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Original Article

The treatment effect of silymarin on heart damage in rats

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Abstract

Aim: In this article, we evaluated the possible cardiac protective effects of silymarin on valproic acid-induced heart injury by histological and biochemical parameters in rat heart.

Material and Methods: The experiment was performed with 21 Sprague Dawley male rats. Rats were divided into three groups: group 1; control, group 2; valproic acid, group 3; valproic acid + silymarin. The groups received valproic acid 500 mg/kg/day per os (p.o.) and silymarin 100 mg/kg/day p.o. for 14 days except the control group.

Results: Silymarin treatment decreased the levels of lactate dehydrogenase, creatine kinase isoenzyme MB, alanine aminotransferase and aspartate aminotransferase significantly ($p < 0.05$). In addition, the increase of malondialdehyde level and decrease of glutathione level by valproic acid were significantly suppressed by silymarin in heart tissue ($p < 0.05$). Histologically, the amount of heart injury was significantly lower in valproic acid + silymarin group and histopathological findings were decreased in valproic acid + silymarin group compared to valproic acid group ($p < 0.005$).

Conclusion: In this study, it was observed that silymarin has a curative effect on valproic acid induced heart damage. In this sense, we believe that our study will be usefull for other studies which will be study with silymarin and valproic acid.

Keywords: Heart damage; rat; silymarin; valproic acid

INTRODUCTION

Epilepsy is a type of disease that brings a significant financial burden on both the health system and patients. On the other hand, valproic acid (VPA) is used to treat psychiatric disorders such as mania, migraine, bipolar and epilepsy due to its therapeutic benefits and low cost (1, 2). Its chemical structure comprises an eight-carbon fatty acid called dipropylacetate. It acts by inhibiting the recovery of gamma aminobutyric acid (GABA) from presynaptic terminals by inhibiting GABA transaminase and increasing synaptic cleavage (2). VPA binds to plasma proteins at high levels when administered at therapeutic concentrations. This makes them prone to fluctuations in therapeutic effects during treatment, more importantly to unpredictable toxicity and drug interactions (3). Therefore, significant side effects have been reported during prolonged treatment with VPA. The main ones are pancreatitis, elevated liver enzymes, leukopenia, thrombocytopenia and cardiovascular disease (CVD) (1). Myocardial infarction (MI), especially known as heart attack, results in death as a result of permanent heart muscle damage (4). It increases serum cardiac enzymes and lipid peroxidation from the first month of chronic treatment. According to histopathological and biochemical studies, VPA causes cardiac necrosis, apoptosis and oxidative stress (5). The production of excess reactive oxygen species (ROS) directly damages cellular macromolecules such as proteins, lipids and DNA. It changes normal signaling pathways by stimulating redox sensitive transcription agents (1). They are also aggressively involved in oxidative heart injury and become the center of cellular damage that seriously affects the myocardium. Various types of inflammatory cells in the heart affected by this ROS formation are cardiomyocytes and endothelial cells (6). Detoxification of ROS requires enzymatic and non-enzymatic antioxidant mechanisms (1).

Silymarin (SLY) is a flavonoid derived from the seeds of the milk thistle plant (Silybum marianum) and widely used as a hepatoprotective agent (7-9). SLY has anti-inflammatory, immunomodulatory, antifibrotic, antioxidant, metal chelation (iron), protein synthesis-stimulating, cardoprotective and neuroprotective effects (6,7). It has been widely used in the treatments of chronic viral hepatitis, acute ischemic stroke and exposure to environmental toxins (10). It stimulates protein synthesis

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and stabilizes membrane phospholipids due to antioxidant properties. It also has a cleansing and stabilizing effect on cytoplasmic membranes to release free radicals (7,10). Additionally, it improves irregular sarcoma, swollen mitochondria, and cardiomyocyte hypertrophy by reducing collagen production in the heart of rats with diabetes mellitus (DM) (11). It binds to receptors in membranes, prevents toxins from binding and reduces drug-induced damage (12). Reacts with ROS, converts them to less reactive compounds, enhances the effects of antioxidants (glutathione) (10).

Our experiment is to determine whether antiepileptic VPA interferes with oxidative metabolism in the heart and to determine the effects of free radical scavenger SLY on this subject (1).

MATERIAL and METHODS

Chemicals

VPA (as Convulex 500 mg capsules) from Liba Co (Turkey) (5). SLY was obtained as Legalon fort (100 mg/kg capsules) from Madaus Co (Turkey) (12). VPA and SLY doses were determined based on previous articles, respectively (5,12). Hydrochloric acid (HCl), thiochleroacetic acid (TCA), thiobarbuturic acid (TBA) and paraffin were obtained from Sigma-Aldrich (USA). Formalin and 5,5 '-dithio-bis-2, -nitrobenzoic acid were obtained from Chem-Impex (USA) and Tekkim (Turkey), respectively. Xylene, hematoxylin-eosin and ethanol were obtained from Merck (Germany).

Animals

In this study, 21 male Sprague-Dawley rats (220-250 g for 8 weeks) from Adiyaman University Experimental Animal Production Application and Research Center were obtained and the experiment was carried out in this center. Ethics committee report; it was obtained from the Laboratory Animals Ethics Committee (Protocol 2019/10) of Fırat University Faculty of Medicine and the study was conducted according to this protocol.

Treatment Protocol

The animals were randomised into three groups, with seven rats in each group, as follows: Control; VPA; VPA + SLY. The control group received 0.5 mL saline i.p. and 1 mL saline orally for 14 days. VPA group received 500 mg/kg/day VPA p.o. for 14 days (5). VPA + SLY group received 500 mg/kg VPA p.o. and 100 mg/kg/day SLY p.o. for 14 days (12). Rats were sacrificed by cervical dislocation under anesthesia with ethyl ether at the end of day 14. Blood samples were collected from the jugular vein, centrifuged at 5,000 x g for 15 minutes, and serum was collected and stored at -86 ºC for biochemical analysis. The heart was excised and stored at -86 ° C until analysis.

Biochemical Evaluation

Serum biomarkers of creatine isoenzyme kinase MB (CK-MB) U/L, lactate dehydrogenase (LDH) U/L, alanine aminotransferase (ALT) U/L and aspartate aminotransferase (AST) U/L heart function were analyzed using routine enzymatic methods with the Olympus 2700 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany) at the Adiyaman University. In addition, the findings were evaluated according to the Reitman-Frankel colorimetric transaminase method (13).

Oxidative Stress Biomarkers

Malondialdehyde (MDA) measurements were made in heart tissue (14). The amount of lipid peroxidation was measured according to the concentration of thiobarbituric acid reactive species MDA was treated with TBA at a pH of 2-3 and at 95 °C for a period of 15 minutes. The residue was centrifuged at 2500 x g for 10 minutes and samples were read by spectrophotometer at a wavelength of 532 nm (15).

Glutathione (GSH) levels in heart tissues were determined according to Sedlak and Lindsay method (16). The sample was eluted with 50% TCA and centrifuged at 1000 x g for 5 minutes. 2 mL Tris-EDTA buffer (0.2 M, PH = 8.9) and 0.1 mL 0.01M 5,5'-dithio-bis-2 by taking 0.5 mL of the supernatant from the supernatant -nitrobenzoic acid was added. The mixture sample was allowed to stand at room temperature for 5 minutes and read by spectrophotometer at 412 nm wavelength.

Histopathological Examinations

During autopsy, the hearts of all rats used in the experiment were taken, made macroscopic and 10% neutral formalin was used for fixation. After fixing the tissues, the formalin was removed by washing in the stream. It was passed through a series of graded alcohols for dehydration and kept in xylene for transparency. It was then put into paraffin. From these obtained blocks, 3-4 micron sections were cut with microtome (RM2125RTS, Leica, Germany) and these sections were stained with Hematoxylin-Eosin for histopathological evaluation. The structural data in the heart tissue of groups were evaluated according to the notes of Refaiy et al. (17). Modified semi-quantitative scale were used for the evaluation of histopathological changes; (0): none, (1): mild, (2): moderate, (3): severe grade. Samples were evaluated and imaged by imaging binocular light microscopy (ECLIPSE Ni-U, Nikon and Tokyo, Japan).

Statistical Analysis

SPSS version 20.0 was used for statistical analysis. Shapiro-Wilk test was used to evaluate normality. One-way ANOVA and post-hoc, LSD were applied for in-group comparisons of parametric data; Kruskal Wallis test was used for non-parametric and biochemical data. The same test was also used for semi-qualified evaluation of histopathological scores and for differences in the data measured between the groups. Mann-Whitney U test was used to compare the two groups. Data were considered statistically significant for $P \leq 0.05$.

RESULTS

Biochemical Evaluation

LDH, CK-MB, ALT and AST levels were significantly increased in VPA group compared to control and VPA + SLY groups (Table 1 and Figure 1). Treatment with SLY

Table 1. Serum biochemical and heart tissue oxidative stress biomarkers of the experimental groups

	Control	VPA	VPA + SLY
	Serum biochemical biomarkers		
LDH (U/L)	1608.28 ± 20.48 [b]	1750.00 ± 44.38 [a,c]	1622.14 ± 33.43 [b]
CK-MB (U/L)	19.86 ± 0.80 [b,c]	50.00 ± 0.81 [a,c]	26.00 ± 1.00 [a,b]
ALT (U/L)	22.14 ± 0.80 [b,c]	105.29 ± 1.56 [a,c]	49.86 ± 2.48 [a,b]
AST (U/L)	320.71 ± 4.77 [b,c]	669.86 ± 6.97 [a,c]	292.00 ± 10.32 [a,b]
	Heart tissue oxidative stress biomarkers		
GSH (µmole/mg)	0.132 ± 0.007 [b]	0.080 ± 0.017 [a,c]	0.168 ± 0.019 [b]
MDA (nmole/mg tissue)	0.118 ± 0.004 [b]	0.159 ± 0.011 [a,c]	0.123 ± 0.003 [b]

Each group represents the mean ± SEM for seven rats. a: Significant from Control; b: Significant from VPA; c: Significant from VPA + SİLY. Abbreviations: VPA: Valproic acid; SLY: Silymarine; LDH, Lactate dehydrogenase; CK-MB, Creatine kinase MB; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GSH, Glutathione; MDA, Malondialdehyde. VPA: 500 mg/kg VPA; VPA+SLY: 500 mg/kg VPA+100 mg/kg SL

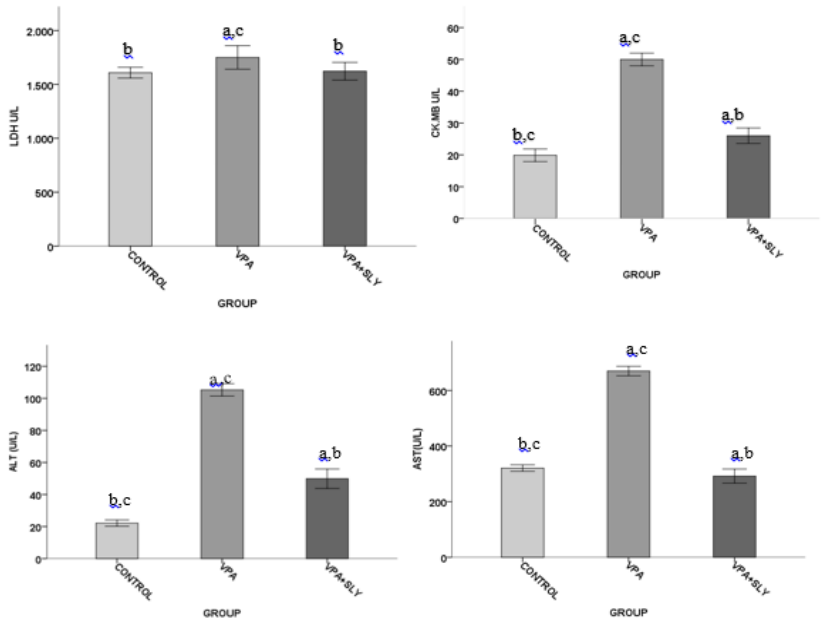

Figure 1. Effects of VPA and VPA + SLY on serum biochemical parameters. Values are means ± SEM (n = 7). [a] $p < 0.05$ vs control, [b] $p < 0.05$ vs. VPA treated rats, [c] $p < 0.05$ vs. VPA + SLY treated rats. LDH; lactate dehydrogenase. CK-MB; creatine kinase MB. ALT; alanine aminotransferase. AST; aspartate aminotransferase.

resulted in a significant decrease in LDH, CK-MB, ALT and AST levels elevated by VPA.

Oxidative Stress Biomarkers

According to the data in Table 1 and significantly higher MDA level and significantly lower GSH levels were detected in the VPA group. SLY treatments resulted in a significant decrease in VPA-induced MDA and a significant increase in VPA-induced GSH (Figure 2).

Histopathological Results

Normal heart histological tissue was confirmed in the control group (Figures 3; a-a1). In VPA group, mononuclear cell infiltrations in myofibrils, lipocytic infiltration and adipocytes, haemorrhagic areas, degeneration in myofibrils were moderately observed (Figure 3; b-b1). These histopathological findings were less common in the SLY group (Figure 3; c-c1). VPA + SLY given group; heart tissue sections; a decrease in histopathological findings was observed compared to VPA group (H – E, a-b-c x20, a1-b1-c1 x40) (Table 2).

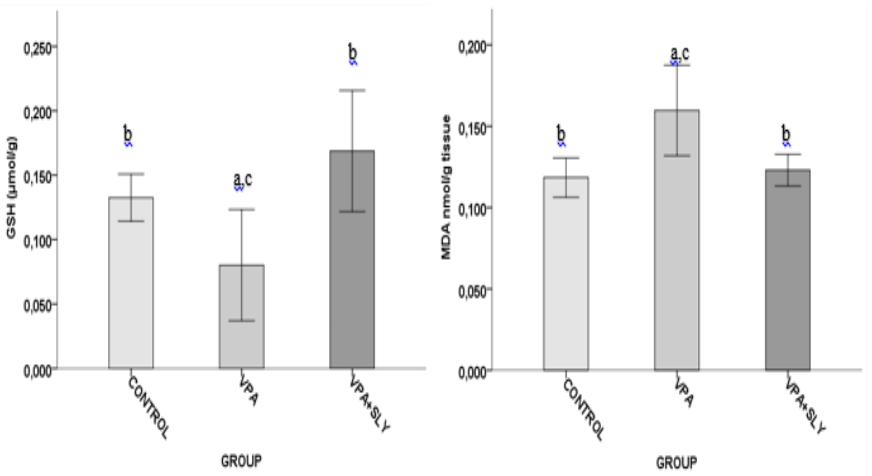

Figure 2. Effects of VPA, VPA + SLY on cardiac lipid oxidation and antioxidant profile of rats after fourteen days. Values are means ± SEM (n = 7). [a] $p < 0.05$ vs. control, [b] $p < 0.05$ vs. VPA treated rats, [c] $p < 0.05$ vs. VPA + SLY treated rats. MDA; malondialdehyde, GSH; glutathione

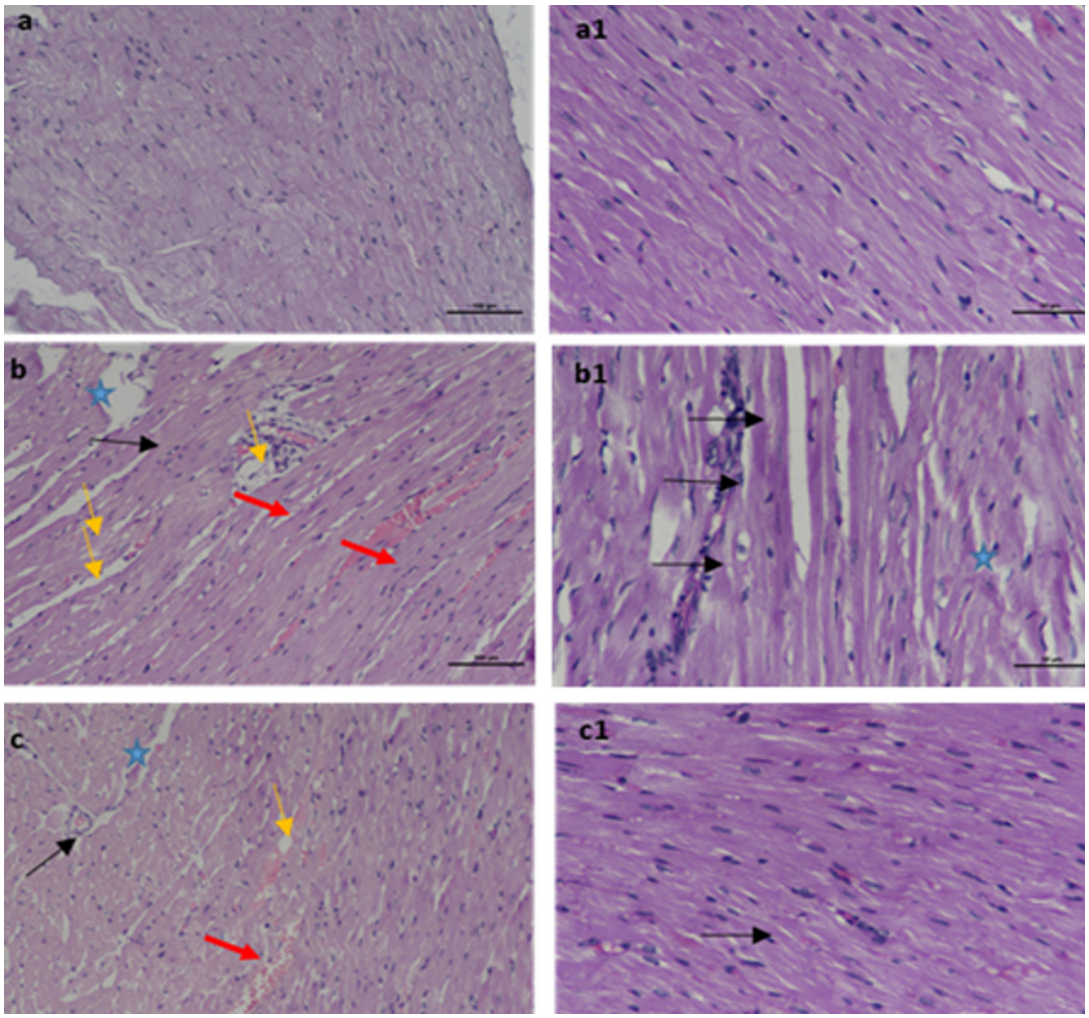

Figure 3. Rat heart tissue section. a-a1; Control group; normal histological appearance is observed in heart tissue sections. b-b1; VPA given to the group; heart tissue sections mononuclear cell infiltrations in myofibrils (black arrows), lipocytic infiltration and adposites (yellow arrows), haemorrhagic areas (red arrows), degeneration in myofibrils (blue star sections), c-c1; VPA + SLY given group; showed decrease of histopathological findings compared to VPA group (H – E, a-b-c x20, a1-b1-c1 x40).

Table 2. Histopathological scoring of heart sections of experimental groups

Parameters/scores	Control	VPA	VPA + SLY
Mononuclear Cell Infiltrations In Myofibrils	–	+++[a]	++[b]
Degeneration of Myofibrils	-	++[a]	+[b]
Lipocytic Infiltration And Adposites	-	++[a]	+[b]
Haemorrhagic Areas	-	+++[a]	++[b]

In according to modified semi-quantitative scale for the evaluation of histopathological changes; (0): none, (1): mild, (2): moderate, (3): severe grade (n = 7). VPA, valproic acid; SLY, slymarin; a: VPA increased heart damage, $p < 0.05$ vs. control group. b: SLY reduced heart damage, $p < 0.05$ vs. VPA group

DISCUSSION

In this study, we investigated the protective effects of SLY against the negative effects of VPA commonly used in the treatment of epilepsy. Heart tissue is highly susceptible to ROS-induced damage due to its high oxidative metabolism and less antioxidant defense than other organs (10). Oxidative stress is considered as one of the factors causing cardiac dysfunction. VPA treatment promotes oxidative damage, causing cardiovascular damage (18). Our study confirms previous studies showing that VPA harms the heart (19). According to recent studies, SLY is an antioxidant and an ROS cleaner. SLY reacts with cell membranes to increase their resistance to harmful effects while at the same time interacting with ROS to form less reactive and toxic compounds (10). To date, different antioxidant agents have been used to improve the toxic effect of VPA. However, there is little data on the protective effect of SLY on VPA-induced heart damage.

In this study, biochemical data increased in VPA group compared to control group, whereas SLY group significantly decreased elevated parameters. Heart damage can lead to irreversible changes in the membrane structure of the heart cells and functions as a result of increased lipid peroxidation, which causes cardiac enzymes to leak, and leaked serum concentrations are indicative of myocyte damage (8). Increased levels of LDH and CK-MB are diagnostic markers of heart damage (20). Increased production of free radicals, particularly superoxide anion radicals, can increase inflammation in the cell wall and cause atrial endothelial dysfunction. This may be the cause of ongoing myocyte degeneration, shortened coronary reserve enzyme leakage, ventricular changes (21). The results show the presence of cytotoxic free radicals, because the breakdown of unsaturated fatty acids in the membrane structure of polyunsaturated fatty acids, which has adverse effects on heart structure and function. In addition, ventricular changes, ongoing myocyte deterioration and limited coronary reserve may be causes of enzyme leakage. SLY stabilizes cardiac membranes and stops enzymes from leaking (8). VPA group compared to control and SLY group; increased levels of lipid peroxide in cardiac tissues; important symptoms of membrane damage in VPA group. SLY preserves membrane integrity by neutralizing cytotoxic free radicals and stabilizing the membrane (22). As a result of VPA application, ALT and AST levels, which are biochemical parameters, were increased. These conditions may have been caused by accumulation of VPA with toxic activity in the liver, which may have led to cellular destruction or elevation in the permeability of hepatic cells (2). Liu Y et al. confirmed that our study with VPA increased ALT and AST (23). SLY acts essentially as an antioxidant, reduces the production of ROS and lipid peroxidation, and increases endogenous concentrations of antioxidant enzymes (24). It is accepted as an indicator of the regeneration ability of damaged hepatocytes (12). These elevated values were reversed by the SLY application and were consistent with the work of Tuorkey MJ et al. (25).

In this study, a significant decrease in GSH level was found in VPA administration. This destructive effect is the result of oxidative stress caused by VPA. GSH is an endogenous antioxidant enzyme. Toxic substances lead to depletion of GSH and related antioxidants, alter cellular redox status and lead to endogenous ROS accumulation (8). SLY prevents lipid peroxidation and increases GSH concentration. Furthermore, the cell membrane regulating effect prevents microsomal defects by protecting the DNA of cardiomyocytes from harmful effects (9).

High levels of MDA induced by VPA show increased lipid peroxidation leading to heart damage and decreased antioxidant protection mechanisms (26). ROS resulting from oxidative stress are increasingly recognized in this toxicity. These oxidize carbohydrates, lipids, proteins and DNA in the heart cell and lead to modification (27). Biochemical findings in our study are consistent with previous studies (1). SLY has antioxidant properties, inhibits lipid peroxidation and increases the activity of antioxidant enzymes, which increases the level of MDA to normal (28).

In this study, we found that VPA caused damage to the heart tissue and partially reduced this damage in the SLY-treated group by histological findings. Our histological findings; confirmed biochemical results and showed that VPA causes heart tissue damage. Histological examination

of VPA group; mononuclear cell infiltrations in myofibrils, degeneration of myofibrils, lipocytic infiltration and adposites, haemorrhagic areas were detected. In a similar study by Meng et al. SLY corrected the histological structure as in this study (12). The reason why people prefer herbal treatment is increasing every day because they find it more reliable and easily accessible. The protective effect of SLY used in many studies is examined with different tissues and different analyzes (29). In our study, we observed the protective effect of SLY by histochemical and biochemical analyzes. Histopathological findings supported the hypothesis that SLY effectively preserves the histological structure and that the endogenous antioxidant defense system is effective against VPA-induced injuries, and at the same time our histological findings supported with biochemical findings, too.

CONCLUSION

Our study demonstrated that SLY improves biochemical and histological disorders in VPA-induced heart injury. In addition, the mechanism of this effect may be inhibition of lipid peroxidation and stimulation of antioxidant enzymes. This suggests that SLY may be effective in the treatment of heart damage. Further studies are needed to fully elucidate the mechanism of the antioxidant effect of SLY.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: Ethics committee report; it was obtained from the Laboratory Animals Ethics Committee (Protocol 2019/10) of Firat University Faculty of Medicine and the study was conducted according to this protocol.

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