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Original Article
Özgün Araştırma

Protective effect of silymarin and boric acid against isoproterenol-induced myocardial infarction in mice

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Abstract: This study aimed to compare the protective effects of separate and combined use of silymarin and boric acid against myocardial infarction induced by isoproterenol. Distilled water was given orally by gavage to the GI and GII groups for fourteen days. The GIII group was received silymarin (100 mg/kg), while the GIV group was given boric acid (100 mg/kg) for 14 days. In the last group, both silymarin and boric acid were given orally (gavage) for fourteen days. On the thirteenth and fourteenth days of the study, while isotonic water was applied to the GI group, isoproterenol (85 mg/kg) was applied to the other groups subcutaneously. Plasma ALT, AST, Ca, CK, CHO, TP, Ing P, IL-6, IL-12, PAL-1, and sPLA2 parameters were evaluated. Histopathological examination was performed on the heart, lung, liver, kidney, spleen, cerebrum, and cerebellum tissues taken from mice. The CK level was found to be significantly lower in the GIII and GV groups compared to the GII group ($P<0.05$). As a result, the increases shown in the plasma AST and CK activities caused due to the myocardial damage induced by isoproterenol were decreased in the silymarin and boric acid applied groups. Therefore it was thought that silymarin and boric acid may contribute to protection against myocardial infarction.

Keywords: Boric acid, Creatinine kinase, Isoproteranol, Myocardial infarction, Silymarin.

Farelerde izoproterenol ile indüklenmiş miyokard infarktüsüne karşı silimarin ve borik asidin koruyucu etkisi

Özet: Bu çalışmada izoproterenol ile oluşturulan miyokard infarktüsüne karşı silimarin ve borik asidin ayrı ve kombine kullanımlarının koruyucu etkilerinin karşılaştırılması amaçlanmıştır. GI ve GII gruplarına on dört gün boyunca gavaj ile oral yolla distile su verildi. On dört gün boyunca; GIII grubuna silimarin (100 mg/kg) verilirken GIV grubuna borik asit (100 mg/kg) verildi. Son gruba ise hem silimarin hem de borik asit on dört gün boyunca oral yolla (gavaj) verildi. Çalışmanın on üçüncü ve on dördüncü günü intraperitoneal yolla; GI grubuna izotonik uygulanırken, diğer gruplara subkutan izoproteranol (85 mg/kg) uygulandı. Plazmadan; ALT, AST, Ca, CK, CHO, TP, Ing P, IL-6, IL-12, PAL-1 ve sPLA2 parametreleri değerlendirildi. Farelerden alınan kalp, akciğer, karaciğer, böbrek, dalak, cerebrum ve cerebellum dokularında histopatolojik inceleme yapıldı. CK düzeyi GII grubuna göre GIII grubunda anlamlı düzeyde azaldığı bulundu ($P<0.05$). Sonuç olarak isoproterenolun sebep olduğu miyokardiyal hasar sonucunda AST ve CK düzeylerinde artışlarına karşı silimarin ve borik asit uygulanan gruplarda azalmalar görüldü. Bu özelliklerinden dolayı, silimarin ve borik asitin; miyokard enfarktüs durumlarına karşı korumaya katkı sağlayabileceği düşünülmektedir.

Anahtar kelimeler: Borik asit, İzoproteranol, Kreatin kinaz, Miyokard enfraktüsü, Silimarin

Introduction

Myocardial infarction is one of the most important causes of death among cardiovascular diseases (Pollard 2000). Generally, the cause of myocardial infarction is the cessation or reduction of blood flow as a result of occlusion of the vessels in a certain part of the heart resulting in necrosis in the cells (Lu et al. 2015, Saleh and Ambrose 2018). Many factors such as genetics, obesity, diabetes, and irregular nutrition trigger this disease (Lu et al. 2015).

Silymarin has been long used in traditional medicine, and extracted from the plant Silybum marianum (L.) Gaertn. It consists of many flavonolignans such as silybin A-B, and has multiple effects such

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as anti-inflammatory and antioxidant (Chambers et al. 2017, MacDonald-Ramos et al. 2021). Although silymarin has been investigated in organ toxicity such as cardiotoxicity and hepatotoxicity, there is not enough data on its effectiveness. For this reason, it is necessary to obtain data with a large scale of studies (Vahabzadeh et al. 2018).

Boron is an important element present in soil, rock, drinking water and plants, and is generally found in the body as boric acid (Murray 1998, Devirian and Volpe 2003, Uluisik et al. 2018, Karimkhani et al. 2020). Boron affects the activity of many metabolic enzymes and cell membranes in humans and animals, and in case of its deficiency, many negative conditions such as a decrease in steroid hormone level and brain functions, growth and development disorders can occur (Devirian and Volpe 2003, Uluisik et al. 2018). In general, although the effects of boron on animals and humans are limited, new studies should be conducted to clarify its positive or negative effects (Uluisik et al. 2018).

Myocardial infarction cases, which are increasing worldwide due to clinical complications, are one of the most important global health problems. Medical difficulties in the treatment of these myocardial infarction cases have led to new searches. For this reason, the demands for preventive or curative herbal medicines and/or supplements containing elements are increasing. In this study, the protective effects of silymarin and/or boric acid against isoproterenol-induced myocardial infarction were investigated.

Material and Method

Animal experimental protocol

Fifty male Balb/c mice (6-8 weeks old) with an average weight of 30±5 g were used in the study. Animals were randomly selected and divided into 5 groups of 10 Balb/c mice each. The first group (GI) was the control group and received sterile distilled water orally via gavage for fourteen days, and physiological isotonic saline was administered subcutaneously in the same amount on the 13th and 14th days of the study. The second group (GII) received sterile distilled water for 14 days via gavage. The third group (GIII) was given silymarin (100 mg/kg, Sigma, Germany) via gavage for 14 days (Papackova et al. 2018). The fourth group (GIV) was given boric acid (100 mg/kg, Merck, Germany) orally for 14 days by gavage (Karimkhani et al. 2020). The last group (GV) was given oral silymarin (100 mg/kg) and boric acid (100 mg/kg) for fourteen days. Except for the first group, all groups received 85 mg/kg isoproterenol subcutaneously on the thirteenth and fourteenth days (Zhou and Ma 2020). Blood was collected from mice under anesthesia [xylazine 10 mg/kg ip. (Xylazinbio 2%®, Bioveta, Czech Republic), ketamine 90 mg/kg ip. (Vetaketam®, Vetagro, Poland)] 24 hours after isoproterenol administration. After blood collection, animals were sacrificed, hearts were taken, and histopathological examinations were made.

Sample collection and biochemical analysis

The blood taken from Balb/c mice was centrifuged at 3000 rpm for 10 minutes at +4C° and their plasma was separated. Plasma alanine aminotransferase (ALT) and aspartate transaminase (AST) activities, creatine kinase (CK), calcium (Ca), cholesterol (CHO), inorganic phosphate (Ing P) (Teco diagnostic, USA), and total protein (TP) (Spinreact, Spain) levels were determined in a spectrophotometer device (Thermo Scientific Multiskan Go, Finland) with commercial test kits.

ELISA measurements

Plasma interleukin-6 (IL-6) levels were determined by a commercially available ELISA kit (FineTest, China). The sensitivity of the assay was defined as 2.813 pg/ml and the detection range was between 4.68-300 pg/ml. The coefficient of variation (CV) for the intraassay and interassay were <8% and <10%, respectively. The plasma levels of interleukin-12 (IL-12) were also detected by ELISA kit (FineTest, China). The analytical sensitivity of the kit was less than 9.375 pg/ml. The detection range was between 15.625-1000 pg/ml. The intraassay and interassay coefficients of variation were less than 8% and 10%, respectively. The plasminogen activator inhibitor 1 (PAI-1) levels were also detected by a commercially available ELISA kit (FineTest, China). The sensitivity of the assay was 0.938 ng/ml, and the intraassay and interassay CV values were <8% and <10%, respectively. The ELISA kit used for the detection of secretory phospholipase A2 (sPLA2) was purchased from Bioassay Technology Laboratory (Shanghai, China) and the sensitivity was 0.25 ng/ml. The intraassay and inter-assay CV values were <8% and <10%, respectively. Results are expressed according to the A_{450} values obtained from plasma samples using the calibration curve.

Histopathological Examination

Cardiac and other aforementioned organs samples were immediately fixed in 10 % formalin solution

(pH 7.4-7.6) after necropsy. The tissue samples were fixed for 48 h. After fixation, the tissues were treated with graded alcohol and xylol series and blocked in paraffin at an automatic tissue processor (Leica TP1020, Germany). Sections of 5 µm thickness were cut from the paraffin blocks (Leica 11EG50H, Germany). The sections were placed on slides, deparaffinated in xylene, and rehydrated using decreasing concentrations of ethanol. One set of slides was hematoxylin and eosin (H&E)-stained for the routine histopathological setting (Luna, 1968). The glass slides were evaluated under a digital optical light microscope and images were taken with a camera attachment (Olympus BX51 digital microscope, Japan). For scoring histopathological findings, each tissue section including several organs was obtained by counting 10 fields at 400x magnification (10 HPF).

Lesion scoring and Statistical analysis

For scoring histopathological findings, each tissue section including several organs were obtained by counting 10 fields at 400x magnification (10 HPFs). Lesions were scored semiquantitatively as (0): no lesion, (1): a few lesion, (2): mild, (3): low moderate, (4): moderate, (5): high moderate, (6): strong. Biochemical data were given as mean ± SE. As the groups showed parametric distribution, OneWay ANOVA was performed, and the Tukey test (post hoc) was used to check the significance of the difference between groups. A value of $P < 0.05$ was considered significant in the tests performed.

Results

Biochemical Findings

Plasma biochemical parameters of the control and treatment groups were given in Table 1. There was no statistical difference between the groups in terms of ALT, Ca, CHO, Ing P, and TP parameters ($P>0.05$). AST activity was found to be significantly lower in the other groups compared to the GII group ($P<0.01$). The CK level was found to be significantly decreased in the GIII and GV groups compared to the GII group ($P<0.05$). The plasma cytokine levels of the control and treatment groups were given in Table 2. There was no statistical difference between the groups in terms of IL-6, IL-12, PAI-1, and sPLA2 parameters ($P>0.05$).

Table 1. The plasma biochemical parameter levels in control and treated groups (n=10).

Parameters	GI	GII	GIII	GIV	GV
ALT (U/L)	30.22±0.89	35.23±4.50	30.18±0.76	30.8±1.15	30.64±1.06
AST (U/L)	42.38±2.42^{a}	71.81±12.13^{b}	39.19±1.30^{a}	37.21±1.42^{a}	38.36±1.63^{a}
CK (U/L)	61.61±13.59^{ab}	87.95±16.67^{a}	40.99±4.29^{b}	59.51±5.32^{ab}	47.32±5.63^{b}
Ca (mg/dl)	7.23±0.04	7.12±0.05	7.20±0.06	7.16±0.05	7.08±0.05
CHO (mg/dl)	106.00±3.22	123.40±9.10	111.60±2.60	115.80±3.30	109.00±2.60
Ing P (mg/dl)	7.10±0.28	6.48±0.25	6.61±0.45	7.21±0.51	6.78±0.38
TP (mg/dl)	3.49±0.09	3.57±0.04	3.32±0.14	3.49±0.08	3.43±0.04

^{a,b,}: The difference between values with different letters on the same line is significant. ($P<0.05$; $P<0.01$).

Table 2. The cytokine levels in control and treated groups (n=10).

Parameters	GI	GII	GIII	GIV	GV
IL-6 (pg/ml)	0.99±0.11	1.02±0.25	1.09±0.24	1.03±0.14	1.03±0.11
IL-12 (pg/ml)	26.20±5.06	23.31±2.11	22.78±1.78	23.47±1.26	23.36±1.91
PAI-1 (ng/ml)	32.47±1.24	33.18±2.44	34.04±5.24	35.61±5.14	33.33±6.35
sPLA2 (ng/ml)	1.68±0.88	2.77±2.39	1.92±1.01	1.63±0.22	1.55±0.59

The difference between values with different letters on the same line is significant. ($P<0.05$)

Macroscopic Findings

The cardiac appearance was covered with some pale foci in experimental groups although there were no findings in heart of the GI group. Lungs were hyperemic and edematous in appearance in the experimental groups. In the GI group, only euthanasia-associated artifacts were observed. Livers, kidneys, and spleens were congested and covered with pale areas in experimental groups in spite of no

lesion in the GI group. In the central nervous system (CNS), no visible findings were seen in all groups.

Histopathological Findings

Heart

In the GI group, there were no findings in heart muscles. In only isoproterenol incude GII group, loss of sitration, cytoplasmic shrinkage as well as karyopyknosis, karyolysis, and other degenerative findings were evidently seen in many areas. In the GIII group and GIV group, karyopyknosis, karyolysis, and cytoplasmic shrinkage were observed in cardiomyocytes in a few areas. In the GV group, the findings were only localized individually in some cardiomyocytes despite not being distinctive lesions. Histopathological images of the heart tissue of the control and treatment groups are given in Figure 1.

Lung

In the GI group, any findings including degeneration in the alveolar epithelium, vascular congestion, and edema were not observed. In the GII group, moderate hyperemia and edema were common in many areas. In the GIII group, these vascular changes were diminished in spite of being milder degree. Edema was also milder in some areas. There were degenerations in the alveolar epithelium of a few areas. In the GIV group, interalveolar capillary vessels and veins were filled in some areas as being the previous group. Additionally, edema was observed in the alveolar lumina. There were degenerations in only a few areas. In the GV group, there were only a few degeneration alveolar epithelium as well as hyperemia was limited in a few areas. Lung tissue histopathological images of the control and treatment groups were given in Figure 1.

Liver

In the GI group, there were no conspicuous findings at neither hepatocytes nor biliary duct or vessels. In other experimental groups, the general changes belonged to acute cell swelling-vacuolar degeneration and necrosis in hepatocytes. The affected cells had swollen/pyknotic nuclei and clear vacuoles of different sizes. Some necrotic cells lost their nuclei and their cytoplasms were dense pink colored. Additionally, sinusoids and central veins were hyperemic in appearance. In GII group animals, both degeneration at alveolar epitheliums of some areas and vascular congestion were founded at some areas in this manner. Such kind of findings and degenerations were observed in lesser areas in the GIII group and in only a few areas in the GIV group. In the GV group, there were a few degenerative changes at hepatocytes were present as many as in the GIII group. Histopathological images of the liver tissue of the control and treatment groups are given in Figure 2.

Kidney

In the GI group, there were no conspicuous findings in terms of degeneration (acute cell swelling and vacuolar degeneration) in tubule epitheliums and/or hypercellularization in glomeruli. In the GII group, severe changes including degeneration were common in spite of the previous experimental group. Karyopyknosis, karyolysis, and shrinkage cytoplasms or small vacuoles in cytoplasms were seen in tubule epitheliums. Some glomerules showed hypercellularity. In the GIII group, tubular degeneration and glomerular hypercellularization as well as milder vascular congestion were seen in many areas. In GIV and GV groups, such kind of milder degenerative changes were seen in only some tubules as well as milder vascular congestion in vessels. Histopathological images of kidney tissue in the control and treatment groups were given in Figure 2.

Spleen

In the GI group, neither hemorrhage nor follicle hyperplasia was observed. Thus, there was not any distinctive lesion in the red and white pulp in control animals. In the remaining groups, the main findings were hyperemia, free erythrocytes at the periphery of the follicle. These findings were relatively more increased in GII group. There were follicle hyperplasia and hemorrhage in some areas in this group. In the GIII group, the lesions were encountered in only a few areas. In GIV group, moderate follicle hyperplasia and milder hemorrhage were observed. In GV group, there were milder follicular hyperplasia apart from only a few areas. Other vascular findings were not attended as being previous groups. Histopathological images of spleen tissue in the control and treatment groups were given in Figure 3.

Central Nervous System

In GI group, no findings were encountered in both cerebrum and cerebellum. In experimental group, degenerations (karyopyknosis and cytoplasmic shrinkage in general) were clearly and severely observed in neurons of brain cortex and in purkinje cells of cerebellum in GII group. In GIII group, alterative lesions were seen similarly to previous group at whole cerebrum; but it was observed in only a fewer area. However, they were encountered at lesser fields in brain cortex despite not being common in cerebellum. In GIV group, there was milder degeneration in only a few areas similar to previous group.

In GV group, the findings were not common in every area of brain. But, when compared between boric acid and other experimental groups, degenerations were more diminished in neurons and purkinje cells accordingly to GII group. Histopathological images of the cerebrum and cerebellum tissue of the control and treatment groups are given in Figure 3. Mean scores of the histopathological findings of the groups were given in Table 3.

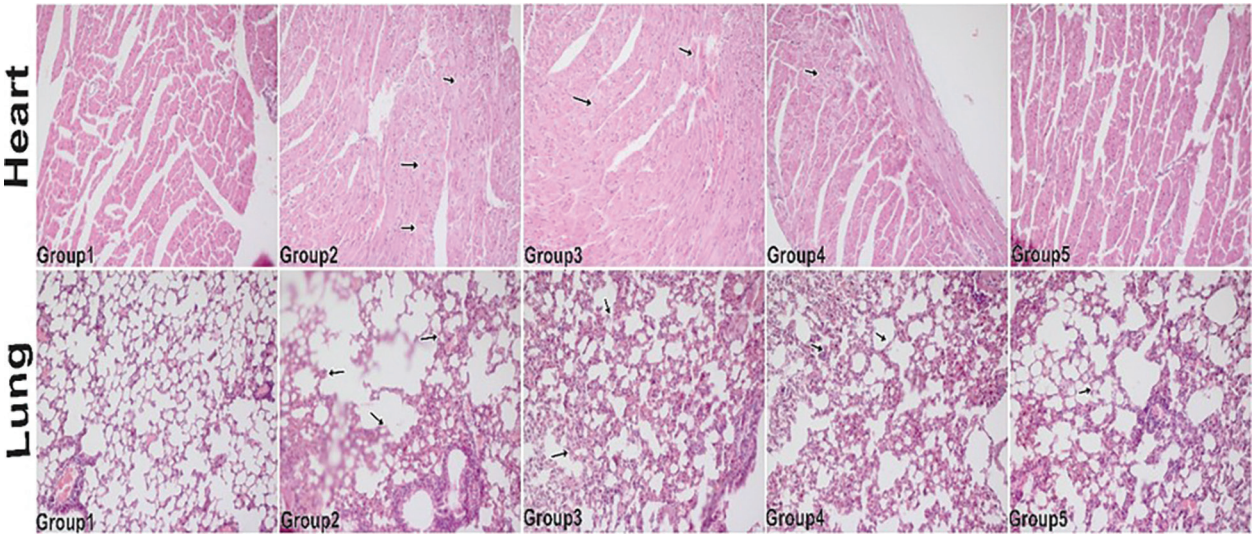

Figure 1. Paranchyme degeneration in cardiac muscles (arrows, x200, H&E staining). There is not any lesion in cardiomyocytes in GI group. There is degeneration in some areas of GII group. There is degeneration in a few areas of GIII group. There is degeneration in only a few areas of GIV group. There is no distinctive lesion in cardiomyocytes of GV group. Paranchyme degeneration in alveolar epitheliums (arrows, x200, H&E staining). There is not any lesion in paranchyme in GI group. There is degeneration in alveol at some areas and moderate vascular congestion as well as edema of GII group. There are degeneration in a few areas, milderm vascular congestion and edema of GIII group. There is degeneration in only a few areas, milder vascular congestion and edema of GIV group. There are only a few degenerative alveolar epitheliums of GV group.

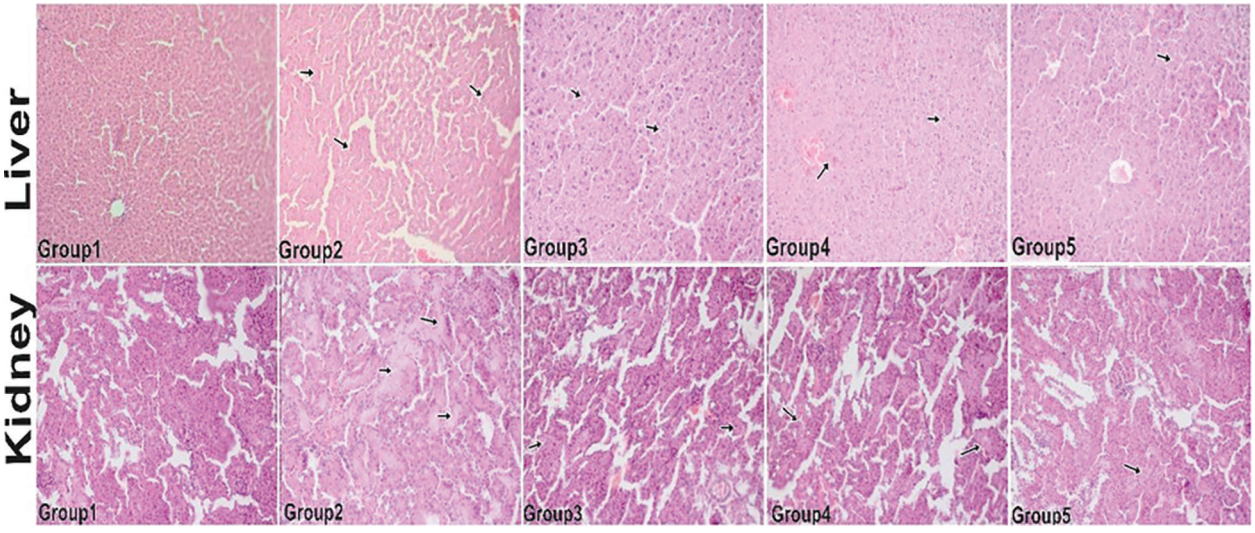

Figure 2. Acute cell swelling and vacuolar degeneration in hepatocytes (arrows, x200, H&E staining). There is not any lesion in hepatocyte in GI group. There is degeneration in alveol at some areas and moderate vascular congestion of GII group. There is degeneration in a few areas of GIII group. There is degeneration in only a few areas, milder vascular congestion and edema of GIV group, There are only a few degenerative hepatocyte of GV group. Acute cell swelling and vacuolar degeneration in renal tubules (arrows, x200, H&E

staining). There is not any lesion in tubule epitheliums in GI group. There is severe degeneration in tubule epithelium of GII group. There is moderate degeneration in a few areas, milderm vascular congestion of GIII group. There is milder degeneration in only a few areas, milder vascular congestion of GIV group. There are only a few degenerative tubule epitheliums in GV group.

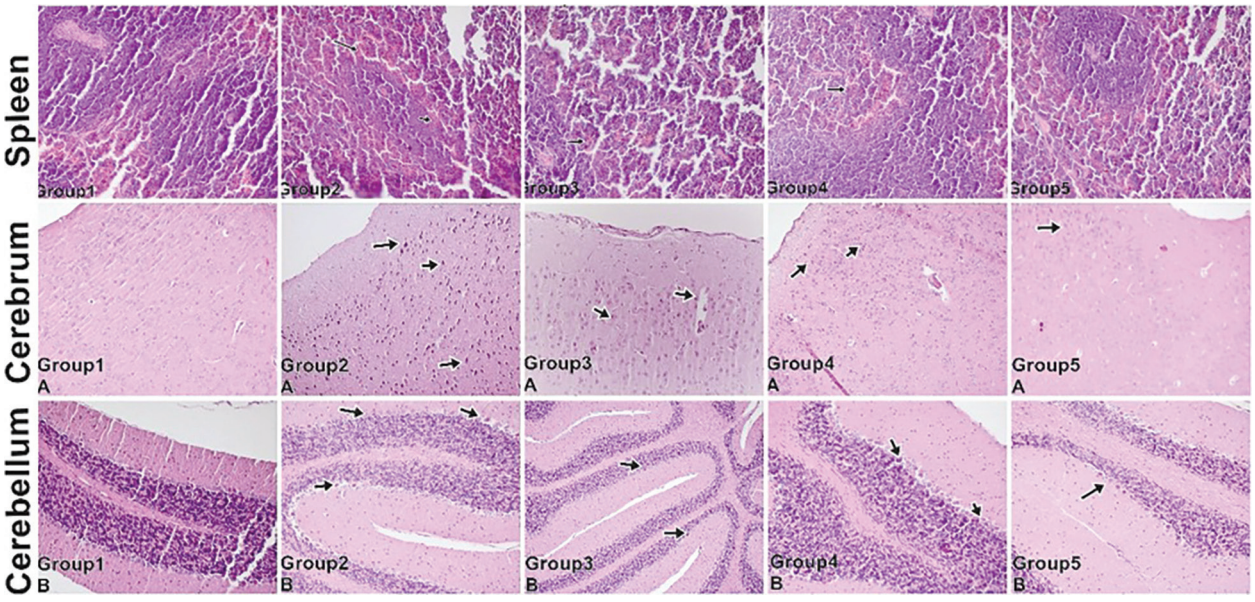

Figure 3. Hemorrhage (arrows) and follicle hyperplasia (asterix) in spleen (x200, H&E staining). There is not any lesion in red and white pulp in GI group. There are follicle hyperplasia and hemorrhage at some areas of GII group. There are lesions in only a few areas of GIII group. Moderate follicle hyperplasia and milder hemorrhage of GIVgroup. There is milder follicle hyperplasia at only a few areas of GV group. Acute cell swelling in cerebrum and cerebellum (x200, H&E). There is not any lesion i neurons in both cerebrum (A) and cerebellum (B) of GI group. There is severe degeneration in neurons of cerebrum (A) and Purkinje cells of cerebellum (B) at some areas of GII group. There is degeneration of neurons in cerebrum (A) and purkinje cells of cerebellum (B) at a few areas of GIII group. GIV: There is milder degeneration in only a few areas of cerebrum (A) and cerebellum (B), GIV group. There are only a few degenerative neurons (A) and purkinje cells (B) of GV group.

Table 3. Mean score ± standard deviation (SD) of histopathological changes at various organs.

Parameters	GI	GII	GIII	GIV	GV
Heart	0	4	3	2	2
Lung	0	4	3	3	2
Liver	0	6	4	3	2
Kidney	0	5	4	1	0
Spleen	0	4	2	2	1
Cerebrum	0	6	3	2	1
Cerebellum	0	5	3	2	1

The mean scores have been presented 0 to 6. Scores has shown number of degeneration and necrosis in organs at high power fielded microscopy.

Discussion and Conclusion

Herbal products, which are used alternatively in the treatment of various diseases, can be also used in the treatment of cardiovascular diseases (Shaito et al. 2020). Especially due to the serious side effects of drugs used for the treatment of cardiovascular diseases, interest in alternative products is increasing (Liu and Huang 2016). For this reason, the increase in the demand for herbal medicinal products, causes new products to be launched around the world. Although the rate of use of supplements increases, there is not enough information about the effects, contraindications, and side effects of these products (Ekor 2014).

Acute myocardial infarction is a cardiovascular disease that can result in death. The use of isoprote-

renol provides a reliable non-invasive model to examine changes in the body during acute myocardial infarction in terms of histopathological and biochemical changes (Pérez-Cao et al. 1994, Karthick et al. 2006). Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, causes significant disruption of the myocardial membrane and production of reactive oxygen species (ROS), which causes necrosis of the heart muscle (Mukherjee et al. 2010, Lalitha et al. 2013). Oxidative stress and inflammation play a central role in the pathogenesis of myocardial infarction (Neri et al. 2015). Excess ROS damages cardiomyocytes worsen their contractile function and increased capillary leakage that causes cardiac pathology (Sugamura and Keaney, 2011). In such cases, cardiac enzymes such as CK, ALT, AST, and LDH, which are defined as biochemical markers for myocardial damage, can be found in the blood, and the enzyme levels in the blood were increased (Mathew et al. 1985, Karthick et al. 2006, Priscilla and Prince 2009, Mert et al. 2018).

Although there were numerical differences in inflammatory cytokines (IL-6, IL-12) and cardiovascular markers (PAI-1 and sPLA-2) among the trial groups, these differences were not statistically significant. According to the biochemical parameters, histopathological findings are mostly concentrated in the heart and accordingly the liver, brain and cerebellum, kidney, and spleen. Low CK levels showing directly cardiac alterations showed most prominent at GIII and GV groups. At this point, the values are found coherently to previous literatures to be related to acute cardiac failure (Devrim et al. 2017, Mert et al. 2018, Zhou and Ma 2020). Elevated AST and CK levels in GII group are related to disruption of histoarchitecture and alterations in cardiomyocytes as well as other tissues. Boric acid, solely, administration and boric acid and silymarin co-administration have been shown great parallelism in especially heart and other organs. Silymarin administration, solely, has not been preventive effective against degeneration and other vascular changes as much as it has been considered in heart, lung, liver. It is concluded that boric acid might be potentfull for prevention of lesions; however, it is though that its combined effect with silymarin is more elevated.

Various studies have been conducted to investigate the therapeutic roles of different applications against myocardial damage caused by isoproterenol. In a study evaluating the protective effect of rutin, a bioflavonoid, in isoproterenol-induced myocardial infarction, it was reported that serum CK, LDH, AST and ALT activity in the isoproterenol group increased significantly compared to the control group, and the enzyme levels that had increased with routine application decreased (Karthick et al. 2006).

In addition, it was shown that the enzymatic activity in the heart of the isoproterenol-treated experimental group was significantly reduced compared to the control group. This has been associated with the fact that isoproterenol causes complex biochemical and structural changes that lead to cell damage and necrosis by depleting the energy reserve of heart muscle cells (Rona 1985, Karthick et al. 2006).

In a study by Nivethetha et al. (2009), the protective effect of M. calabra plant extract against the cardiotoxic effects of isoproterenol was investigated and an increase in serum AST values was reported in the isoproterenol group compared to the control group. Devrim et al. (2017) also examined the protective effects of periostin against the cardiotoxic effect of isoproterenol in their study on rats and reported that periostin application caused a decrease in CK, AST, ALT and LDH levels, which were increased with isoproterenol application. Similarly, Mert et al. (2018) reported that AST, CK and LDH levels were higher in rats with myocardial damage caused by isoproterenol compared to other groups, and kefir used for protective purposes significantly reduced the toxic effects of isoproterenol and caused a decrease in enzyme levels. In these studies, they reported that isoproterenol application induced AST, ALT and CK levels, possibly due to the increase in the activities of innervated lysosomal enzymes (Abbas 2016, Devrim et al. 2017, Mert et al. 2018).

In this study, the increase in AST and CK enzyme levels after isoproterenol application is compatible with the studies of Abbas 2016, Devrim et al. 2017 and Mert et al. 2018. The decrease in AST and CK enzyme levels in the treatment groups after isoproterenol application is thought to be due to the decrease in the activities of lysosomal enzymes. Significant increases in plasma CK level, which is used as a sensitive biomarker especially in skeletal muscle and myocardium injuries, have also been reported in other studies in rats administered isoproterenol (Tiwari et al. 2009, De Sánchez et al. 2012, Mert et al. 2018) and this is in agreement with the data of this study.

Various studies have revealed that substances with antioxidant activity may be important for therapeutic use in preventing expected heart damage, and may reduce the incidence and mortality from heart disease (McCullough et al. 2012). The increase in AST and CK levels as a result of myocardial dama-

ge induced by isoproterenol (the strongest sympathomimetic drug that affects beta-adrenergic receptors) improved by silymarin and boric acid, whose therapeutic effectiveness was evaluated within the scope of the study, therefore it can be suggested that the silymarin and boric acid have positive effects against excessive increases in enzyme levels. It is thought that it may contribute to protection against myocardial infarction conditions.

Ethical Statement: The study was carried out after the animal experiment approval of the Kırıkkale University Local Ethics Committee (Decision no: 2021/03 - 10).

Financial Support and Conflict of interest: This research no people/organizations that financially support, and the authors do not have any interest-based relationships.

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