ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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An Investigation of the Effects of Duloxetine on the Heart

Duloksetinin Kalp Üzerindeki Etkilerinin Araştırılması

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The histochemical and immunohistochemical results of K-43, TnT, TnI antibodies of this research were presented as a poster at the 14th National Histology and Embryology Congress, 10th-13rd May 2018, Antalya, Turkey.

ABSTRACT Objevtive: The present study was designed to examine the effects of high dose and low dose duloxetine on Cx43, a gap junction (GJ) protein; on S100A, cardiac contractility component and on certain early cardiac impairment parameters as TnI and TnT. **Material and Methods:** The study was performed with 24 male Wistar Albino rats by generating control group (1 ml solvent), low-dose duloxetine (LDD) group (10 mg/kg) and high-dose duloxetine (HDD) group (100 mg/kg). After the experimental procedure, the results concerning blood pressure and heart rates of the anesthetized rats were recorded and HE staining and immunostaining of Cx43, S100A, TnI and TnT were applied to the heart tissue sections. **Results:** In the control group, Cx43 was stained as marked streaks between cardiomyocytes; TnI, TnT and S100A were stained as homogenous and dark brown in cytoplasm. Nevertheless, intensity of Cx43 immunostaining showed significant increase in duloxetine- treated groups ($p<0.001$). TnT ($p=0.024$, $p=0.004$, $p<0.05$) and S100A ($p<0.001$) immunostaining were low in high dose group compared to control group and low dose group. TnI immunostaining also demonstrated significant decrease in high dose duloxetine group as compared to the control group ($p<0.001$). There was no statistically significant difference between groups in terms of heart rate, ECG parameters, MDA, SOD, GSH and CAT levels ($p>0.05$). **Conclusion:** Our study reveals that duloxetine induced an increase in the immunostaining of Cx43, and a decrease in the immunostaining of TnI, TnT and S100A, also known as early cardiac parameters. Furthermore, duloxetine was observed to show no effect on oxidant and antioxidant parameters. While heart rate remained unchanged, mean blood pressure decreased in high dose duloxetine group. In the light of the study outcomes, it has been concluded that antidepressants must be administered more advertently in depression patients with heart disorders.

Keywords: Rat; heart; antidepressant; duloxetine; immunohistochemistry

ÖZET Amaç: Bu çalışmada düşük ve yüksek doz duloksetinin, gap junction bileşenlerinden Cx43, kardiyak kontraktilite komponenti S100A ve kardiyak kasılma belirteci olarak kullanılan TnI ve TnT gibi erken kardiyak hasar parametreleri üzerindeki etkilerinin değerlendirilmesi amaçlanmaktadır. **Gereç ve Yöntemler:** Wistar Albino cinsi 24 adet erkek sıçan; kontrol (1 ml çözücü), düşük doz duloksetin (DDD) (10 mg/kg) ve yüksek doz duloksetin (YDD) (100 mg/kg) şeklinde üç gruba ayrıldı. Deney protokolü bitiminde anestezi altındaki sıçanların kan basıncı ile kalp hızı sonuçları kaydedildi ve elde edilen doku kesitlerine H-E boyama yöntemi, Cx43, S100A, TnI ve TnT immün boyama yöntemleri uygulandı. **Bulgular:** Kontrol grubunda Cx43 kardiyomiyositlerin bağlantı bölgelerinde belirgin çizgilenmeler şeklinde, TnI, TnT ve S100A ise sitoplazma içerisinde homojen ve koyu kahverengi olarak boyandı. Diğer yandan, duloksetin uygulanan gruplarda Cx43 immünreaktivite şiddetinde artış gözlendi ($p<0,001$). TnT ($p=0,024$, $p=0,004$ $p<0,05$) ve S100A ($p<0,001$) immünreaktivitesi yüksek doz duloksetin grubunda, kontrol ve düşük doz duloksetin grubuna göre daha azalmış olarak tespit edildi ($p<0,001$). TnI immünreaktivitesinin yüksek doz duloksetin grubunda, kontrol grubu ile karşılaştırıldığında azaldığı gözlendi ($p<0,05$). Ortalama kan basıncının doza bağlı olarak azaldığı diğer yandan kalp hızı, EKG parametreleri ve MDA, SOD, GSH ve CAT seviyelerinde gruplar arasında istatistiksel olarak anlamlı bir değişiklik olmadığı tespit edildi ($p>0,05$). **Sonuç:** Çalışmamız duloksetinin erken kardiyak hasar parametreleri olarak bilinen Cx43 immünoreaktivesinde artışa, TnI, TnT ve S100A'da ise azalmaya neden olduğunu gösterdi. Ayrıca oksidan- antioksidan parametreler ile kalp hızı üzerine herhangi bir değişikliğe neden olmadığı, ancak doza bağlı olarak ortalama kan basıncını azalttığı gözlendi. Bütün bunlar göz önüne alınarak, kalp rahatsızlığı olan depresyon hastalarında, antidepresanların da daha dikkatli uygulanması gerektiği sonucuna varıldı.

Anahtar Kelimeler: Sıçan; kalp; antidepresan; duloksetin; immünohistokimya

Antidepressant drugs administered in depression treatment function as enzyme or receptor inhibitors and reuptake inhibitors. Antidepressants are classified as monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs).[1]

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It is considered that the drugs which exert their actions through the receptors of SSRIs, SNRIs, dopamine and other serotonin inhibitors are safer and more tolerable.[2,3] Although not prevalent, it is reported that those drugs possess cardiovascular side effects in some cases since they affect noradrenergic system and, as a result, serotonin and norepinephrine reuptake inhibitors increase blood pressure.[4]

This study was aimed to investigate the effects of duloxetine, a SNRI group drug, on heart through immunohistochemical and biochemical parameters. Biomarkers detected in serum in the diagnosis of myocardial damage are recommended in a clinical trial.[5] Several researchers associated myocardial damage resulting from various pathologic effects with structural disorders of gap junction responsible for intercellular transmission.[6,7] Cx43 isoform is by far the most abundant connexion type in intercellular communication between ventricular cardiomyocytes.[8-10] Certain researchers prefer cardiac troponins considering their myocardial tissue specificity as markers although several researchers prefer S100A required for cardiac stimulation and contraction.[5-11] On the contrary, no study has been found in the literature investigating the effect of duloxetine on certain immunohistochemical markers as Cx43, S100A, TnI and TnT. In addition, the originality of this study, thus, lies in the fact that the effect of low and high doses of duloxetine on blood pressure (systolic, diastolic and mean) and heart rate with all those parameters were investigated.

MATERIAL AND METHODS

STUDY POPULATION

Male Wistar Albino rats (n=24) weighing 250-300 grams (g) and procured from Inonu University Experimental Animals Research Centre, were used. The rats were randomly assigned to 3 groups (8 rats/group): control group, low-dose duloxetine group (LDD) and high-dose duloxetine group (HDD).

1 ml distilled water as a solvent via oral gavage was administered to the control group during the experiment. LDD group received 10 mg/kg duloxetine soluble in distilled water and HDD group received 100 mg/kg duloxetine soluble in distilled water.[12-14]

After the experimental procedure was carried out, one of the carotid arteries was cannulated in order to monitor systolic, diastolic and mean blood pressure and heart rates of anaesthetized rats. Blood pressure and heart rates were recorded by Biopac MP-100 Data Acquisition System. At the end of 21 day- procedure, the hearts of the rats, sacrificed through high dose ketamine (50 mg/kg) and xylazine (5 mg/kg), were removed. HE staining and immunostaining of Cx43, S100A, TnI and TnT (Table 1) were applied to serial sections received from paraffin blocks.

Ethical approval was granted by the Faculty of Medicine Experimental Animals Ethics Committee of İnönü University (22.06.2016, 2016/A-88).

BLOOD PRESSURE, HEART RATE AND ECG

One of the carotid arteries of rats, having received intraperitoneal (i.p.) injection of ethyl carbamate (Üretan®) (1.2 g/kg) to provide anaesthesia, was cannulated in order to determine systolic, diastolic and mean blood pressure (BP) and heart rates (HR). In case of a bleeding or rupture in one of the carotid arteries, another artery was used as a substitution. 3-lead ECG electrodes were employed to observe the changes in ECG. BP, HR and ECG values were recorded by Biopac MP-100 Data Acquisition System for 3 minutes. After the experimental protocol, blood sample and heart tissue were taken from vena

TABLE 1: Primary antibodies used for immunohistochemistry.

Primary antibody	Manufacturer
Connexin 43	Abcam, Abcam plc 330 Cambridge Science Park Cambridge CB4 0FL UK
Troponin I	Abcam, Abcam plc 330 Cambridge Science Park Cambridge CB4 0FL UK
Troponin T	Thermo, Thermo Fisher Scientific Anatomical Pathology Tudor Road, Manor Park Runcorn, Cheshire WA7 1TA, UK
S100A	Thermo, Thermo Fisher Scientific Anatomical Pathology 93-96 Chadwick Road, Astmoor Runcorn, Cheshire WA7 1PR, UK

cava inferior. The durations of PR, QRS and QT from ECG measurements were evaluated based on Lambeth Conventions.[15]

HISTOPATHOLOGICAL ANALYSES

The heart tissue removed at the end of the experiment was fixed in %10 formaldehyde solution. The tissues were placed in ascending degrees of alcohol in dehydration step for tissue processing. During clearing stage, the tissues were processed by using xylene. The subsequent procedure was that processed tissue specimens were embedded in molten paraffin wax. The sections of 4 to 5 µm in thickness were taken from the paraffin blocks prior to staining with hematoxylin and eosin (H&E). H&E staining method was, then, applied to the tissues in order to identify the general morphological structure.

Heart sections were inspected in terms of the existence of congestion- haemorrhage, interstitial edema and degenerate cardiomyocytes (intensive eosinophilic cytoplasm, pyknotic nucleus) in myocardium. Each area was labelled according to the severity of change (0; no change, 1; slight change, 2; moderate change, 3; severe change).

The analyses were performed with the assistance of Leica DFC- 280 research microscope and Leica Q Win Image Analysis Software (Leica Micros Images Solutions Ltd., Cambridge, The UK).

IMMUNOHISTOCHEMICAL (IHC) ANALYSES

The deparaffinised and rehydrated tissue sections were heated in 0.01 M citrate buffer solution (pH 6.0) for 15-20 min in 2100 Antigen Retriever incubator for immunohistochemical analyses. The tissue specimens were treated with 3% hydrogen peroxide for 12 min for the purpose of blocking endogenous peroxidase activity. Protein block (Ultra V Block) was applied to the sections, having been washed in PBS, for 5 min. Subsequently, the tissues were incubated with primary antibody (Table 1) for 60 min at 37˚C. Mouse monoclonal antibody connexin43 the reactivity of which has been observed in rat, mouse and human tissues, Troponin I, S100A and rabbit polyclonal Troponin T antibodies were diluted 1:200. Biotinylated seconder antibody was added to the sections, having washed in PBS, for 10 min at 37˚C. This was followed by incubating the sections with streptavidin peroxidase for 10 min at 37˚C. After the chromogen application, the sections stained with hematoxylin were coverslipped with water- based mounting medium. Staining was labelled semi- quantitatively based on the prevalence (0: 0-25%, 1: 26-50%, 2: 51-75%, 3: 76-100%) and severity (0: no detection, 1: slight detection, 2: moderate detection, 3: severe detection) of immunostaining. Total staining score was calculated by the multiplication of prevalence and severity.[16]

BIOCHEMICAL ANALYSES

In order to be used in enzyme reactivity measurement, 0.1 g tissue was homogenized in ice- cold isolation medium in 1 ml PBS (pH 7.4) by IKA- Werke T-25 homogenizer. The homogenates were sonified for 10 sec twice in Sonics VCX130 sonificator. The homogenates were centrifuged for 10 min at 10000 x g at +4˚C by Nüve NF800R microcentrifuge tool, thereby discarding supernatants. The identification of enzyme activities was carried out by the supernatants obtained. Total Glutathione (GSH), superoxide dismutase (SOD), Catalase (CAT) and malondialdehyde (MDA) levels were measured.

STATISTICAL ANALYSIS

The statistical analyses were conducted with software programme. Non-parametric techniques were used since data did not show the normal distribution. The comparison between groups was conducted through Mann-Whitney U test and Wilcoxon test was used for comparing two paired groups. The data were presented as descriptive statistics and median (minimum-maximum). The statistical significance level was set at $p < 0.05$ and $p < 0.001$.

RESULTS

HISTOPATHOLOGIC FINDINGS

Cardiac muscle fibres were horizontally and regularly-laid within longitudinal planes in HE staining in control group. Transverse cardiomyocyte banding and intercalated discs were particularly observed (Figure 1 and Figure 2). The tissue sections of LDD groups were similar to those in the control group (Figure 3 and Figure 4). On the other hand, slight degenerative changes were detected in myocardia of

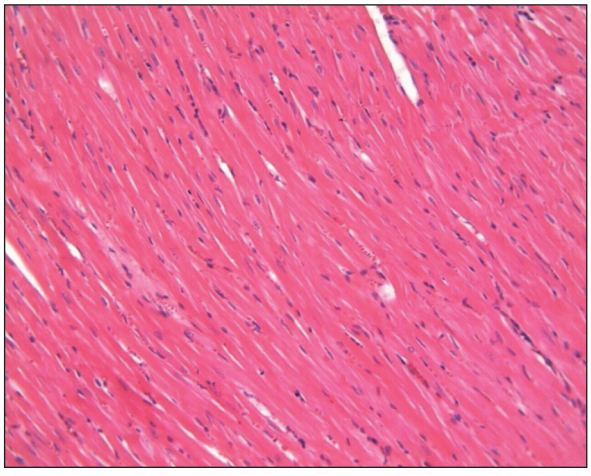


FIGURE 1: In the control group, normal appearance of the heart muscle fibers in the longitudinal plane using the H&E staining method (x200).

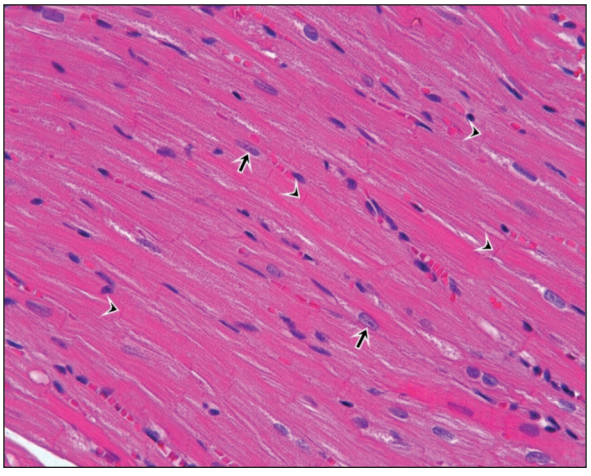


FIGURE 2: In the control group, normal appearance of the heart muscle fibers in the longitudinal plane using the H&E staining method. Intercalated discs (arrowheads) and cardiomyocyte nuclei (arrows) are clearly observed (x400).

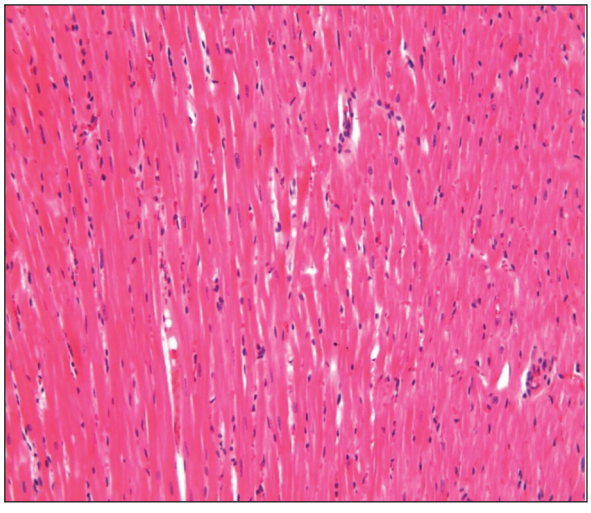


FIGURE 3: In LDD group, the appearance of heart muscle fibers in the longitudinal plane by H&E staining method (x200).

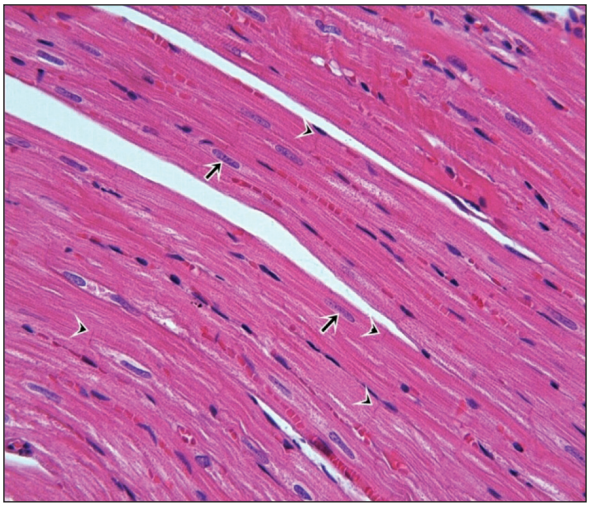


FIGURE 4: In LDD group, the appearance of the heart muscle fibers in the longitudinal plane is similar to the control group by H&E staining method. The arrowheads show the intercalated discs and the arrows show the cardiomyocyte nuclei (x400).

rats exposed to high dose duloxetine (Figure 5 and Figure 6). The interstitial edema was the most evident among those changes. When compared to control group, interstitial edema was determined to show significant increase in this group.

The scoring and p values representing histopathologic changes in myocardial tissue were presented in Table 2.

IMMUNOHISTOCHEMICAL FINDINGS

Cx43 immunostaining was clearly observed as dark brown in intercalated discs (Figure 7 A,B). Intercalated discs showed staining in the form of smooth streaks in control group although it was observed that intercalated disc contours were damaged in duloxetine groups. In addition, the severity of Cx43 immunostaining was observed to increase dramatically in duloxetine groups (Figure 8A,B and Figure 9A,B). It was determined that the changes in Cx43 staining were statistically significant in duloxetine groups compared to control group ($p<0.001$). Nevertheless, it was revealed that Cx43 staining immunostaining was statistically similar between duloxetine groups ($p=0.625$, $p>0.05$).

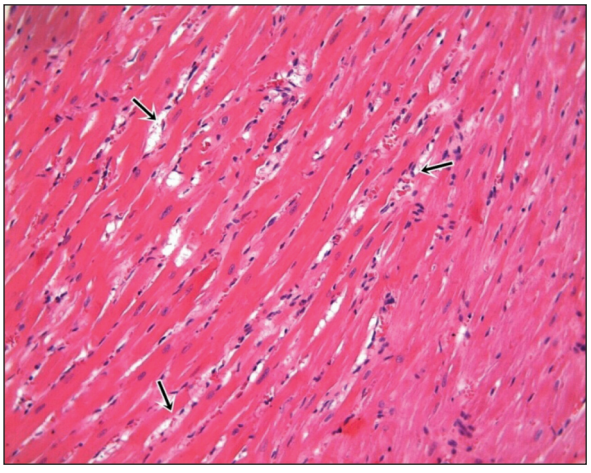


FIGURE 5: In HDD group, apperance of heart muscle fibers in longitudinal plane by H&E staining method. In contrast to the control group, mild edema (arrows) is observed between cardiomyocytes (x200).

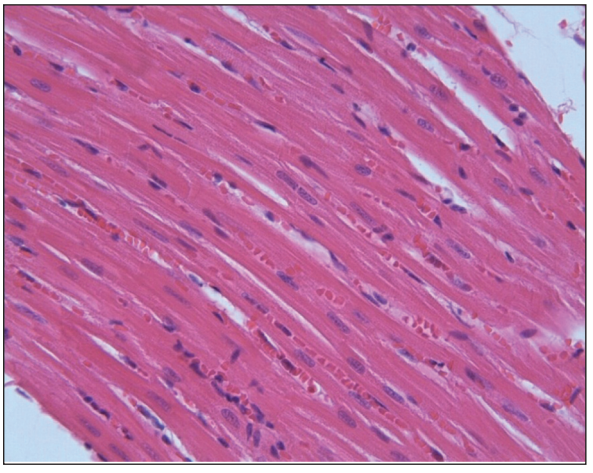


FIGURE 6: In HDD group, appearance of heart muscle fibers in longitudinal plane by H&E staining method (x400).

TABLE 2: Histopathological score results in myocardial tissue [median (minimum-maximum)].

	Congestion- hemorrhage	Interstitial edema	Degenerate cell
Control	0.0 (0.0-3.0)	0.0 (0.0-2.0)	0.0 (0.0-3.0)
LDD	0.0 (0.0-3.0)	0.5 (0.0-3.0)	0.0 (0.0-3.0)
HDD	1.0 (0.0-3.0)	1.0 (0.0-3.0)	0.0 (0.0-3.0)
	p values		
	Congestion-Hemorrhage	Interstitial edema	Degenerate cell
C-LDD	0.910	0.088	0.307
C-HDD	0.081	0.001	0.787
LDD-HDD	0.070	0.118	0.156

*Mann Whitney U test was used.
LDD: Low-dose duloxetine; HDD: High-dose duloxetin.

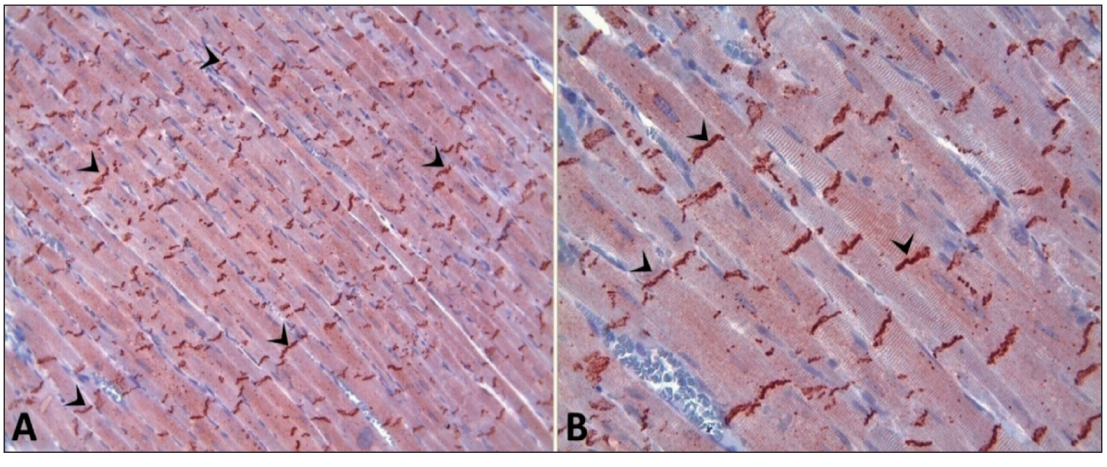


FIGURE 7: In the control group, The CX43 immunostaining (arrowheads) on intercalated discs is observed in the plane parallel to the long axis of cardiomyocytes. A: x200, B: x400.

Troponin T was dark brown in cytoplasm of cardiomyocytes (Figure 10A,B). The intensity of TnT immunostaining was similar to those in control group and low- dose duloxetine group (p=0.742, p>0.05) although TnT immunostaining was lower in high- dose duloxetine group than in the control group and low-

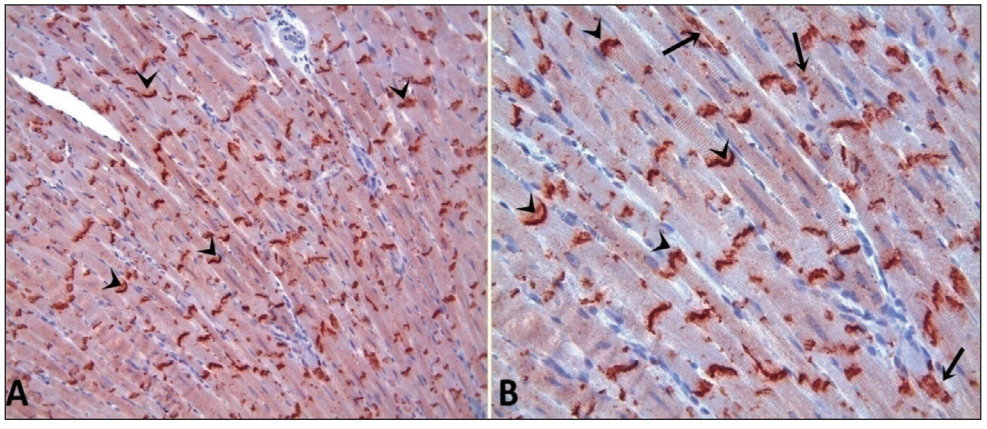


FIGURE 8: In LDD group, the presence of CX43 immunostaining is also noticeable in the cytoplasmic areas of the cardiomyocytes (arrows), where the contours of the intercalated discs are disturbed (arrowheads). A: x200, B: x400.

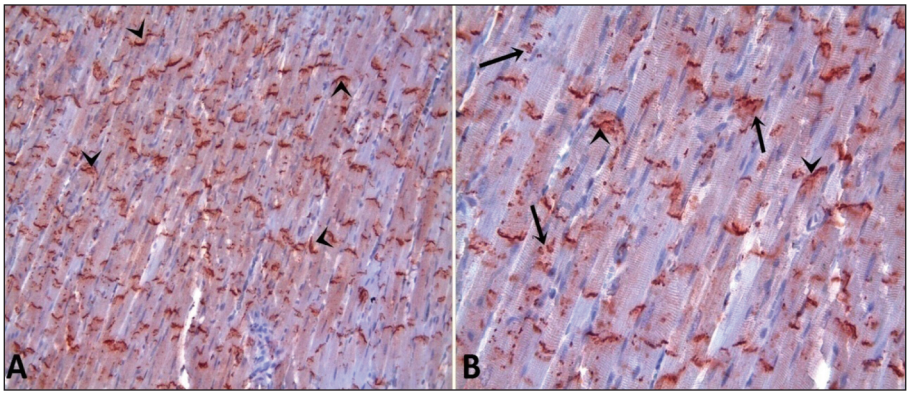


FIGURE 9: In HDD group, CX43 immunostaining is similar to LDD group. The impaired intercalated disc contours (arrows) and the existence of CX43 in cytoplasmic areas of cardiomyocytes (arrowheads) are significant. A: x200, B: x400.

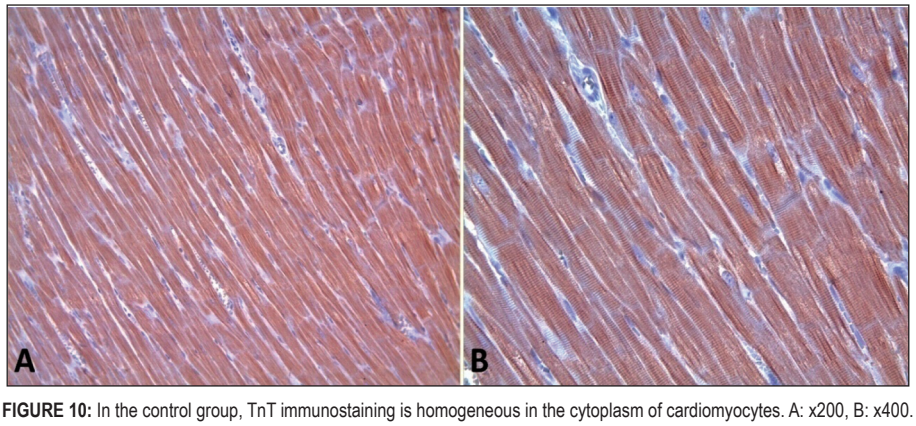


FIGURE 10: In the control group, TnT immunostaining is homogeneous in the cytoplasm of cardiomyocytes. A: x200, B: x400.

dose duloxetine group (Figure 11A,B and Figure 12A,B).

It was seen that Tnl immunostaining showed a homogenous distribution on cardiomyocytes cytoplasm in the control group (Figure 13). It was indicated that TnI reactivity was decreased induced by duloxetine and it was statistically significant compared to the control group ($p=0.000$, $p=0.004$, $p<0.05$). However, immunostaining in HDD group was observed to be lower compared to LDD group although the difference be-

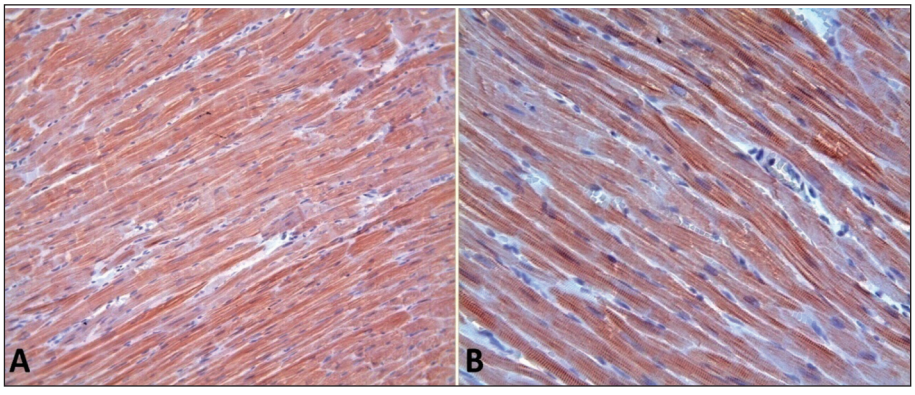


FIGURE 11: In LDD group, TnT immunostaining is similar to the control group. A: x200, B: x400.

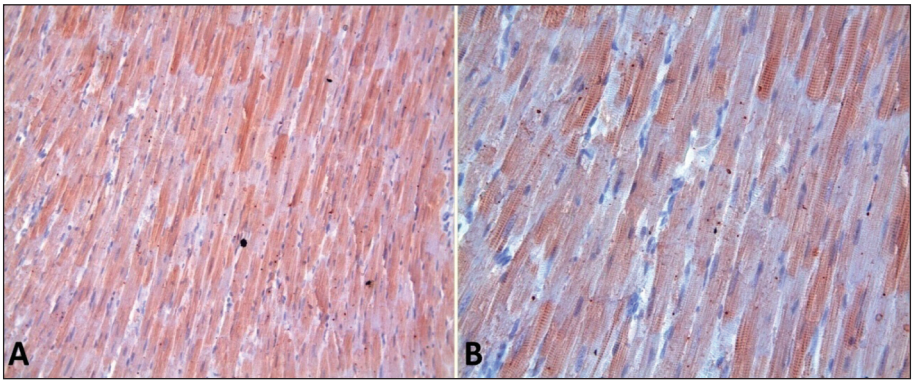


FIGURE 12: In HDD group, TnT immunostaining is significantly decreased in this group compared to the control and LDD groups. A: x200, B: x400.

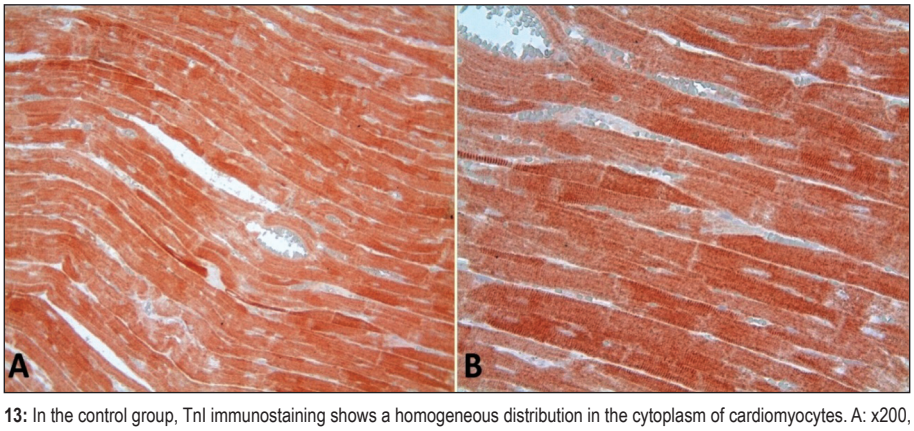


FIGURE 13: In the control group, TnI immunostaining shows a homogeneous distribution in the cytoplasm of cardiomyocytes. A: x200, B: x400.

tween duloxetine groups was not statistically significant (Figure 14A,B and Figure 15A,B).

The staining score of S100A immunostaining was statistically similar to the scores of control group and LDD group (Figure 16A,B and Figure 17A,B). On the other hand, immunostaining was dramatically reduced resulting from high- dose duloxetine. The decrease in HDD group was found to be statistically significant compared to control group and LDD group (Figure 16A,B, Figure 17A,B and Figure 18A,B). Cx43, TnI, TnT and S100A staining scores and p values of the groups were presented in Table 3.

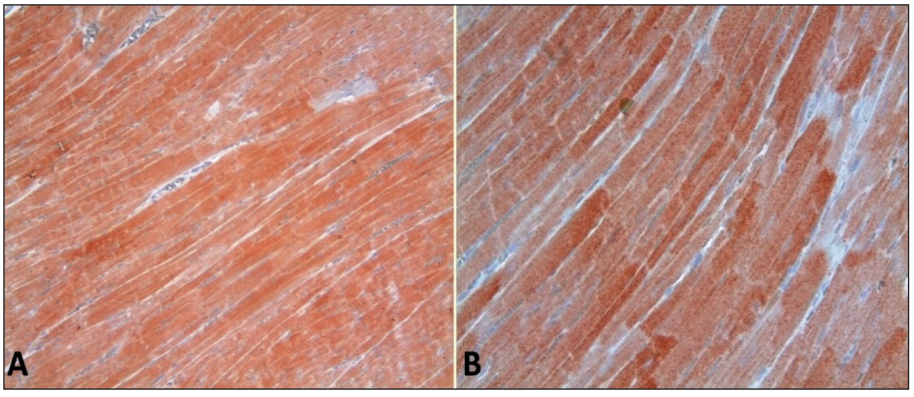


FIGURE 14: In LDD group, TnI immunostaining decreased significantly compared to the control group. A: x200, B: x400.

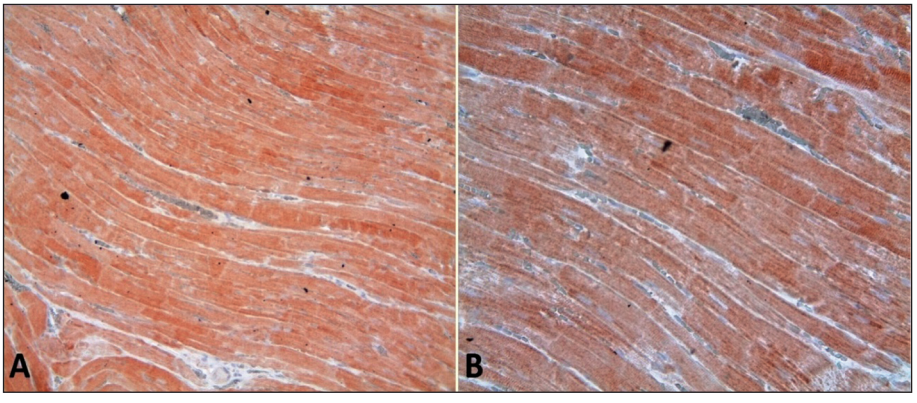


FIGURE 15: In HDD group, TnI immunostaining decreased significantly com pared to the control group, similar to the LDD group. A: x200, B: x400.

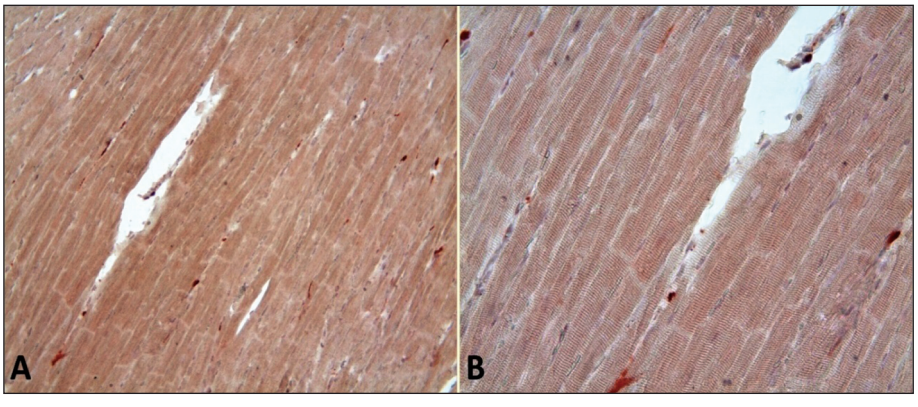


FIGURE 16: In the control group, S100A immunostaining is clearly observed in the cytoplasm of cardiomyocytes, especially at the level of A band. A: x200, B: x400.

BODY WEIGHTS

There was no significant difference between control and LDD groups based on body weight when compared to before and after the experiment. However, after the experiment, it was indicated that there was a significant decrease in body weight in HDD group compared to prior to the experiment (Table 4 and Graphic 1).

BIOCHEMICAL FINDINGS

MDA levels have been determined as 13.83±2.77 nmol/mg in control group, 13.58±3.54 nmol/mg in LDD group and in HDD group. MDA levels were found statistically similar in the groups ($p>0.05$). GSH, SOD and CAT levels were 51.21±4.62 nmol/mg, 165.57±53.49 U/mg, 29.84±2.42 U/mg in control group; 51.27±3.37 nmol/mg, 164.82± 22.56.49 U/mg,

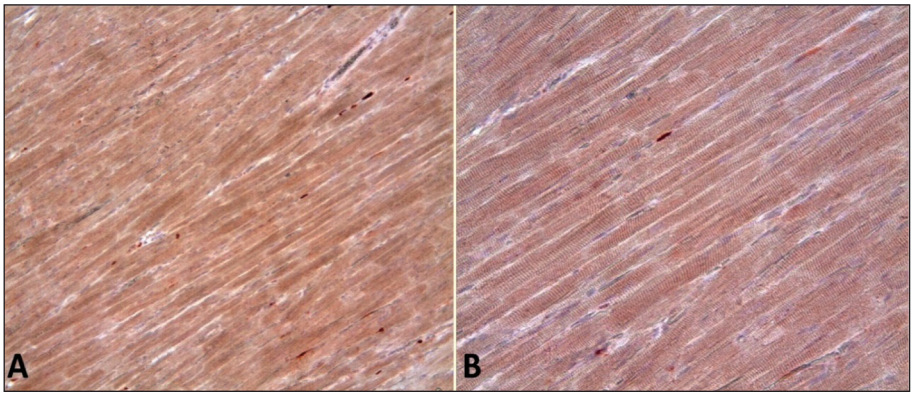


FIGURE 17: In LDD group, S100A immunostaining is similar to the control group. A: x200, B: x400.

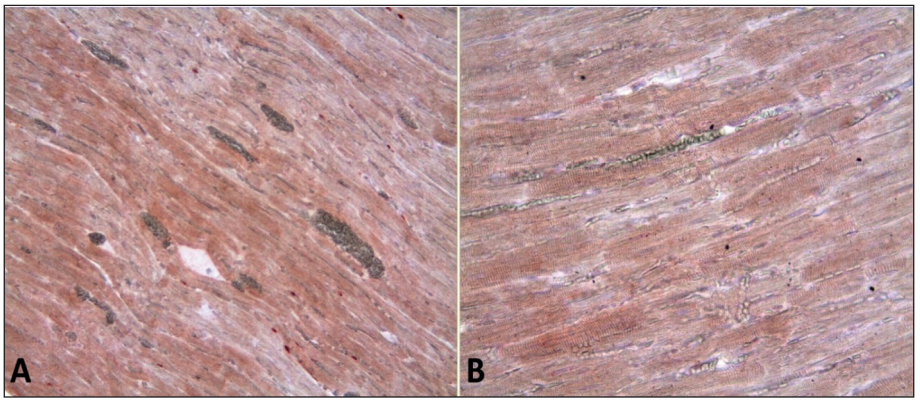


FIGURE 18: In HDD group, S100A immunostaining decreased significantly compared to the control and LDD groups. A: x200, B: x400.

TABLE 3: Immunostaining score results for each antibody in myocardial tissue.

	C43	TnT	TnI	S100A
Control	1.0 (1.0-9.0)	6.0 (1.0-12.0)	6.0 (0.0-12.0)	4.0 (0.0-12.0)
LDD	3.0 (1.0-9.0)	6.0 (0.0-12.0)	4.0 (0.0-12.0)	4.0 (0.0-12.0)
HDD	3.0 (1.0-16.0)	4.0 (1.0-12.0)	2.0 (0.0-9.0)	2.0 (0.0-6.0)
		p values		
	C43	TnT	TnI	S100A
C-LDD	p<0.001	0.742	0.004	0.016
C-HDD	p<0.001	0.004	p<0.001	p<0.001
LDD-HDD	0.625	0.024	0.269	p<0.001

*Mann Whitney U test was used

29.36±3.69 U/mg in LDD group 51.38±3.24 nmol/mg, 146.75±32.28 U/mg, 29.50± 5.26 U/mg in HDD group respectively. No statistically significant difference was revealed among the groups pertaining to these parameters (p>0.05). The results of biochemical analysis were displayed in Table 5.

BLOOD PRESSURE, HEART RATE AND ECG

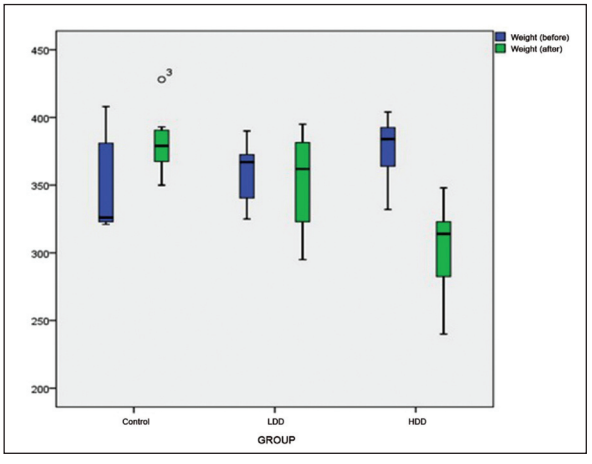
Heart rate was measured as 352.6±54 in control group, 323.3±63 in LDD group and 361.3±85 in HDD group. There was no significant difference among groups in terms of heart rate. On the other hand, mean blood pressure was measured as 72.3±8.8 mmHg in control group, 65.1±3.1 mmHg in LDD group and 52.7±6.4 mmHg in HDD group. Besides, mean blood pressure was determined to show a significant decrease in HDD group in association with the dosage (p<0.001). There was no significant change in control, LDD and HDD groups concerning ECG parameters (PR, QRS and QT) (p<0.001). The

TABLE 4: P values for body weights before and after groups.

Groups	Group mean of body weights (before)	Group mean of body weights (after)	p value
Control Group	351.857	381.857	0.063
LDD Group	358.285	351.571	0.499
HDD Group	376.142	301.857	0.018

* Wilcoxon test was used.

LDD: Low-dose duloxetine; HDD: High-dose duloxetin.



GRAPHIC 1: Comparison of body weights before and after groups (g).

LDD: Low-dose duloxetine; HDD: High-dose duloxetin.

effect of duloxetine on hemodynamic parameters is presented in Table 6. The effect of duloxetine on cardiac rhythm is given in Table 7. Data on ST depression, branch block, and T negativity are presented in Figure 19, Figure 20, Figure 21.

DISCUSSION

Antidepressant drugs used in depression treatment are the set of drugs known as MAOIs, TCAs, SSRIs and SNRIs.[17] SNRIs inhibit the reuptake of both 5-HT and NE from synaptic cleft, thereby causing an increase in neurotransmitters. In the literature, there have been numerous studies asserting that SNRIs are safer than SSRIs.[18-20] The duloxetine used in our study is a potent selective serotonin and norepinephrine reuptake inhibitor. Duloxetine is used to treat symptoms associated with chronic musculoskeletal pain, fibromyalgia, diabetic peripheral neuropathic pain, anxiety disorders and major depressive disorder.[21]

In the present study, it was determined that low-dose duloxetine did not affect body weight although high-dose duloxetine led to weight loss.

The daily therapeutic dosage of duloxetine (60-120 mg), one of the emerging antidepressants, are reported to be better- tolerated. However, clinical studies have revealed that duloxetine causes certain side effects as dose- related nausea, loss of appetite, con-

TABLE 5: MDA, SOD CAT and GSH results of the groups.

	MDA nmol/mg	GSH nmol/mg	SOD U/mg	CAT U/mg
Control	14.4 (10.5-16.8)	50.2 (47.4-58.6)	179.7 (127.8-183.8)	30.1 (27.3-33.0)
LDD	13.9 (7.8-16.6)	50.9 (47.6-57.1)	166.0 (134.1-194.3)	30.9 (24.9-33.3)
HDD	13.6 (13.0-14.7)	50.0 (48.1-55.8)	152.1 (113.2-178.5)	29.9 (23.8-34.9)

*Mann Whitney U test was used. No statistically significant difference was revealed among the groups pertaining to these parameters (p>0.05).

MDA: Malondialdehyde; GSH: Glutathione S-Transferases; SOD: Superoxide dismutase; CAT: Catalase; LDD: Low-dose duloxetine; HDD: High-dose duloxetin.

TABLE 6: The effects of duloxetine on hemodynamic parameters.

Groups	Heart Rate (beats/min)	Systolic Blood Pressure (mm Hg)	Diastolic Blood Pressure (mm Hg)	Mean Blood Pressure (mmHg)	PR Milliseconds	QRS Milliseconds	QT Milliseconds
Control	352.6±54	114±17.8	44.9±13.3	72.3±8.8	43.7±7.3	77.4±15	110.6±14
LDD	323.3±63	104±14.2	43.1±12.4	65.1±3.1	46.3±7.3	66.6±11.6	110.6±12
HDD	361.3±85	93.3±14.0[a]	45.7±11.8	52.7±6.4[a]	43.1±8.1	75.6±11.4	111.1±14.3
Significant Mann Whitney U Test Results							
Groups				**z**	**r**	**U**	**p**
Mean Blood Pressure: Control/HDD				-2.853	-0.390	115.000	0.003*
Systolic Blood Pressure: Control/HDD				-4.859	-0.348	520.000	0.021*

*Mann Whitney U test was used. a: Significant compared to control (p<0.001).

PR: p,r dalgaları; QRS: q,r,s dalgalarının oluşturduğu kompleks; QT: q,t dalgaları; LDD: Low-dose duloxetine; HDD: High-dose duloxetin.

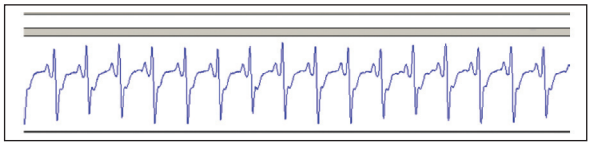


FIGURE 19: ST Depression image is given.

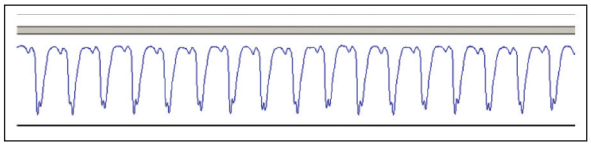


FIGURE 20: Branch block image is given.

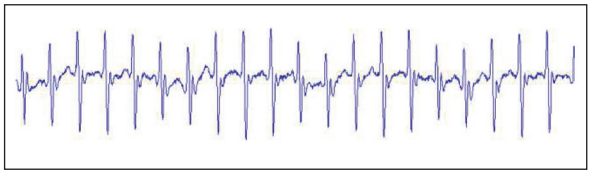


FIGURE 21: T Negativity image is given.

TABLE 7: The effect of duloxetine on cardiac rhythm.

Groups	ST Depression	Branch Block	T Negativity
Control	-	-	-
LDD	-	-	1
HDD	1	1	2

For all groups n=7.
ST: Segment t; T: EKG'deki T dalgası; LDD: Low-dose duloxetine; HDD: High-dose duloxetin.

stipation, xerostomia and numbness.[20] Another common side effect resulting from antidepressant use is the change in body weight.[22]

Antidepressants are considered to cause weight loss by affecting gastrointestinal system through several physiological mechanisms such as inhibition of gastric acid secretion, delayed gastric emptying and intestinal smooth muscle contraction.[23] Khaksara et al. revealed a decrease in body weight of rats following 4-week-fluoxetine treatment.[24] In addition, James et al. reported potential weight loss in the early periods of treatment while frequently observing weight gain in long-term treatment.[22]

In the present study, it was revealed that duloxetine decreased blood pressure depending on the dosage; however, no significant effect on heart rate was found.

The emerging drugs as serotonin norepinephrine reuptake inhibitors are the most- preferred treatment options thanks to their effectiveness, cardiovascular safety profiles and high tolerance levels.[1] On the contrary, certain researchers have stated that such drugs affect blood pressure and heart rate and cause arrhythmia.[25]

A clinical study performed on 8504 patients using duloxetine by Wernicke et al. showed that there was no significant difference in heart rate in duloxetine group compared to the placebo group.[19] Yamazaki-Hashimoto, et al. stated that therapeutic doses of fluvoxamine, one of SSRIs, cause hypotensive effect by inhibiting Na and Ca channels and supressing atrioventricular and intraventricular transmission. The same study suggested that low-dose fluvoxamine did not have an effect on blood pressure and heart rate although medium- dose decreased heart rate while having no impact on blood pressure and high- dose decreased blood pressure and heart rate. Cardiac sodium (Na) channels are of fundamental importance for the rapid upstroke of the action potential.[26] Stoetzer et al. showed that duloxetine potentially inhibits cardiac Na channel in the cardiomyocytes of rats. To sum up, it is acknowledged that hypotensive effect of duloxetine is likely associated with the inhibition of cardiac voltage-gated Na channel.[27] Domenic et al. and Sharma et al. reported that duloxetine contributed to an increase in systolic and diastolic blood pressure and in heart rate.[28]

In the present study, it was observed that both the severity and intensity of Cx43 secretion were significantly increased in low-dose and high-dose duloxetine group compared to control group.

The intercalated disc (ICD) is situated at the end of a cardiomyocyte and ensures connection between neighbouring cardiomyocytes. The transverse and lateral segments of intercalated disc are gap junctions.[29] Cx43, the main constituent of gap junction, is the most abundantly expressed protein in the cardiomyocytes.[30] Required for the normal ventricular function, Cx43 has a relatively half-life compared to other intermembrane proteins.[31] Recent studies have indicated that changes in the amount and distribution of Cx43 affect transmission, induce arrhythmia and un-

coordinated contraction and even contribute to the disorder of myocardial function.[8,31] Fatemi et al. demonstrated an increase in Cx43 secretion in prefrontal cortex and hippocampus following a chronic treatment of fluoxetine, one of SSRIs, through Western- Blot technique.[32] Viczenczova et al. who investigated Cx43 distribution in cardiac damage reported that Cx43 distribution was increased in lateral surfaces of cardiomyocytes after single-dose injection of isoproterenol causing myocardial ischemia.[33] It has been acknowledged that c-Fos/Ap-1 signalling pathway plays a major role in increasing Cx43 secretion by antidepressants. Ligand binding causes an activation of Braf, a peripheral membrane protein, through MAPK signalling pathway. Braf activates activator protein-1 (AP-1) by the phosphorylation of MEK-1 and MEK-2 through ERK-1 and ERK-2. Thus, AP components lead to an increase in Cx43 transcription factor by penetrating Jun and c-Fos cell nuclei.[34,35]

In this study, it was found that TnT expression was similar in control group and low- dose duloxetine group. In contrast, TnT expression was observed to decline in high-dose duloxetine group compared to the control group and low- dose duloxetine group. In addition, TnI expression was revealed to be decreased in low and high-dose duloxetine groups compared to control group. No statistically difference regarding TnI expression between duloxetine- treated groups was found.

There are numerous tissue and serum biomarkers in clinical trials to diagnose myocardial damage caused by drugs and other cardio-toxic agents.[36,37] Troponin is considered as the biomarker of choice due to its high clinical sensitivity and absolute myocardial tissue specificity.[38] Recent studies have indicated that the measurements of cardiac troponin T and I are of pivotal role in determining the risk of myocardial infarction and myocardial ischemia.[39] American College of Cardiology and European Society Cardiology acknowledged that cardiac troponin supplanted CK-MB parameters in the diagnosis of the patients with myocardial infarction.[38] Ricchiuti et al. found a decrease in myocardial tissue and an increase in serum in terms of troponin in experimental subjects with experimental myocardial infarction.[38,40] It has been acknowledged that cardiac troponins cause an increase in serum concentration by being elevated locally from cardiomyocytes.[40] William et al. revealed that the decrease in TnI and TnT in the tissue resulting from the severity of myocardial damage caused by cardio-toxic agents were correlated with their experimental studies.[39,40] A case study has reported that cardiac troponin I and T are highly effective methods in cardiac risk assessment in patients on chronic dialysis.[41]

It was observed in this study that low- dose duloxetine did not show an effect on the intensity of S100A protein staining in myocardial tissue although high- dose duloxetine led to a significant decrease in the intensity of S100A protein staining.

S100A protein is another early marker of cardiac damage.[40] Nineteen members of S100A protein family have been isolated.[42] It is reported that the heart muscle consists of the highest level of S100A with 1.8 pg/mg protein.[43] This protein was observed to localize in sarcoplasmic reticulum in cardiac tissue and actin- myosin filaments.[44] Recent studies have demonstrated that the level of S100A1 protein in myocardium takes a major role in response to cardiac damage and in the protection of the heart. However, the functional role of S100A protein has not been fully understood yet.[43,44]

S100 proteins having their basic effects through Ca are of two binding areas showing affinity to Ca. Ca ions are involved in the regulation of certain cellular processes as muscle contraction, cellular division, energy metabolism, proliferation, gene transcription and differentiation. The damage in calcium homeostasis and change in the secretion of proteins which bind calcium conduce to cardiomyopathy and myocardial hypertrophy or myocardial ischemia.[43] Abnormality in Ca metabolism is one of the underlying causes of myocardial damage. The deterioration of cytoskeleton organization is prevalent in impaired myocardium. This situation is accompanied by various Ca binding proteins. Inamato associated the differences in S100 secretion with cardiomyocyte damage. It was reported in their study that the severity of S100A4 and S100A1 secretion was increased in rats given isoproterenol causing cardiac hypertrophy.[45] Rohde stated that S100A was released from cardiomyocytes into interstitial area and had an anti-fibrotic influence by

affecting cardiac fibroblasts.[46] The decrease in S100 proteins in myocardial tissue has an effect on myofilament calcium sensitivity, thereby causing an impairment of cardiac contractility and the performance of heart contraction. In light of above-mentioned findings, it may be concluded that that changes in S100A secretion towards damage in myocardial tissue are one of the reasons affecting cardiac contraction.[47]

CONCLUSION

The current study offers insights into the changes in myocardial tissue cause by duloxetine showing antidepressant effects by inhibiting reuptake of serotonin and norepinephrine from synapses through early cardiac damage parameters.

Duloxetine did not cause histopathologic change in ECG results (PR, QRS and QT), antioxidant and oxidant parameters and myocardial tissue (except interstitial edema). On the other hand, the expression of Cx43 which is essential for transmission and coordination between cardiomyocytes has been observed to show significant increase based on the dose whereas the intensity of TnI and TnT used as cardiac contraction markers decreased. Furthermore, S100A expression was observed to decrease significantly in merely HDD group. In addition, duloxetine was determined to induce a decrease in mean blood pressure and a slight increase in heart rate.

Depression is accompanied by other diseases since it develops as a component, a complication or a result of another disease. Depression is a risk factor for heart attack, coronary artery diseases and cardiovascular-related diseases such as early death. Therefore, it is of high importance that cardiovascular profile of a patient must be considered while administering an antidepressant drug.

The study outcomes highlight the necessity of considering antidepressant use since it poses cardiovascular risk although recent antidepressants (SNRIs) are mostly well-tolerated. Additionally, we are of the opinion that our study will contribute to further research which will discover molecular and cell basis of antidepressant drugs via cardiac profile to prevent side effects of the treatment.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Işıl Eranıl, Nigar Vardı;* ***Design:*** *Nigar Vardı;* ***Control/Supervision:*** *Nigar Vardı;****Data Collection and/or Processing:*** *Işıl Eranıl, Onural Özhan, Azie Yıldız;* ***Analysis and/or Interpretation:*** *Hakan Parlakpınar, Nigar Vardı, Onural Özhan;* ***Literature Review:*** *Işıl Eranıl, Nigar Vardı;* ***Writing the Article:*** *Işıl Eranıl;* ***Critical Review:*** *Nigar Vardı, Azibe Yıldız;* ***References and Fundings:*** *BAP Birimi.*

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