animal models (Revis et al. 1981; Subramanyam et al. 1992), and environmental exposure to cadmium has been suggested to be particularly toxic to heart and blood vessels (Kopp et al. 1982; Jamall et al. 1989), in part because of the ability of Cd^{+2} to increase free radical generation (Wang et al. 2004).

Bioimpedance cardiography or impedance cardiography (ICG) (thoracic electrical bioimpedance-TEB) is a non-invasive, indirect means of evaluating hemodynamic parameters. The method makes it possible to denote stroke volume and cardiac output. The technique employs a small unnoticeable alternating current flow introduced across the thorax. The resultant changes in thoracic impedance are related in part to changes in volume and velocity of blood flow in the thoracic aorta during systole and diastole. Impedance to electrical current decreases during systole due to increased blood volume, flow velocity, and alignment of red blood cells. The pulsatile impedance changes directly reflect ascending aortic flow and, thus, left ventricular function. The impedance changes during a cardiac cycle are used to determine stroke volume (SV), which can be used to calculate cardiac output (CO), when coupled with the simultaneous measurement of heart rate (HR). When combined with heart sounds and electrocardiogram signals, TEB provides a continuous comprehensive hemodynamic profile for safety Pharmacology/General Pharmacology evaluations (De Pasquale and Fossa 1996).

The aim of the present study was to determine the systemic hemodynamics induced by acute and chronic Cd^{+2} intoxication in the cardiovascular system of rats using TEB and to compare the acute and chronic effects of Cd^{+2} intoxication on the activities of antioxidant enzymes catalase (CAT), GSHPx, superoxide dismutase (SOD) as well as MDA in rat erythrocytes. Also, in this study, ultrastructural changes in the cardiovascular system of rats were evaluated.

Materials and methods

Animals and experimental protocol

All procedures were approved by the Medical Faculty Experimentation Ethics Committee of Mersin University and followed the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

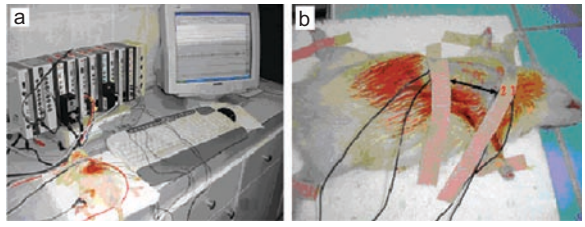
In this study, 38 healthy Wistar male albino rats, aged 3 months, were used. Animals were housed at 23 ± 2°C under a reversed dark–light-cycle (dark: 9 am to 9 pm) and fed on standard rat pellets (Tavas animal food product Co., Turkey) and tap water ad libitum. They were randomly divided into three groups: control (C), acute (A), chronic (Ch), C containing 15 animals, A containing nine animals, and Ch containing 14 animals. Weights of individual rats were recorded weekly.

The chronic group was administered by oral gavage an aqueous solution of $CdCl_2$ for 60 days, at a dose of 15 mg Cd^{+2}/kg/day. The acute group was administered by oral gavage an aqueous solution of $CdCl_2$ with a single dose of 15 mg Cd^{+2}/kg. The control group was administered by oral gavage 0.9% saline for 60 days, at a dose of 1 ml/day.

Determination of hemodynamic (stroke volume and cardiac output) parameters

Thoracic electrical bioimpedance (TEB)

Prior to TEB recordings the rats were anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi Ilac Sanayi ve Ticaret A.S., Istanbul, Turkey), administered intramuscularly. All the rats were placed in a standard position during the TEB recordings (Figures 1A and B). Then the neck and the thorax region were shaved. TEB recordings were made by using an EBI100C module in BIOPAC MP 100 acquisition system (Santa Barbara, USA) and hemodynamic parameters were recorded on day 0 (first measurement) and day 60 (second measurement) days in control and chronic groups, but were recorded on day 0 (first measurement) and day 2 (second measurement) days in the acute group.



Electrodese 1 and 4 apply AC across thorax
Electrodese 1 and 3 detect voltage changes

$$(1)\ SV = VEPT.VET.\frac{dZ/dt_{max}}{Z_0}$$

VET=ventricular ejectin time
dZ_{max}/dt = maximum rate of impedance change
Z_0=total thoracic steady state basal impedance
VEPT=volume of electrically participating tissue

$$(2)\ VEPT = \frac{Rho.L^2}{Z_0} = \frac{L^3}{4.25}$$

Figure 1. (A) Thoracic electrical bioimpedance (TEB) recordings were made by using EBI100C module in BIOPAC MP 100 acquisition system (Santa Barbara, USA). (B) TEB involved placement of surface electrode pairs laterally on the rat's neck and on the thorax. A high-frequency (70 kHz), low-amplitude (2.5 mA) electrical current was applied to the outer sensors (most superior sensors on the neck and most inferior sensors on the thorax. Voltage changes across the thorax were detected by the inner set of electrodes. Equation (1) was used for deriving stroke volume for the calculation of cardiac output. Equation (2) was used to estimate the volume of electrically participating tissue of the thorax, where Rho was the resistivity of blood and L was the distance between the inner set of electrodes.

The hemodynamic parameters that were recorded on day 0 served as the baseline data.

A total of four electrodes are placed laterally on the rat's neck and thorax and a high-frequency (70 kHz), low-amplitude (2.5 mA) electrical current applied to the outer sensors (most superior sensors on the neck and most inferior sensors on the thorax, as depicted in Figure 1B). The inner sensors measure the pulsatile changes in voltage that occur due to pulsatile changes in thoracic impedance related to the change in the size of the thoracic aorta and reflecting stroke volume. Impedance within the thorax is related to the conduction properties of the tissue and volume of the path of resistance (De Pasquale and Fossa 1996). Using the change in voltage across the thorax to measure the change in thoracic impedance, equations (1) and (2) of Figure 1 describe the components of the equation by Bernstein (1986) used to derive stroke volume. SV (ml/beat) can be calculated as the product of four components: volume of electrically participating tissue (VEPT in ml), which is solely dependent on L, the distance between the inner electrodes (equation (2) of Figure 1); ventricular ejection time (VET in s); maximum rate of change in impedance (dZ_{max}/dt in Ω/s); and steady-state basal impedance, Z_0 (Ω) (De Pasquale and Fossa 1996). The recording system recognized and eliminated the impedance changes arising from ventilation and body movements. Finally, to record HR, three ECG electrodes were placed in a Lead I configuration on the thorax.

ECG, heart sounds, and TEB signals were continuously recorded in 60 s epochs. HR, Z_0, dZ_{max}/dt, and VET were derived from the event waveforms (Figure 2). SV was calculated using equations (1) and (2) of Figure 1. CO was determined using the equation CO (ml/min) = SV (ml/beat) × HR (beats/min).

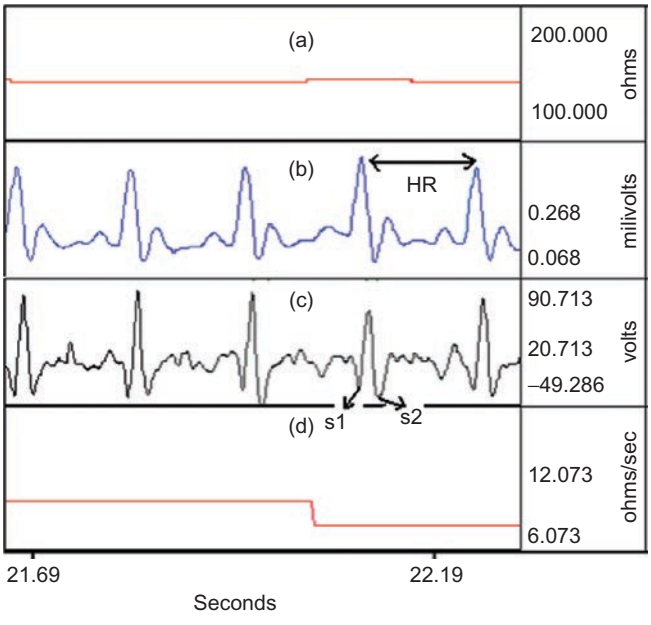


Figure 2. The simultaneous recordings of thoracic electrical bioimpedance (TEB), electrocardiogram (ECG), and heart sounds from each rat. (A) Z_0 (steady-state basal impedance). (B) ECG. Heart rate (HR) was detected by R-R interval detection method in ECG. (C) Heart sounds. Ventricular ejection time (VET) was determined as the duration of electro-mechanical systole. So, VET was measured as the time intervals between the first (S1) to second (S2) heart sounds using the heart-sounds trigger pulses. (D) Maximum rate of impedance change (dZ_{max}/dt).

Determination of biochemical parameters

In the control and chronic groups, 60 days (2 days in the acute group) after the beginning of the experiment, the rats were sacrificed by cardiac blood aspiration and the whole heart and aorta were obtained by dissection and were immediately weighted.

Whole blood was centrifuged after coagulation and serum was separated immediately. The soft tissues were washed thoroughly in physiological saline and weighed. The biological material not used immediately after the collection was frozen at –20°C until further analysis. In the materials collected at the end of the experiment (from the beginning after 60 days in control and chronic groups, after 2 days in acute group), concentration of Cd^{+2} was determined.

Cadmium concentration

The heart, the aorta, and the blood of rats were dried and mineralized in an electrical muffle furnance. Cd^{+2} concentration was determined by flameless atomic absorption spectrometry (GFAAS Atomic Absorption Spectrophotometer–Varian) with electrothermal atomization in a graphite cuvette. The cathode lamp for cadmium was operated under standard conditions using its resonance line of 228.8 nm. The Cd^{+2} concentration was expressed in μg/l in blood or μg/g of wet tissue weight.

Enzyme assessments

After blood samples of all groups were collected into citrate (3.5 mg/mL blood) containing glass tubes, erythrocyte sediments were prepared for the analyses as described by Beutler (1975). The blood with anticoagulant was centrifuged at 480×g for 10 min; and plasma samples were removed. Plasma samples were stored at –20°C until analyzed. Erythrocytes were washed three times with physiological saline solution and then hemolysed by diluting 50-fold with deionized water. Hemolysate was then centrifuged at 22,000 × g for 30 min and supernatant was removed. Analyses were carried out in this hemolysed supernatant fraction. After samples were obtained, they were immediately prepared for the analysis. All the procedures were performed at +4°C throughout the experiments. All samples were studied in the same batch.

SOD, GSHPx, and CAT enzyme activities were measured as described in Durak et al. (1996), Paglia and Valentine (1967), and Aebi and Bergmeyer (1974). respectively. Activities of the enzymes except SOD were expressed as international unit/milliliter (IU/ml). One unit of SOD activity was defined as the enzyme protein amount causing 50% inhibition in nitrobluetetrazolium (NBT) reduction rate and results were expressed as unit/milliliter (U/ml) erythrocyte pellet. The GSHPx activity method is based on the measurement of the absorbance decrease at 340 nm due to the consumption of NADPH and that of CAT is based on the measurement of the absorbance decrease due to H_2O_2 consumption at 240 nm.

MDA method was based on the spectrophotometer absorbance measurement of the pink colored product of thiobarbituric acid–malondialdehyde complex formation, and results were expressed as nmol/ml (Van Ye et al. 1993).

Sample preparation for electron microscopy

For transmission electron microscopic (EM) evaluation of the heart and aorta tissue samples were fixed with 2.5% gluteraldehyde, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol series, cleared with propylene oxide, and embedded in epon. Thin sections (50–70 nm) were cut by a microtome (Leica UCT-125) and contrasted with uranyl acetate and lead citrate. Sections were examined and photographed under an electron microscope (JEOL JEM-1011).

Statistical analyses

Descriptive statistics (mean ± standard deviation) were calculated in each group for all hemodynamic and biochemical parameters. Shapiro-Wilk test was used to determine whether all parameters were normality distributed or not and it was found that all parameters were normally distributed.

Statistical analyses of hemodynamic parameters

The paired samples *t*-test was used to test the null hypothesis, which is used to test if the average of the differences between a series of paired observations was zero for all hemodynamic parameters in this study. One-way analysis of variance (ANOVA**)** was used to test the differences between the groups for the first measurements of all the parameters. Data were analyzed by using the SPSS 11.5 statistical packet program. Statistica 6.0 packet program is used in figures for differences. The results were considered statistically significant if p values were less than 0.05.

Statistical analyses of biochemical parameters

Variance analyses were used to test the differences between the groups for each biochemical parameter. One-way ANOVA was used for parameters to provide homogeneity of variances and Welch test was used for parameters to provide heterogeneity of variance. Tukey test was used for multiple comparisons in one-way ANOVA and Games-Howell test for multiple comparisons in Welch test statistics.

Results

Evaluation of hemodynamic parameters by thoracic electrical bioimpedance

Hemodynamic parameters by TEB were recorded on admission (Figure 2). Data analyses were conducted using SPSS (V 11.5). The means and standard deviations for the first and the second measurements of hemodynamic parameters of all groups by TEB and weights of the rats are presented in Table 1. Inter group analysis revealed no statistically significant difference ($p > 0.05$) before cadmium treatment (first measurements) in all parameters.

HR, Z_0, dZ/dt_{max}, VET, and VEPT were derived from the TEB waveforms. SV was calculated using equations (1) and (2) of Figure 1. CO was determined using the equation CO (ml/min) = SV (ml/beat) × HR (beats/min). Among the control group data, there was no significant difference between the first and second measurements (Table 2 and Figure 3). Among the parameters of the acute group, only the rat weights were a little elevated, but there was no significant difference for the other parameters (Table 2 and Figure 3). Among the chronic group data, Z_0, VET, dZ_{max}/dt, SV, and CO was elevated, whereas there was no significant difference between the first and second measurements of HR and VEPT (Table 2 and Figure 3). Also, among the chronic group data, weight was decreased at the second measurement compared to the first measurement (Table 2 and Figure 3).

In the chronic group rats, higher values for SV and CO in second measurement compared to first measurement correlated with cadmium intoxication (Table 1). As seen in Table 1, the mean SV of first and second measurements are 0.14 ± 0.02 ml/beat and 0.21 ± 0.03 ml/beat, respectively, and the mean CO of first and second measurements are 55.25 ± 6.77 ml/min and 84.02 ± 13.16 ml/min, respectively.

Biochemical parameters

Cadmium concentrations in the heart, the aorta, and the blood of rats exposed for a single dose (15 mg Cd^{+2}/kg) or 60 days at a dose of 15 mg Cd^{+2}/kg/day of cadmium are presented in Table 3.

In heart and blood of rats exposed to Cd^{+2} for a single dose (acute group) or 60 days (chronic group), the Cd^{+2} concentration was significantly increased, in comparison to the rats in the control group as well as to the aorta of rats exposed for 60 days to the same Cd^{+2} dose. However, exposure for 60 days caused a significant 5-fold increase in concentration of Cd^{+2}

Table 1. Means and standard deviations for the first and second measurements of hemodynamic parameters of all groups by TEB and weights of the rats.

		W (g)	VEPT (ml)	Z_0 (Ω)	VET (s)	dZ_{max}/dt (Ω/s)	SV (ml/beat)	HR (beats/min)	CO (ml/min)
Control	1. Measurement	352.93 ± 26.40	26.24 ± 6.03	142.20 ± 14.37	0.085 ± 0.005	10.37 ± 3.10	0.16 ± 0.01	394.27 ± 10.21	61.14 ± 5.13
	2. Measurement	353.47 ± 51.02	23.70 ± 9.52	142.40 ± 14.48	0.086 ± 0.005	12.33 ± 4.48	0.15 ± 0.02	403.60 ± 10.00	62.24 ± 5.43
Acute	1. Measurement	353.89 ± 44.42	21.76 ± 7.02	144.78 ± 10.87	0.080 ± 0.004	11.00 ± 2.78	0.13 ± 0.02	431.11 ± 30.70	54.67 ± 6.04
	2. Measurement	355.78 ± 44.59	20.92 ± 6.53	148.00 ± 10.91	0.080 ± 0.004	12.22 ± 3.46	0.13 ± 0.02	442.33 ± 36.26	57.92 ± 5.97
Chronic	1. Measurement	344.21 ± 35.66	21.87 ± 6.52	145.29 ± 11.08	0.085 ± 0.005	9.14 ± 7.42	0.14 ± 0.02	410.43 ± 19.45	55.25 ± 6.77
	2. Measurement	275.43 ± 20.08	26.02 ± 10.51	160.14 ± 13.25	0.090 ± 0.008	15.71 ± 5.11	0.21 ± 0.03	395.86 ± 20.69	84.02 ± 13.16

W (weight in g); VEPT (volume of electrically participating tissue in ml); Z_0 (steady-state basal impedance in Ω); VET (ventricular ejection time in sec); dZ_{max}/dt (maximum rate of change in impedance in Ω/s); SV (stroke volume in ml/beat); HR (heart rate in beats/min); CO (cardiac output in ml/min).

in the heart, in comparison to acute exposure (Table 3). Also, exposure for 60 days caused a significant 0.5-fold increase in concentration of Cd^{+2} in the blood, in comparison to acute exposure (Table 3).

CAT, GSHPx, SOD, and MDA values in all groups are presented in Table 4. As seen from the table, values of CAT, GSHPx, and SOD enzyme activities and MDA level in erythrocytes significantly increased in the chronic group when compared to the rats in the control group ($p<0.05$). In the acute group when compared to the rats in the control group there was a statistically significant increase in parameters only such as CAT enzyme activity and MDA level ($p<0.05$). However, GSHPx and SOD levels did not show a statistically significant difference in the acute group when compared to the rats in the control group (Table 4).

Table 2. Results of significant values of differences between the first and second measurements for each group.

Groups (paired differences)	W	VEPT	Z_0	VET	dZ_{max}/dt	SV	HR	CO
Control	*	*	*	*	*	*	*	*
Acute	0.005	*	*	*	*	*	*	*
Chronic	0.0001	*	0.004	0.002	0.030	0.0001	*	0.0001

* No statistical significant differences between first and second measurements ($p>0.05$).W (weight in g); VEPT (volume of electrically participating tissue in ml); Z_0 (steady-state basal impedance in Ω); VET (ventricular ejection time in s); dZ_{max}/dt (maximum rate of change in impedance in Ω/s); SV (stroke volume in ml/beat); HR (heart rate in beats/min); CO (cardiac output in ml/min).

Table 3. Accumulation of cadmium in the heart, the aorta and the blood of the rats acute and chronically exposed to cadmium. Concentration of Cd^{2+} in the heart, aorta, and blood of the control rats (C); after 'a single dose' exposure –15 mg Cd^{+2}/kg by oral gavage (A); after 'chronic' exposure –15 mg Cd^{+2}/kg/day by oral gavage (Ch). $p \le 0.05$.

Groups	Heart (μg/g)	Aorta (μg/g)	Blood (μg/l)
Control	0.17 ± 0.04	0.19 ± 0.08	9.8 ± 2.7
Acute	0.53 ± 0.31*	0.22 ± 0.10	16.3 ± 1.6*
Chronic	2.64 ± 0.78*	1.18 ± 0.43*	23.7 ± 6.2*

* $p \le 0.05$.

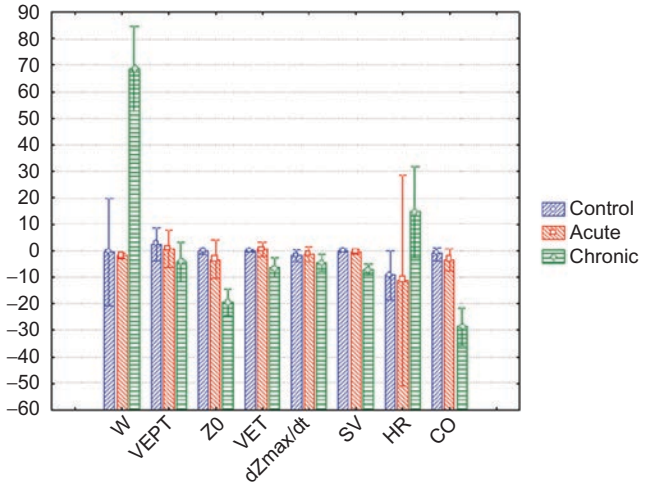


Figure 3. Differences between the first and second measurements of hemodynamic parameters by TEB and weights of all groups of rats. W (weight in g); VEPT (volume of electrically participating tissue in ml); Z_0 (steady-state basal impedance in Ω); VET (ventricular ejection time in s); dZ_{max}/dt (maximum rate of change in impedance in Ω/s); SV (stroke volume in ml/beat); HR (heart rate in beats/min); CO (cardiac output in ml/min). Results between the first and second measurements of VET and SV parameters of all groups by TEB of rats were multiplied by 1000 and by 100, respectively.

Ultrastructural examination

Heart muscle cells had normal morphological features in the control group. Myofibrils, sarcomeric structures, mitochondria, and other sarcoplasmic organelles were detected as normal. It was observed that the nucleus had normal morphological characteristics in this group (Figure 4).

Although some heart muscle cells had normal morphological features, there were degenerative changes in some heart muscle cells in the acute group. Thinning and breaking off in some myofibrils in some heart muscle cells were determined. Furthermore, expansions in sarcoplasmic reticulum cisternaes and perinuclear cisternaes were encountered. The nucleus and mitochondria had normal morphological features in the acute group (Figure 5).

There were widespread degenerative changes in heart muscle cells of the chronic group. Myofibrils were thin and disintegrate and normal myofibril organization was completely corrupted. Degenerative mitochondrial changes were evident in this group. Furthermore, expansions in sarcoplasmic reticulum cisternaes and intracellular vacuoles were determined. The nucleus had normal morphological features in this group (Figure 6).

Endothelial cells and smooth muscle cells in the aorta tissue samples had normal morphological features in all groups (Figure 7).

Discussion

In the present study, the effects of cadmium intoxication on hemodynamic parameters were studied in Wistar male albino rats. In this study, the TEB method was used to determine cardiac output and related variables. Through the use of TEB, the state of the circulatory system and trends in changes in hemodynamic parameters can be assessed easily, quickly, cheaply, and, most importantly, non-invasively. The method makes it possible to denote SV and HR. In many clinical studies, TEB has been compared to invasive methods (thermodilution and the Fick method) (De Maria and Raisinghani 2000; Greenberg et al. 2000; Drazner et al. 2002; Van De Water et al. 2003) and non-invasive methods

Table 4. Mean ± SD of values of CAT, GSHPx, and SOD enzyme activities and MDA level in erythrocytes.

Groups	CAT (IU/ml)	GSHPx (IU/ml)	SOD (U/ml)	MDA (nmol/ml)
Control	29.65 ± 23.73	0.043 ± 0.027	3.16 ± 4.17	5.31 ± 1.20
Acute	81.83 ± 34.44*	0.050 ± 0.024	10.71 ± 8.66	9.71 ± 3.21*
Chronic	120.95 ± 83.05*	0.323 ± 0.058*	11.09 ± 7.19*	7.22 ± 2.34*

* $p<0.05$.

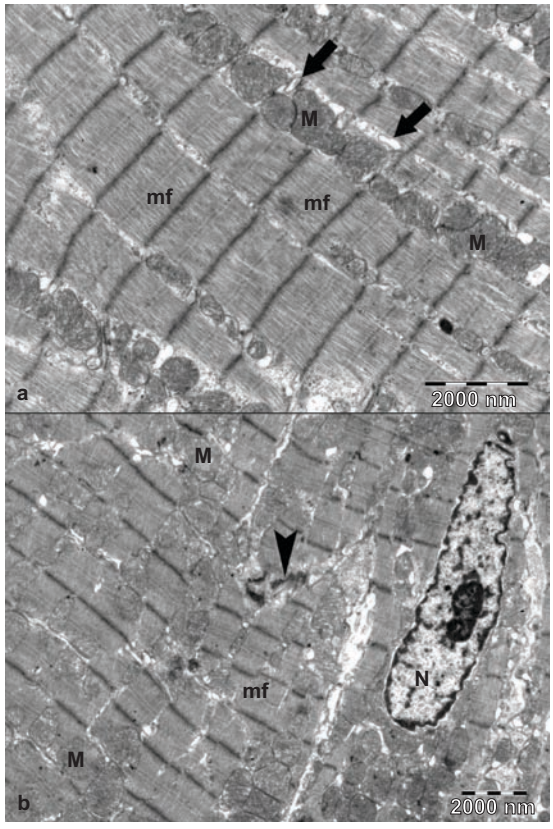


Figure 4. The heart muscle cells had normal morphological characteristics in electronmicrographs of the control group and the miyofibrils were observed to show a regular arrangement. Mitochondria (M), myofibril (mf), sarcoplasmic reticulum (arrow), nucleus (N), intercellular junction complex (discus intercalaris) (arrow head).

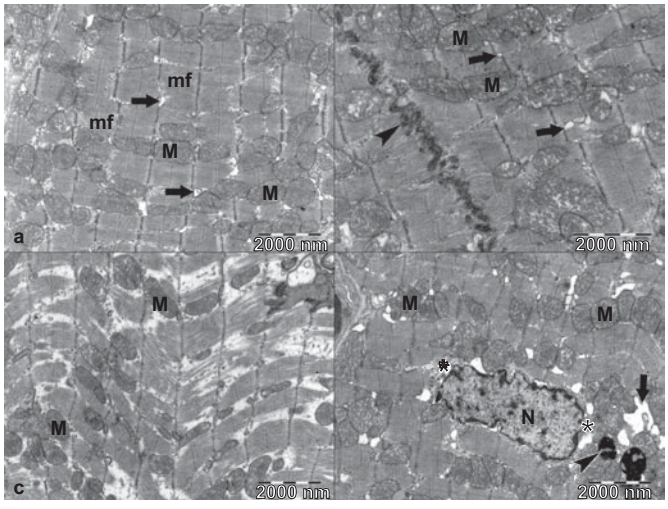


Figure 5. (A, B) Some heart muscle cells in the acute group observed to possess normal morfologic characteristics in the electronmicrograph. Mitochondria (M), myofibril (mf), sarcoplasmic reticulum (arrow), intercellular junction complex (discus intercalaris) (arrow head). (C, D) On the other hand, degeneration in miyofibrils, expansions in sarcoplasmic reticulum cisternaes (arrow) and perinuclear cisternaes (asterisk) were observed in some heart muscle cells of the acute group. Mitochondria (M), lipofuscin granule (arrow head), nucleus (N).

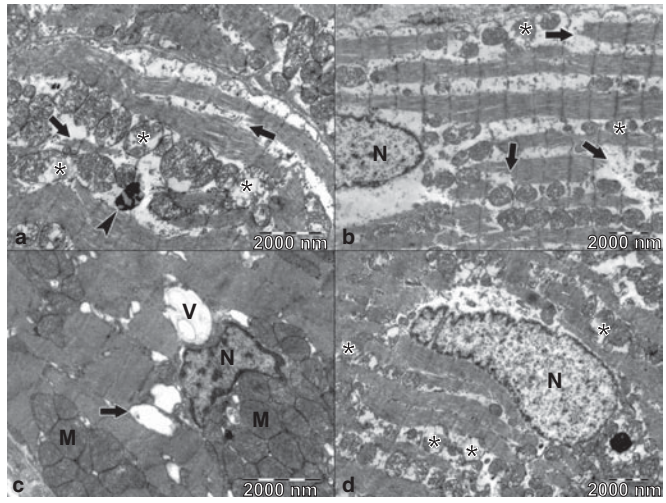


Figure 6. (A, B) Degeneration in miyofibrils (arrow) and degenerative alteration in mitochondria (asterisk) were followed in heart muscle cells of the chronic group in the electronmicrograph. Nucleus (N), lipofuscin granule (arrow head). (C, D) Expansions in sarcoplasmic reticulum cisternaes (arrow) and intracellular vacuols (V) were observed. Nucleus (N), Mitochondria (M), degenerative alteration in mitochondria (asterisk).

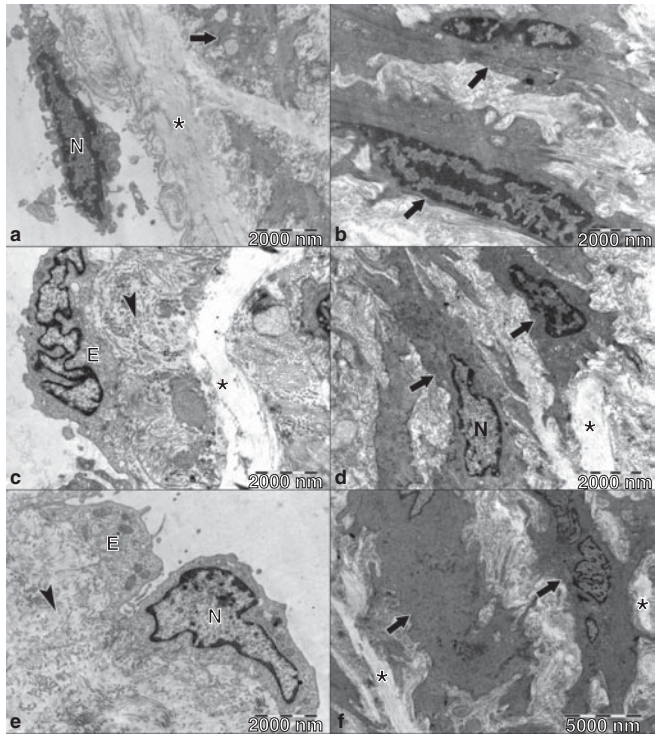


Figure 7. (A, B) Control group, (C, D) acute group, (E, F) chronic group. Endothelial cells and smooth muscle cells in the aorta tissue samples had normal morphological features in all groups in the electronmicrograph. Endothelial cell nucleus (N), elastic fibers (asterisk), smooth muscle cell (arrow), endothelial cell (E), connective tissue (arrow head).

(echocardiography) (De Maria and Raisinghani 2000; Parrott et al. 2004). The results obtained by TEB and invasive and non-invasive methods were determined to be close. There have been several experimental studies comparing cardiac

output values obtained by bioimpedance with other methodologies. Spinale et al. (1990) performed simultaneous measurements of cardiac output in anesthetized pigs during positive inotropic stimulation and preload reduction using bioimpedance and thermodilution or echocardiography, yielding correlation coefficients of 0.92 and 0.81, respectively. This group has also reported studies on anesthetized dogs using calcium chloride infusion to alter hemodynamics, demonstrating a correlation of 0.92 between bioimpedance (TEB) and thermodilution (Spinale et al. 1988). Tremper et al. (1986) compared bioimpedance and thermodilution measurements on anesthetized dogs for cardiac outputs ranging from 1–6 l/min, reporting a correlation coefficient of 0.84.

Thermodilution and echocardiography are not applicable for use together with bioimpedance in the same time on such small animals like rats. Moreover, because the cardiac output measurements obtained by using bioimpedance were favorable when compared with the values obtained by other methods such as thermodilution (Tremper et al. 1986; Spinale et al. 1988; 1990) and echocardiography (Spinale et al. 1990), in this study, we were unable to perform simultaneous measurements using these techniques along with bioimpedance.

In this study, when the first measurements of hemodynamic parameters of all groups were compared, there was no statistically significant difference between the parameters in all the groups ($p > 0.05$). However, when compared to the first measurements, in the second measurements, there were statistically significant differences in W, Z_0, VET, dZ_{max}/dt, SV, and CO parameters in the chronic group and only in the W parameter in the acute group (Tables 1 and 2). Hence, it can be stated that Cd^{+2} intoxication caused the differences between the results of the first and the second measurements in the acute and chronic groups.

In our study, Cd^{+2} caused loss of weight in the living rats in the chronic group. In another study, each rat was given 15 mg Cd^{+2}/kg/day for 30 days and it was found that even this Cd^{+2} exposed period caused loss of weight in the rats (Zikic et al. 1998).

Slama et al. (2003) determined CO values of the rats to be 73 ± 4 ml/min by echocardiography Doppler technique and 69 ± 4 ml/min by thermodilution. In another study on rats, CO values were determined to be 67.4 ± 15.7 ml/min using the Fick method (Ledingham and Lees 1981). In our study, TEB was used and CO values were determined to be 57.44 ±± 6.57 ml/min (mean ± SD of the first measurements of all the three groups in 38 rats) (Table 1). So, it can be seen that the CO results in our study are similar to the CO values of the rats in the other studies given above. VEPT, Z_0, dZ_{max}/dt, and VET values are rarely used in clinical evaluations. VEPT, Z_0, dZ_{max}/dt, and VET values of the rats could not be found in the literature. In our study, the values of VEPT were 23.57 ± 6.64 ml; the values of Z_0 were 143.95 ± 12.20 Ω; the values of dZ_{max}/dt were 10.07 ± 5.02 Ω/s; the values of VET were 0.080 ± 0. 005 s (mean ± SD of the first measurements of all three groups in 38 rats).

In our study, in the chronic group rats, higher values for SV that was calculated using Z_0, VEPT, VET, and dZ_{max}/dt parameters and CO that was calculated using HR and SV parameters in second measurement compared to the first measurement correlated with cadmium intoxication (Tables 1 and 2, Figures 1 and 2). However, HR was not modified. As seen in Table 1, in the chronic group rats, the mean SV of the first and the second measurements are 0.14 ± 0.02 ml/beat and 0.21 ± 0.03 ml/beat, respectively; the mean CO of the first and the second measurements are 55.25 ± 6.77 ml/min and 84.02 ± 13.16 ml/min, respectively, and the mean HR of the first and the second measurements are 410.43 ± 19.45 beats/min and 395.86 ± 20.69 beats/min, respectively.

In another study that Boscolo and Carmignani (1986) reported, 20 μg/ml of cadmium was added to the drinking water of the male rabbits for 9 months and HR values of the rabbits were determined by a Beckman cardiotachometer coupler triggered by the R-peak of the lead II electrocardiogram and SV was obtained by integrating the pulsatile aortic blood flow using a Biotronex apparatus (Boscolo and Carmignani 1986). Then, CO was calculated using the equation $SV \times HR \times 10^{-3}$ ml/min. The values of HR, SV, and CO in the control group reported 163 ± 6 beats/min, 0.98 ± 0.076 ml/beat, and 157 ± 11 ml/min, respectively. Boscolo and Carmignani (1986) stated that there was no statistically significant difference in HR values of Cd^{+2} given group compared to control group. In our study, 15 mg Cd^{+2}/kg/day was given to each rat by oral gavages for 60 days and there was no statistically significant difference in HR values in the first and second measurements. Although the rats in our study were exposed to Cd^{+2} for a shorter period (9 months/2 months), HR results in our study are similar to the results of the study given above. So, it can be claimed that Cd^{+2} is not effective on HR. But there are also studies that state that Cd^{+2} increased (Puri 1997) or decreased HR (Carmignani and Boscolo 1984).

Cardiac output is one of the most important hemodynamic parameters in the cardiovascular system. Blood pressure is related with CO and peripheral vascular resistance. So, change in CO can affect blood pressure. In the same way, CO is affected by HR and SV. So, any change in one of these parameters causes a change in blood pressure. The increase in intravascular volume and sympathetic activity cause the amount of the blood to increase by increasing CO. In order to control blood pressure, peripheral vascular resistance is tried to be decreased by baroreflex mechanism. However, hypertension is developed by any deterioration in this mechanism (Zungur and Yıldız 2004). There is no specific reason that causes hypertension. However, increased CO causes hypertension (Zungur and Yıldız 2004). In this study, because of increase in the value of CO in the chronic group (cadmium intoxication), hypertension related to Cd^{+2} can be said to develop in the rats in this group. However, it is not possible to interpret on this subject definitely, as blood pressure and peripheral vascular resistance values were not measured in this study.

In our study, there was no change in HR, but there was an increase in SV at the end of a long-term-Cd^{+2} exposure and this increased CO values of the rats in the chronic group when compared to the control and the acute groups ($p < 0.05$). The acute group was exposed to 15 mg/kg/rat of cadmium in a single dose. On the other hand, the chronic group was exposed to 15 mg/kg/rat of cadmium for 60 days. This means that the difference between these two groups can be related to the period of Cd^{+2} exposures. A study carried out by Nishiyama et al. (1990) suggested that the rats were given 50 μg/g cadmium for 45 consecutive days and no difference in CO values was determined when compared to the control group, but an increase in blood pressure was determined because of Cd^{+2} accumulation in the aorta. Our biochemical results showed that there was an accumulation of Cd^{+2} in the aorta of the chronic group. Hence, blood pressure may have increased in our study. In our study, each rat was given 15 mg/kg (15 μg/g), which was one third of the dose given by Nishiyama et al. (1990). However, the exposure period in our study was 15 days longer than Nishiyama et al.'s. The difference between the results of these two studies may be related to the period and the Cd^{+2} dose given.

The mechanisms by which cadmium may affect cardiovascular regulation are controversial. In this study, it was determined that long-term Cd^{+2} exposure caused an increase in SV and so CO. This result may have been related to the increase in contractions in heart muscle by the myogenic effect of Cd^{+2}. A lot of research has been carried out to determine the effects of Cd^{+2} on contraction mechanism of smooth and skeletal muscles. The studies on aorta reported that cadmium-accumulation in aortas increased vasocontractility (Sakurada and Wakabayashi 1999), but the studies on myocardium reported that Cd^{+2} decreased the contractions (Asqrimsson et al. 1995). It was determined that the contractions in the mouse corpus cavernosum decreased by the neurogenic effect of Cd^{+2} (Göçmen et al. 2000). Also, in another study, it was reported that the contractions in the rat detrusor muscle decreased by the myogenic effect of Cd^{+2} (Bayazit et al. 2002).

Studies on the phrenic nerve-diaphragm preparation of the mouse showed that Cd^{+2} increased the contractions (Fu and Lin-Shiau 1985). By the use of conventional microelectrodes, it was found that Cd^{+2} not only depolarized the muscle membrane, but also induced spontaneous action potentials at a high frequency (Fu and Lin-Shiau 1985). It was reported that increased Na^{+} permeability of the muscle membrane was the essential step bringing about spontaneous contractions. The binding of Cd^{+2} to thiol groups of the membrane was closely related to the induction of these effects (Fu and Lin-Shiau 1985).

It is widely accepted that the activation of cardiac contraction is the result of Ca^{+2}-induced Ca^{+2} release from the sarcoplasmic reticulum and that the source of the activator Ca^{+2} is entry via voltage gated L-type Ca^{+2} current (I_{Ca}) (Wasserstrom and Vites 1999). In a study about the effects of Cd^{+2} on canine cardiac sarcolemmal vesicles, it was found that Cd^{+2} stimulated Ca^{+2} efflux, possibly via divalent cation-Ca^{+2} exchange (Trosper and Philipson 1983). In another study, the effect of Cd^{+2} on the $Ca^{(2+)}$-ATPase activity of sarcoplasmic reticulum from rabbit muscle was studied and, as a result, ATP hydrolysis was found to be inhibited by Cd^{+2} (Hechtenberg and Beyersmann 1991). Also, in another study, it was reported that Cd^{+2} increased intracellular Ca^{+2} in muscle by preventing the inositol 1,4,5-trisphosphate ($InsP_3$) hydrolysis (Vergara et al. 1985). It was known that exogenous $InsP_3$ releases calcium from skinned muscle fibers at relatively high doses under normal conditions (Vergara et al. 1985). Hence, even if Cd^{+2}, a voltage gated L-type Ca^{+2} channel antagonist, blocks Ca^{+2} influx, it has been reported that Cd^{+2} causes contractions to develop or increase by increasing the amount of intracellular Ca^{+2} by the other mechanisms indicated above. The mechanisms which play a role in the increase of carbachol-induced responses of isolated mouse esophageal striated muscle by cadmium was investigated by Kıroglu (2006). The results show that cadmium may increase the carbachol contractions by a myogenic effect or by interacting with intracellular and extracellular calcium concentration or by inhibition of sarcoplasmic reticulum calcium ATPase and increasing intracellular calcium concentration or by bounding to thiol groups or reducing the thiol reservoir and by functioning like calcium and binding to calmoduline.

Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids and altering the antioxidant system of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles. In our study, Cd^{+2} dose exposed to rats can be claimed to have induced lipid peroxidation. The antioxidant enzymes and other antioxidants provide protection to the cells against oxidative damage. In our study, when SOD activity, which developed against this damage as an enzymatic defense system, was observed, it was found that SOD activity in the chronic group significantly increased ($p < 0.05$) compared to the control group. Also, SOD activity was seen to have increased in the acute group when compared to the control group. However, this increase was not statistically significant ($p > 0.05$). In this study, MDA levels (an index of lipid peroxidation) and CAT activity, which changes H_2O_2 molecules into water and molecular O_2, were found to have increased both in the acute and chronic groups when compared to the control group ($p < 0.05$). Sarkar et al. (1995) reported that after the rats were injected with 0.4 mg/kg body wt Cd^{+2}, there was a significant increase in SOD activity on heart within 24 h of Cd^{+2} intoxication when compared to the control group.

It is important for an organism to have GSHPx and this enzyme activity can show a deviation from normal values related to changes in physiology and various diseases. Findings obtained in this study indicated that an increase in the GSHPx activity in the chronic group when compared to the control was statistically significant ($p < 0.05$). Yin et al. (1991) suggested that rats were exposed to 5 mg/kg/day Cd^{+2} for 4 weeks and GSHPx activity in the experimental group was found to have increased significantly when compared

to the control group. However, Stajn et al. (1997) reported in the Cd^{+2} given groups that GSHPx was significantly reduced when compared to the control.

It was shown that exposure to Cd^{+2} induced a significant decrease ($p < 0.05$) in SOD and CAT activities in red blood cells of rats (Jemai et al. 2007). However, Cd^{+2} had no effect on glutathione peroxidase (GSH-Px) activity.

In another study on rats, Koyu et al. (2006) showed that MDA levels were increased and SOD and CAT activities were decreased in cadmium-exposed group compared to control group, so these findings suggest the role of oxidative mechanisms in cadmium-induced tissue damage.

Sarkar et al. (1998) studied in rat erythrocytes that cadmium induced lipid peroxidation (LPO) and the activity of antioxidant enzymes after the administration of a single dose of $CdCl_2$ (0.4 mg/kg body wt, i.p.). As a result, Cd^{+2} intoxication increased erythrocyte LPO along with a decrease in superoxide dismutase (SOD) up to 3 days of Cd^{+2} treatment. The decrease in erythrocyte catalase (CAT) activity was marked within 9 h of Cd^{+2} intoxication. After 3 days of Cd^{+2} treatment, LPO decreased towards normal, along with an increase in erythrocyte SOD and CAT activity (Sarkar et al. 1998).

As a result, in our study, long-term Cd^{+2} exposure was found to increase in SOD enzyme activity by increasing lipid peroxidation to induce SOD, which is responsible for providing the dismutation of superoxide radical to hydrogen peroxide and oxygen, and accelerate the transformation of superoxide radical into hydrogen peroxide. Hydrogen peroxide is a combination of reactive oxygen having a toxic effect on cells. Hydrogen peroxide is removed from the ambient by catalase and glutathione peroxidase. In the same experimental group (chronic group), the increase in CAT, providing the decomposition hydrogen peroxide into water and oxygen, and glutathione peroxidase activity suggested the accumulation of hydrogen peroxide in cells. Hence, Cd^{+2} exposed to rats can be claimed to have induced lipid peroxidation in cells. Also, the findings have been supported by the histopathologic evidence.

In our study, by observing heart tissue under an electron microscope, it was determined that heart muscle cells of the control group had normal morphological characteristics. On the other hand in the acute group, while some heart muscle cells had normal morphological characteristics, some cells were determined to have degenerative changes. The degenerative changes, which were observed in some heart cells of the acute group, were found to have increased in rats exposed to Cd^{+2} for 60 days. In this group, blood vessels in the connective tissue among heart muscle cells still had their normal morphological structures. The histopathological findings obtained in this study are in accordance with the biochemical findings. This means Cd^{+2} accumulated in the heart tissues of the rats both in the acute and chronic groups (Table 3), and caused damage in the heart cells.

The electron microscopic findings of aorta tissues in the control and acute groups suggested that smooth muscle cells on aorta tissue wall had normal morphological characteristics. In fact, the biochemical findings of these groups showed that there was no Cd^{+2} accumulation in aorta tissue (Table 3). In the chronic group, aorta cells were observed to have normal morphological characteristics on electron microscopy, but the biochemical results of that group showed that there was Cd^{+2} accumulation in aorta tissue (Table 3).

In another study on rats, the harmful effects of Cd^{+2} on the heart were investigated (Jamall et al. 1989). Male rats were fed on an adequate-copper low-selenium feed supplemented with 50 ppm dietary cadmium for 7 weeks. When the Cd^{+2} group was compared to the control group, the Cd^{+2} group was determined to have a significant increase in heart Cd^{+2} level. Also, cadmium treatment resulted in histopathological lesions in rat heart tissues. In another study, it has been reported that MDA levels were increased ($p < 0.001$) in cardiac tissue and developed more histopathological lesions in the Cd^{+2} group than those of the control group (Mollaoglu et al. 2006). In our study, both in the acute and chronic groups, there was a significant increase in MDA when compared to the control group. The reason of the histopathological effects on heart tissue can be the increase in MDA. However, in aorta tissue, although the MDA value was increased, no histopathological effects related to Cd^{+2} were observed.

In conclusion, all of our findings indicate that Cd^{+2} toxication can cause deformation in heart muscle cells due to an increase in free radicals and lipid peroxidation. Also, we have confirmed that a long-term-Cd^{+2} exposure increased SV and CO, but did not change the HR.

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