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The beneficial effects of ivabradine against myocardial damage induced by isoproterenol in rats

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Abstract

We aimed to investigate the potential benefits of two doses of ivabradine (IVA)-an If sodium channel blocker against isoproterenol (ISO)-induced myocardial damage in rats.The rats were randomly divided into 4 groups: Control group (n=8); Saline was administered, ISO group (n=12); 150 mg/kg ISO was administered for 2 days, ISO+IVA (1 mg/kg) group (n=12); 1 mg/kg IVA was administered for 4 days in addition to ISO. ISO+IVA (10 mg/kg) group (n=12): 10 mg/kg IVA was administered for 4 days in addition to ISO. Thereafter, hemodynamic, histopathological, and biochemical studies were performed. In the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups, ISO-induced myocardial changes including an increase in density of granulation tissue and degenerated cardiomyocyte were equally decreased. HR was mildly reduced, and arterial blood pressures were slightly increased in the IVA-treated groups versus the ISO group. In the hearts of IVA-treated groups, malondialdehyde (MDA) levels were significantly reduced and glutathione (GSH) level and catalase (CAT) activity were mildly increased compared to the ISO group. Elevation of GSH and CAT activity were more pronounced in the ISO+IVA (10 mg/kg) group. Our results indicate that both 1 mg/kg and 10 mg/kg doses of IVA are effective against heart damage induced by ISO via its negative chronotropic, anti-oxidant (dose-dependent), anti-inflammatory and anti-degenerative properties.

Keywords: Anti-degenerative, anti-inflammatory; anti-oxidant, isoproterenol, ivabradine, myocardial damage

Introduction

Heart failure (HF), which is common worldwide, is still the leading death reason and causes hospitalization [1]. The prevalence of HF is about 2% in adults and about 10% among the elderly [2]. In this context, it is essential to research for pharmacological agents for HF treatment.

Isoproterenol (ISO) is a nonselective β receptor agonist. It shows positive inotropic and chronotropic effects; therefore it causes an ischemia-like condition. Additionally, ISO-induced changes including impaired antioxidant defense, lipid peroxidation, myocyte damage, and contractile dysfunction play important roles in the development of acute myocardial infarction (MI). Because the pathophysiological changes caused by ISO are close to human MI, pharmacological agents that reduce ISO damage have the potential to be beneficial in the treatment of acute MI [3].

IVA, an *If* sodium channel blocker, is a Food and Drug

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Administration (FDA)-approved drug indicated in the treatment of stable and symptomatic chronic HF patients under the following conditions: left ventricular ejection fraction (LVEF) ≤35%, sinus rhythm, resting HR≥70 beats per minute (bpm), and use of beta-blockers (BBs) at maximally tolerated doses or contraindication to BBs. It reduces heart rate (HR) without significantly changing other hemodynamics and has advantages of not affecting the gastrointestinal and bronchial muscles [4,5]. Additionally, IVA has been reported to preserve coronary reserve by reducing HR and it is stated that this is associated with decrease in perivascular collagen in infarcted rats [6]. Also, HR reduction by IVA has been shown to ameliorate diastolic dysfunction and heart fibrosis in rabbits [7]. Simko et al. [8] reported that IVA (10 mg/kg/day) reduces HR and alleviates left ventricle (LV) remodelling and systolic arterial pressure (SAP) decrease, increases survival rate and prolongs average survival time in rats with ISO-induced cardiac damage. In addition, oxidative stress is a pathogenetic factor for cardiac damage [8]. In this regard, IVA has been reported to exert antioxidant effects on mice aortic wall [9]. Although IVA has been previously reported to reduce ISO-induced myocardial injury [8], whether this amelioration is due to IVA's antioxidant properties, histopathological effects of IVA on the hearts of ISO-treated subjects and 1 mg/kg dose of IVA have not been evaluated. We used 1 mg/kg/d and 10 mg/kg/d doses of IVA in this study to investigate whether its potential cardioprotective effects is dose-dependent.

In this context, we sought to determine the potential benefits of IVA treatment at the two doses in the heart damage caused by ISO. To reveal the mechanisms of these impacts of IVA, the study was enriched with biochemical, histopathological, and hemodynamic studies.

Material and Methods

Ethics approval

This study was conducted under the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines [10]. The ethical approval of Inonu University Local Ethics Committee for Animal Experiments (protocol number: 2019/A-22) was obtained.

Animals

We used a total of 44 five-month-old *Wistar albino* male rats (288-547 g) produced in Laboratory Animal Production and Research Center of Inonu University. The housing and feeding conditions of the rats were as follows: plastic cages (50x35x20 cm; 4 animals per cage) and *ad libitum* feeding with standard rat chow and fresh tap water, 12-hour light/dark cycles, controlled room temperature (21±2°C) and relative humidness (60±5%).

Experimental design

The simple and random grouping of the rats was done in this way:

1. Control group (n=8): Animals were treated with saline.
2. ISO group (n=12): The rats were administered intraperitoneally (ip) with 150 mg/kg/d ISO for 2 consecutive days.
3. ISO+IVA (1 mg/kg) group (n=12): The rats were administered ip with 150 mg/kg/d ISO for 2 consecutive days. Starting simultaneously with the first ISO injection, 1 mg/kg/d IVA was administered perorally (po) to the rats for 4 consecutive days.
4. ISO+IVA (10 mg/kg) group (n=12): The rats were administered ip with 150 mg/kg/d ISO for 2 consecutive days. Starting simultaneously with the first ISO injection, 10 mg/kg/d IVA was administered po to the rats for 4 consecutive days.

The dosages of ISO (CAS: 7683-59-2, Sigma Chemical Co., St Louis, MO) and IVA (Coralan®, Servier Corp, Istanbul, TÜRKİYE) were selected as reported earlier [8,11,12].

Hemodynamic assessment

On the 5th day of the study, the experiment was terminated and the animals were weighed and anesthetized. Then, the SAP and diastolic arterial pressure (DAP) were determined invasively with a cannula inserted into the carotid artery. The Biopac MP-100 Data Acquisition system (Biopac Systems, Inc., Santa Barbara, CA, USA) was utilized to record SAP, DAP and HR. Calculation of the mean arterial pressure (MAP) was done in this way: 2/3DAP+1/3SAP.

After the hemodynamic assessment, blood samples were collected, centrifuged for obtaining serum. Then, hearts were excised, and heart weight (HW) was detected. HW/Body weight (BW), an indicator of cardiac remodeling [13], was calculated. After the heart tissues were removed and weighed, they were symmetrically divided into two sections. One of the sections was stored for biochemical analysis, while the other part was held in 10% formaldehyde to evaluate histopathologically. The serum and heart samples were kept at -80°C till analyzed.

Biochemical analyses of heart

Malondialdehyde (MDA) levels were studied as defined before [14]. Concisely, 10% homogenate that formed from the heart tissue was used in the analysis. After vortexing and warming the test tubes with appropriate solutions, the samples were centrifuged. Determination of the MDA concentrations were performed according to the absorbances of the samples at 535 nm and 520 nm in the spectrophotometer. The consentrations were achieved by standart graph and represented as nmol/g wet tissue.

Glutathione (GSH) levels were assayed as previously reported [15]. Briefly, the supernatant obtained from the centrifugation of the 10% homogenate of the heart tissue was used to prepare the sample for analysis. After vortexing the test tubes with appropriate solutions, the color intensity was read at 410 nm in the spectrophotometer. The outcomes were achieved by standart chart and represented as nmol/g wet tissue.

Superoxide dismutase (SOD) activity was detected based on a previously determined method [16]. Briefly, the supernatant obtained from the centrifugation of the 10% homogenate of the heart tissue was used to prepare the sample for analysis. After adding the chloroform/ethanol mixture and centrifugation, the top

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clear white chloroform phase was used for CuZn-SOD analysis. After incubation, $CuCl_2$ was added to tubes and the reaction was stopped. The absorbance of the blank and the samples was recorded at 560 nm and the enzyme activity was determined. The results was presented as U/g protein.

Catalase (CAT) activity was determined as earlier reported [17]. Briefly, the supernatant obtained from the centrifugation of the 10% homogenate of the heart tissue was used for analysis. After adding the supernatant to tubes, absorbance was read at 240 nm. After following the absorbance reduction for 90 seconds by reading every 15 seconds, the absorbance value was recorded. The time interval of the decrease in linear absorbance was detected. The results was expressed as K/g protein.

Biochemical analyses of serum

The serums at -80°C were used for biochemical analysis after thawing at +4°C. Afterwards, the measurement of serum levels of renal function parameters (blood urea nitrogen [BUN] and creatinine [Cr]) and a cardiac enzyme (creatine kinase-MB [CK-MB]) were conducted at The Central Laboratory (Abbott Architect c16000) of Turgut Ozal Medical Center in the Inonu University.

Histopathological analysis

The heart tissues were fixed in 10% formaldehyde and embedded in paraffin. After tissue processing, 4-5 µm-thick sections were mounted on slides, and stained with hematoxylin and eosin (H&E).

Myocardial tissues were evaluated by an investigator blinded to the experimental groups for cardiomyocyte degeneration, interstitial edema and granulation tissue (myofibril loss, swelling, vacuolization, dense eosinophilic cytoplasm, pycnotic nucleus). The randomly selected ten areas were examined and the areas were scored as 0: no change, 1: mild, 2: moderate, 3: severe change, according to the degree of histological changes. For analysis, Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) and Leica DFC-280 research microscope were utilized.

Statistical analysis

The necessary power and sample size was determined by an available software [18]. The minimum sample size was revealed as at least 6 in each group (18 in all), taking into account type I error (alfa)=0.05, power (1-beta)=0.8, and effect size=0.82 for HR.

Considering histopatological data, it was found that measurable variables in all groups did not show normal distribution according to normality tests. Therefore, Kruskal Wallis analysis was performed using a KruskalWallis software [19]. For intergroup comparison, Mann-Whitney-U test was used. $p<0.05$ was accepted as significant. Data were showed as median (minimum-maximum).

For other data, statistical analysis was performed using the Kruskal-Wallis H test [19]. The data were indicated as median (min-max). For multiple comparisons, Conover test was used. $p<0.05$ was expressed as significant.

Results

BW, HW and HW/BW

All groups were similar regarding the BW, HW, and HW/BW values (Table 1).

Hemodynamics

HR was found to be mildly reduced in the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups versus the ISO group. SAP, MAP, and DAP were mildly decreased in the ISO group contrary to the Control group. Besides, arterial blood pressures were mildly increased in the IVA-treated groups versus the ISO group (Table 2).

Table 1. Summary of BW, HW, and HW/BW values

Parameters	Groups				p-value
	Control	ISO	ISO+IVA (1 mg/kg)	ISO+IVA (10 mg/kg)	
BW(pretest) (g)	367.5 (288-500)	417 (360-502)	413 (372-476)	408 (334-547)	0.27
BW(posttest) (g)	370 (288-509)	436.5 (346-498)	388.1 (357.5-480.5)	386.5 (325-468.5)	0.39
HW(g)	1.665 (1.19-2.22)	2.035 (1.69-2.18)	1.81 (1.41-2.24)	2.115 (1.33-2.67)	0.08
HW/BW(posttest)	0.004 (0.004-0.005)	0.004 (0.004-0.006)	0.005 (0.003-0.006)	0.005 (0.004-0.008)	0.56

BW: body weight, HW: heart weight, ISO: isoproterenol, IVA: ivabradine

Table 2. The effect of the IVA treatment on hemodynamics

Parameters	Groups				p-value
	Control	ISO	ISO+IVA (1 mg/kg)	ISO+IVA (10 mg/kg)	
HR (beats/min)	368.5 (269-415)	345 (264-484)	296 (99-396)	302 (87-435)	0.11
SAP (mmHg)	114.76 (97.27-136.11)	102,35 (87.6-141.87)	109.27 (71.12-142.25)	121.29 (67.4-144.71)	0.39
DAP (mmHg)	105.15 (84.52-132.82)	98,57 (75.34-180.98)	105.94 (69.22-138.24)	101.11 (65.59-137.73)	0.97
MAP (mmHg)	107.74 (88.77-133.92)	96.58 (0-155.2)	107.05 (69.85-139.58)	104.18 (66.19-138.37)	0.79

HR: heart rate, SAP: systolic arterial pressure, DAP: diastolic arterial pressure, MAP: mean arterial pressure, ISO: isoproterenol, IVA: ivabradine

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Biochemical findings

Lipid peroxidation and antioxidant parameters of heart is available in Table 3.

MDA was significantly lower in the ISO group versus the Control group. Also, MDA was significantly lower in the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups compared to the ISO group. In addition, GSH was slightly reduced in the ISO group versus the Control group. In addition, GSH was slightly increased in the ISO+IVA (1 mg/kg) group and significantly elevated in the ISO+IVA (10 mg/kg) group contrary to the ISO group. SOD was slightly reduced in the ISO group and markedly declined in the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups contrary to the Control group. Also, SOD was significantly lower in the ISO+IVA (10 mg/kg) group contrary to the ISO group. CAT was sligthly reduced in the ISO group versus the Control group. Besides, CAT was mildly elevated in the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups contrary to the ISO group (Table 3). The elevation in the ISO+IVA (10 mg/kg) group was greater than that in the ISO+IVA (1 mg/kg) group.

All groups were similar regarding the BUN, Cr and CK-MB serum levels (Table 4).

Table 3. Comparison of heart lipid peroxidation and antioxidant contents between the groups

Parameters	Groups				p-value
	Control	ISO	ISO+IVA (1 mg/kg)	ISO+IVA (10 mg/kg)	
MDA (nmol/g wet tissue)	106.93 (98.6-179.52)	99.28[a] (74.46-137.7)	75.14[a,b](65.28-80.58)	77.52[a,b] (70.04-88.4)	<0.001
GSH (nmol/g wet tissue)	442.61 (341.88-484.33)	386.65 (319.5-604.39)	463.98 (421.25-512.82)	506.72[c,d] (354.09-588.11)	0.035
SOD activity (U/g protein)	1001.03 (866.35-1081.39)	868.51 (801.01-1251.56)	841.17[a] (751.8-997.5)	734.71[a,b] (627.49-910.64)	0.006
CAT activity (k/g protein)	2000.02 (966.3-2593.28)	1914.68 (640.04-2717.88)	2011.88 (1655.13-3094.41)	2378.26 (829.72-3280.06)	0.445

[a] marked decline (p<0.05) vs control group; [b] marked decline (p<0.05) vs ISO group, [c] marked rise (p<0.05) vs ISO group; [d] Marked rise (p<0.05) vs control group, MDA: malondialdehyde, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, ISO: isoproterenol, IVA: ivabradine

Table 4. Comparison of BUN, Cr and CK-MB serum levels among the groups

Parameters	Groups				p-value
	Control	ISO	ISO+IVA (1 mg/kg)	ISO+IVA (10 mg/kg)	
BUN (mg/dL)	22.5 (16.37-29.6)	25.74 (17.27-32.37)	27.58 (21.43-30.06)	24.68 (19.93-26.63)	0.17
Cr (mg/dL)	0.8 (0.65-1.17)	0.77 (0.48-1.07)	0.81 (0.71-1.13)	0.78 (0.65-0.91)	0.83
CK-MB (U/L)	1000 (975.8-1000)	1000 (1000-6613.6)	1000 (1000-1000)	1000 (1000-1000)	0.23

BUN: blood urea nitrogen, Cr: creatinine, CK-MB: creatine kinase-MB, ISO: isoproterenol, IVA: ivabradine

Histopathological findings

Myocardial histopathology was evaluated. The Control group had a normal view of the myocardium except minimal changes (Figure 1A). Besides, significant impairment was observed in the myocardium of ISO group. Among these changes, the most striking was the granulation tissue (Figure 1B). In some sections of ISO group, normal myocardial tissue left its place to granulation tissue. Other significant changes observed in the ISO group were increased intensity of cardiomyocyte degeneration and interstitial edema (Figure 1B) (Table 5).

The histopathological data are summarized in Table 5. Briefly, in the ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups, interstitial edema continued similarly to the ISO group, while the density of granulation tissue and degenerated cardiomyocyte significantly decreased versus the ISO group (Figure 1C and 1D) (Table 5). Besides, ISO+IVA (1 mg/kg) and ISO+IVA (10 mg/kg) groups were found to be similar in the all parameters.

Table 5. Comparison of myocardium in terms of histopathological score

Groups	Granulation tissue	Cardiomyocyte degeneration	Interstitial edema
Control	0.0 (0.0-0.0)	0.0 (0.0-1.0)	0.0 (0.0-0.0)
ISO	1.0 (0.0-3.0)[a]	1.0 (0.0-3.0)[a]	0.0 (0.0-2.0)[a]
ISO+IVA (1 mg/kg)	0.0 (0.0-3.0)[b]	1.0 (0.0-3.0)[b]	0.0 (0.0-2.0)
ISO+IVA (10 mg/kg)	0.0 (0.0-3.0)[b]	1.0 (0.0-3.0)[b]	0.0 (0.0-2.0)

[a] marked rise (p<0.0001) vs control group, [b] marked drop (p<0.05) vs ISO group, ISO: isoproterenol, IVA: ivabradine

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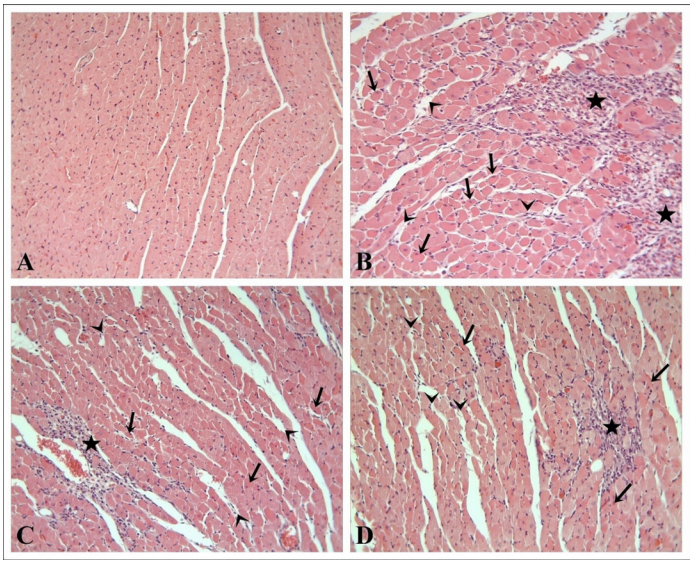

Figure 1. The histopathological effects of IVA on myocardium of the rats, **1A.** Control group: The normal histological appearance of the myocardium. **1B.** ISO group: Appearance of dense granulation tissue (stars), degenerated cardiomyocytes (arrows) and interstitial edema (arrowheads). ISO+IVA (1 mg/kg) **1C.** and ISO+IVA (10 mg/kg) **1D.** groups: Granulation tissue (star) and degenerated cardiomyocytes (arrows) declined markedly contrary to the ISO group ($p<0.05$). Interstitial edema (arrowheads) is still present. (H&E X 20)

Discussion

We intended to research the impacts of 1 mg/kg and 10 mg/kg oral IVA in myocardial damage caused by ISO in rats. ISO is one of the most used agents to induce myocardial damage [11]. Myocardial damage caused by ISO occurs due to many pathogenetic factors including ischemia, hypoxia, increased energy consumption, oxidative stress, calcium overload and metabolic changes [8,20]. Previous studies reported histopathological changes in myocardium related ISO administration. Ardjmand et al. [21] reported ISO-induced myocyte necrosis, edema and inflammatory cell infiltration in the myocardial tissue of rats. Concordantly, Ulutas et al. [22] showed that hearts of ISO-treated rats exerts degenerative changes including granulation tissue, cardiomyocyte degeneration and interstitial edema. Similarly, in our study, administration of ISO significantly led to granulation tissue, increased degenerated cardiomyocyte density and interstitial edema in the myocardium.

According to our current knowledge, the histopathological effects of IVA in ISO-induced cardiac damage have not yet been reported. In our study, we observed that IVA reduces the density of granulation tissue and degenerated cardiomyocytes in the hearts of ISO-treated rats. These findings adresses anti-inflammatory and anti-degenerative effects of IVA. Dai et al. [20] showed that IVA (po, 10 mg/kg/day) attenuates the cardiomyocyte necrosis and apoptosis through promoting the autophagy by inhibition of the PI3K/AKT/mTOR/p70S6K pathway in rats with MI. Also, Kara et al. [23] reported that IVA treatment alleviates the elevation of plasma p-selectin and vascular cell adhesion molecule-1 levels and reduction of the nitric oxide (NO) content in the aortas of diabetic rats. According to these reports, an explanation for our finding is that IVA may attenuated the heart damage characterized by myocardial degeneration and inflammation via autophagy stimulation and inhibition of the adhesion molecule expression. Our results should be confirmed by further analysis that reveal autophagy and inflammation.

High baseline HR is linked to cardiovascular death in HF patients. This situation is estimated to be due to impairment of contractility, energy deficiency, endothelial dysfunction and decreased myocardial energy supply [24]. Cardioprotective effects of IVA-a selective *If* current blocker [8]- provided by its HR-lowering effect were previously shown. In this regard, The SHIFT study (event-driven, multinational, randomised, double-blind, placebo-controlled, parallel-group clinical trial) demonstrated that IVA treatment reduces relative risk of the cardiovascular death or hospital admission for worsening HF by 18% compared to the placebo in patients with moderate-to-severe chronic HF and LV systolic dysfunction [25]. Experimentally, Dedkov et al. [6] reported that IVA-induced HR reduction preserved the coronary reserve via decreasing perivascular collagen in infarcted rats. Also, Busseuil et al. [7] determined that HR decline caused by IVA alleviated the diastolic dysfunction and heart fibrosis in rabbits. Similarly, in our study we observed that HR was mildly decreased in the IVA-administered groups. This effect of IVA was most likely due to its blockage on the *If* current.

ISO-dependent reduction in blood pressure has been demonstrated earlier. Maleki Dizaji et al. [26] observed that ISO causes a slight reduction in SAP, MAP, and DAP in rats. Similarly, in our study, ISO led to a mild decrease in SAP, MAP, and DAP. Concordantly, Goyal et al. [3] reported that ISO reduces the SAP, MAP, and DAP in male Wistar rats. In their study, ISO led to ventricular dysfunction evidenced by left ventricular end-diastolic pressure (LVEDP) increase, maximal positive and negative rate of developed left ventricular pressure (±LVdP/dtmax). Regarding the effects of IVA on blood pressure, Simko et al. [8] reported that IVA alleviates the SAP decrease caused by ISO. Similarly we observed that IVA mildly reverses the ISO-induced reduction of the SAP, MAP, and DAP. As mentioned before, Kara et al. [23] reported that IVA treatment alleviates the reduction of the NO content in the aortas of diabetic rats. Considering this, in our study, IVA may have ameliorated ISO-induced reduction of blood pressure by reducing peripheral vascular resistance (PVR) via elevation of vascular NO level. Another explanation is that IVA-induced HR reduction may decreased the heart's need for oxygen and increased coronary blood supply. These effects may ameliorated blood pressure by improving cardiac contractility. Our results can be confirmed by future studies involving further analyzes such as echocardiography and NO measurement.

It is well known that oxidative stress is an crucial pathogenetic element of ISO-induced myocardial damage [8]. MDA is one of the markers of lipid peroxidation caused by reactive oxygen species (ROS) [26]. SOD and CAT protect the cell against oxidative stress by scavenging free radicals [27]. Shaikh et al.

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[27] reported that ISO leads to an elevation in cardiac MDA level and a decrease in activity of cardiac SOD and CAT. Unlike, Kalkan et al. [11] and Zhang et al. [28] have reported that ISO does not change cardiac MDA levels. In our study, ISO caused a significant decrease in cardiac MDA level and a mild reduction in GSH, SOD and CAT levels. Concordantly, Maleki Dizaji et al. [26] reported that cardiac MDA level is mildly reduced by ISO despite histopathological evidences of cardiac damage including cardiomyocyte necrosis and disintegration. Also, Khatua et al. [29] showed that ISO causes mild reduction in cardiac MDA level despite a marked decrease in GSH level and activities of glutathione peroxidase and CAT in heart. Our findings, which are similar to those in these works, suggest that MDA may be decreased due to over activation of the antioxidant system in the early period of ISO treatment. Additionally, several studies [30-33] that show no change or even a decrease in MDA under oxidative stress circumstances support the reduction of ISO-induced MDA in our study. These differences between the studies may be related to the oxidative stress period at which the MDA value was analyzed. Clinical data also support these variations. Regarding MDA levels in individuals with diabetes, a clinical disease linked to oxidative stress, conflicting results, such as increase, no change, or decrease, have been described in the literature [34-36]. Our observations on MDA might also be explained by the possibility that ISO lowers MDA through an unidentified mechanism. Another possible explanation is that, not just MDA is a product of lipid peroxidation. There are other products of lipid peroxidation, such as propanal, hexanal, and 4-hydroxynonenal [37]. The levels of mentioned lipid peroxidation products can also be measured to clarify our finding regarding MDA. MDA is known to be enzymatically converted to its metabolites including malonic semialdehyde, acetaldehyde, acetate, acetyl CoA and ultimately CO2+H2O, respectively. In these processes, mitochondrial aldehyde dehydrogenase, decarboxylase, acetyl CoA synthase, and tricarboxylic acid cycle enzymes are involved [37]. The possible acceleration of MDA metabolism by ISO via an unidentified mechanism may explain our findings.

Based on our findings and the results of the works of Maleki Dizaji et al. [26] and Khatua et al. [29], the reduced activities of SOD and CAT in our study can be attributed to the fact that there is no longer a need for the activation of the antioxidant system in this late period in which MDA is found to decrease. An explanation for the reduction of GSH may be it's consumption during defense against oxidative stress.

Antioxidant properties of IVA have been reported earlier. In this context, Custodis et al. [9] observed that IVA reduced the NADPH oxidase activity, superoxide production, and lipid peroxidation in aortic wall of mice. Additionally, Li et al. [38] showed that IVA reduces the ROS production caused by low shear stress in endothelial cells. By this study, we determined that both 1 mg/kg and 10 mg/kg doses of IVA reduces the MDA level equally. This observation indicates lipid peroxidation-reducing effect of IVA. In addition, we observed that IVA leads to elevation of GSH level and CAT activity. Elevation due to 10 mg/kg dose of IVA was more pronounced. Our results suggest that cardioprotective effects of IVA are associated with its antioxidant properties that are more obvious at 10 mg/kg dose.

Conclusion

Consequently, in the present study, four-day IVA administration at both 1 mg/kg and 10 mg/kg doses alleviated the heart damage through its negative chronotropic, anti-oxidant (dose dependent), anti-inflammatory and anti-degenerative effects in ISO-treated rats. Nevertheless, further animal and human research is required to confirm our results regarding the beneficial impacts of IVA against myocardial damage.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
The ethical approval of Inonu University Local Ethics Committee for Animal Experiments (protocol number: 2019/A-22) was obtained.

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