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Cardioprotective Effect of *Pistacia vera L.* (Green Pistachio) Hull Extract in Wistar Albino Rats with Doxorubicin-Induced Cardiac Damage

ABSTRACT

Background: *Pistacia vera* L. (green pistachio) has been shown to increase antioxidant capacity and protect against cardiovascular diseases and cancer. This study investigated the protective effect of the *Pistacia vera* L. hull in rats with experimental cardiac damage induced by doxorubicin.

Methods: Sixty adult Wistar albino rats were randomly divided into 5 groups (n = 12). Sham, doxorubicin, doxorubicin + *Pistacia vera* L. extract 50 mg/kg, doxorubicin + *Pistacia vera* L. extract 100 mg/kg, and *Pistacia vera* L. extract 100 mg/kg. Biochemistry parameters, total antioxidant status, total oxidant status, oxidative stress index, 8-hydroxy-deoxyguanosine, and caspase 3/7 values were measured in serum samples. Excised heart tissues were examined histopathologically.

Results: The groups were statistically significantly different in 8-hydroxy-deoxyguanosine, caspase 3/7, total antioxidant status, total oxidant status, oxidative stress index, and basal biochemical parameter values ($P < .05$, $P < .001$). In group II, 8-hydroxy-deoxyguanosine, caspase 3/7, and total oxidant status values increased while the total antioxidant status value decreased ($P < .001$). In the treatment groups (group III and group IV), 8-hydroxy-deoxyguanosine and caspase 3/7 values decreased compared to group II ($P < .001$). While total oxidant status and oxidative stress index values decreased in the treatment groups, total antioxidant status values increased ($P < .001$). The histopathological examination of the heart revealed fewer areas of focal necrosis in the treatment groups compared to group II.

Conclusion: In this study, the cardioprotective effect of *Pistacia vera* L. hull extract was investigated in vivo. It was shown that *Pistacia vera* L. hull extract reduced apoptosis and deoxyribonucleic acid damage in the face of cardiac damage and had antioxidant activity. Future studies will increase our knowledge on this subject.

Keywords: *Pistacia vera* L. (green pistachio) hull extract, doxorubicin, cardiac damage, apoptosis, antioxidant

INTRODUCTION

Doxorubicin (DOX) is a broad-spectrum antibiotic, an anthracycline group chemotherapeutic agent that is frequently used in cancer treatment.[1] It has been shown that anthracyclines induce apoptotic cell death,[2] and DOX causes the formation of reactive oxygen radicals in the cell.[3-5] Oxidative damage to membrane lipids and other cellular components is considered a major factor in the toxicity of DOX and other anthracycline antibiotics. Accordingly, many researchers have ascertained that the use of natural and synthetic antioxidants can be protective against oxidative stress caused by DOX and other cytotoxic drugs.[5-7] Today, the importance of using antioxidant molecules in the treatment of diseases caused by free radicals makes new natural antioxidant sources even more crucial.

The use of antioxidants has been shown to play a positive role in the treatment of the most common diseases such as cardiovascular disease (CVD) and cancer, and studies on healthy people and people with heart disease have revealed that antioxidants reduce free radicals and protect low-density lipoprotein (LDL) against oxidation.[8] Thanks to its phenolic content, *Pistacia vera* L. (*P.v.L.*) is among the top

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50 foods with antioxidant potential.[9,10] *Pistacia vera* L. is one of the most water- and fat-soluble antioxidants among all tree nuts.[11] Anthocyanin-containing *P.v.L.* contains important bioactive polyphenols such as transreservatrol[12,13] and isoflavones with anti-cancer potential.[14] Phenolic compounds play a critical role in maintaining the balance between oxidants and antioxidants in the body.[15] With its monounsaturated fat, vitamin, and antioxidant content, *P.v.L.* has been found to be beneficial in anti-inflammatory activity, glycemic control, protection of endothelial function, and prevent low oxidation in various studies. It has been suggested to have a protective role against LDL in atherogenesis and chronic diseases such as cancer and CVD.[16,17]

In this study, the protective effect of *P.v.L.* (green pistachio) hull with high antioxidant properties was investigated in Wistar albino rats, in which an experimental cardiac injury model was created with DOX.

METHODS

Ethical Approval

This study was conducted with the approval of the Scientific Committee – dated August 11, 2021, 2021/008/07 session number 01-08 – and financial support with the Scientific Research Projects Coordination Unit (BAP) of our university with project number 21152. Wistar albino adult female and male rats deemed suitable for the study were obtained from the University Experimental Animal Research Center.

Establishment of Study Groups and Experimental Cardiac Injury Model

In this study, 60 adult Wistar albino rats (average weight: female rat 250-300 g, male rat 450-500 g) were randomly divided into 5 equal groups [6 males, 6 females (n=12)]: group I (sham), group II (DOX), group III [treatment group I (DOX + *P.v.L.* hull extract 50 mg/kg)], group IV [treatment group II (DOX + *P.v.L.* hull extract 100 mg/kg)], group V (*P.v.L.* hull extract 100 mg/kg). The reason why we use both genders in rats, both male and female, is: heart diseases are found in people of both sexes, and one of the risk factors is gender. However, we could not obtain a statistically significant result in the evaluation made between rats according to their gender, so we presented the data in the general table without gender discrimination. Previously, male and female rats were treated with *P.v.L.* The response to this question was evaluated and compared separately. In order for the rats to adapt to the changing environmental conditions, they were housed for 5 days under routine housing conditions (temperature 22 ± 2°C, 50% relative humidity, 12 hours of light and 12 hours of darkness, in type 3 cages with a transparent visible interior, designed to add standard rat chow, and ad libitum water) without any experimental intervention. All rats were fed with tap water and standard rat chow under standard conditions. The feeding of the rats was completely stopped 12 hours before the intervention. Female rats included in the study were placed in separate cages from male rats after the completion of the lactation period. During the study, experimental protocols were applied to rats grouped as male and female in separate cages. Due to this, vaginal plaque was not observed in female animals, and vaginal smear was not taken. Cardiac injury models were established as in previous study.[18] For the experimental cardiac injury model, only food and water were given to group I for 15 days. In group II, DOX (2.5 mg/kg/day (15 days), a total dose of 37.5 mg/kg was administered intraperitoneally (IP). Group III was given only DOX for the first 7 days, then DOX and *P.v.L.* hull extract 50 mg/kg/day as gavage for 7 days. Group IV was given only DOX for the first 7 days, then DOX and *P.v.L.* hull extract 50 mg/kg/day as gavage for 7 days. In total, the duration of treatment was 15 days. Group V was given water for the first 7 days and *P.v.L.* hull extract by gavage for the next 7 days (Table 1). In total, the treatment lasted 15 days. At the end of the experiment (day 16), all rats were sacrificed under deep anesthesia (ketamine 90 mg/kg and xylazine 10 mg/kg IP), and their blood and all tissues were stored under appropriate conditions (Figure 1). Rats' daily weight, feed, and water intake were followed up and noted. In the DOX-administered groups, the rats lost weight, their nutrition (feed and water consumption) decreased, they had diarrhea and nosebleeds and became weak. One male rat died in group II and group III.

Preparation of Doxorubicin

Doxorubicin was injected from a vial of 50 mg active ingredient as 2.5 mg in 1 mL per 1 kg. It was diluted by adding 20 mL into the 50-mg vial. It was adjusted as 2.5 mg in 1 mL per 100 g weight and administered to rats as 0.1 mL (10 IU) with an insulin injector IP.

Preparation of Pistacia vera L.

Pistacia vera L. hull samples to be used in the study were dried in a dark environment in July 2020. The green mesocarp hull of *P.v.L.* was powdered with a blender, and 50 g of powdered *P.v.L.* hull samples was incubated in methanol (MeOH) : water (1 : 1, v/v) at 40°C overnight. The samples were homogenized in small amounts using an ultrasonicator for 15 min. Then the samples were filtered with a Whatman filter, and the alcohol solvents were evaporated with the help of a rotary evaporator at a temperature not exceeding 40°C. Crude MeOH extracts were powdered in a lyophilizer. Then, this dry crude MeOH extract was dissolved in water and given to the rats.

Blood Plasma and Research Method

The blood taken from the heart and veins of the deeply anesthetized rats was transferred to yellow-capped biochemistry tubes without anticoagulant. These blood samples were centrifuged at 4000 revolutions per minute for 10 min, and the plasma was transferred to Eppendorf tubes to be stored at –80°C until the day of the experiment. On the experiment

HIGHLIGHTS

- Doxorubicin (DOX) is a drug used for chemotherapy but still causes heart damage. Many antioxidants are being tested to prevent damage.
- *Pistacia vera* L. is a powerful antioxidant substance, and we found that it reduces the effects of DOX.
- In the study on rats, it was seen that it reduced the damage especially through 8-hydroxy-deoxyguanosine, pro-brain natriuretic peptide, caspase 3, and caspase 7.

Table 1. Working Groups

Control and Experimental Groups	Injection (IP)	Gavage To Be Applied Once a Day Every Day
Group I (Sham) (n = 12) (female: 6, male: 6)	-	-
Group II (DOX) (n = 12) (female: 6, male: 6)	DOX 2.5 mg/kg/day (15 days)	-
Group III (DOX + *P.v.L.* 50 mg/kg) (n = 12) (female: 6, male: 6)	DOX 2.5 mg/kg/day (15 days)	After the 7th day, *P.v.L.* 50 mg/kg was added to the treatment
Group IV (DOX + *P.v.L.* 100 mg/kg) (n = 12) (female: 6, male: 6)	DOX 2.5 mg/kg/day (15 days)	After the 7th day, *P.v.L.* 100 mg/kg was added to the treatment
Group V (*P.v.L.* 100 mg/kg) (n = 12) (female: 6, male: 6)	-	*P.v.L.* 100 mg/kg (7 days)

DOX, doxorubicin; IP, intraperitoneally; *P.v.L.*, *Pistacia vera* L.

day, these samples were thawed at room temperature, and the parameters were analyzed.

Our study protocol was carried out in 3 steps. In the first step, basal biochemical parameters were measured using Siemens brand commercial kits. Alanine aminotransferase (ALT), urea, and creatinine (Cr) parameters were measured in Atellica Solution device; pro-brain natriuretic peptide (pro-BNP) was measured in the AQT90 Flex device, and troponin I and creatine kinase (CK-MB) values were measured in the Advia Centaur XP immunoassay System device. In the second step, caspase 3/7 and 8-hydroxy-deoxyguanosine (8-OHdG) (BT LAB, Cat. no E0031Ra) were measured with enzyme-linked immunosorbent assay. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values were measured. Total antioxidant status and TOS levels of the samples were measured using Rel Assay brand commercial kits. Total antioxidant status levels

Figure 1. Laparotomy and sternotomy procedures of the rats.

Table 2A. Group I (Sham) Statistical Analysis by Gender

	Group I (Sham) Male			Group I (Sham) Female			
	Min	Max	SD	Min	Max	SD	P[a]
8-OHdG (ng/mL)	0.35	0.69	0.57 ± 0.16	0.23	0.67	0.43 ± 0.16	.202
Caspase 3 (ng/L)	0.88	1.32	1.2 ± 0.18	0.66	1.32	1.07 ± 0.24	.243
Caspase 7 (ng/L)	0.62	0.97	0.79 ± 0.13	0.51	0.92	0.76 ± 0.15	.917
TAS (mmol Trolox equivalent/L)	1.43	1.85	1.62 ± 0.18	1.55	2.03	1.71 ± 0.19	.465
TOS (μmol H_2O_2equivalent/L)	11.3	14.5	12.58 ± 1.17	10.2	15.6	13.09 ± 2.37	.753
OSI (AU)	0.66	0.91	0.79 ± 0.11	0.65	0.97	0.77 ± 0.13	.675
Urea (mg/dL)	23.54	42.8	37.24 ± 8.09	29.96	35.01	32.25 ± 2.35	.115
Creatinine (mg/dL)	0.3	0.6	0.4 ± 0.12	0.3	0.5	0.44 ± 0.09	.448
e-GFR (mg/dL)	112	148	134.4 ± 14.86	120	148	128 ± 12.33	.448
ALT (U/L)	42	78	55.8 ± 13.35	34	48	42.2 ± 6.3	.047
CK-MB (U/L)	0.01	0.04	0.03 ± 0.01	0.01	0.03	0.02 ± 0.01	.501
Troponin-I (pg/ML)	7.6	16.95	12.65 ± 4.48	9.74	58.7	21.5 ± 20.9	.754
Pro-BNP (ng/L)	40	60.3	50.96 ± 7.38	42.6	60	49.74 ± 8.12	.754

[a]Mann–Whitney U-test. $P < .05$, $P < .001$. Sham male (n = 6), Sham female (n = 6).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

Table 2B. Group II (DOX) Statistical Analysis by Gender

	Group II (DOX) Male			Group II (DOX) Female			
	Min	Max	SD	Min	Max	SD	P[a]
8-OHdG (ng/mL)	1.17	1.35	1.31 ± 0.09	1.56	2.14	1.86 ± 0.29	.180
Caspase 3 (ng/L)	2.26	2.61	2.42 ± 0.18	1.24	2.95	2.32 ± 0.74	.564
Caspase 7 (ng/L)	1.75	2.24	1.94 ± 0.21	1.74	2.11	2.02 ± 0.19	.767
TAS (mmol Trolox equivalent/L)	0.66	0.97	0.82 ± 0.14	0.76	1.1	0.94 ± 0.15	.386
TOS (μmol H_2O_2 equivalent/L)	15.4	17.4	16.27 ± 1.02	15.3	19.09	17.67 ± 1.65	.245
OSI (AU)	1.58	2.64	2.03 ± 0.44	1.74	2.08	1.9 ± 0.17	1.000
Urea (mg/dL)	14.74	35.4	23.11 ± 9.64	21.1	51.36	32.03 ± 13.27	.245
Creatinine (mg/dL)	0.3	0.6	0.48 ± 0.13	0.3	0.6	0.43 ± 0.13	.554
e-GFR (mg/dL)	112	148	125 ± 15.79	132	148	140 ± 9.24	.134
ALT (U/L)	16	35	26.75 ± 7.89	22	32	27.5 ± 4.43	1.000
CK-MB (U/L)	0.01	0.05	0.03 ± 0.02	0.02	0.08	0.05 ± 0.03	.243
Troponin-I (pg/mL)	8.14	27.55	20 ± 8.36	7.93	24.7	16.96 ± 7.23	.386
Pro-BNP (ng/L)	62	69.9	65.33 ± 3.96	59	69.5	64.9 ± 4.51	.773

[a]Mann–Whitney *U*-test. $P < .05$, $P < 0.001$. Group II (DOX) male (n = 6), group II (DOX) female (n = 6).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

in tissues were expressed as Trolox equivalent/L, and TOS results as μmol H_2O_2 equivalent/L.[19,20] Oxidative stress index values, an indicator of oxidative stress, were expressed in arbitrary units (AU). In the third stage, the cardiac tissues of the rats were examined histopathologically. Finally, all data were analyzed statistically.

Histopathological Examination of Cardiac Tissue

Cardiac sections taken from rats were placed in 10% formaldehyde. Then, the samples taken from these sections were placed in a tissue-processing device (Leica Bond-Max Immunohistochemistry) for 4 h and embedded in paraffin after tissue processing. Sections of 4 μm thickness were taken from these samples and stained with hematoxylin-eosin stain. The presence of necrosis, inflammation, and edema in the cardiac tissue was examined under a microscope, and the findings were evaluated as mild–moderate–severe. In order to calculate the focal necrosis areas in the groups, all samples were examined histopathologically under a NiU microscope with 40× magnification. The mean area of necrosis was calculated for each rat.

Statistical Analysis

The compliance of the obtained data to normal distribution was checked with Kolmogorov–Smirnov and Shapiro–Wilk tests. Independent samples *t*-test was used for normally

Table 2C. Group III (DOX + *P.v.L.* 50 mg) Statistical Analysis by Gender

	Group III (DOX + *P.v.L.* 50 mg) Male			Group III (DOX + *P.v.L.* 50 mg) Female			
	Min	Max	SD	Min	Max	SD	P[a]
8-OHdG (ng/mL)	1.1	1.27	1.16 ± 0.07	1.15	1.6	1.38 ± 0.19	.47
Caspase 3 (ng/L)	1.75	2.24	1.97 ± 0.2	1.86	2.11	2.01 ± 0.1	.671
Caspase 7 (ng/L)	1.16	1.49	1.35 ± 0.13	1.32	2.01	1.63 ± 0.3	.117
TAS (mmol Trolox equivalent/L)	1.14	2.21	1.76 ± 0.41	0.9	1.2	1.06 ± 0.14	.280
TOS (μmol H_2O_2 equivalent/L)	12.1	16.5	15.3 ± 1.85	12.3	15.6	13.98 ± 1.22	.173
OSI (AU)	0.73	1.06	0.89 ± 0.15	1.12	1.59	1.33 ± 0.17	.009
Urea (mg/dL)	29.96	85.6	55.12 ± 22.2	32.1	104.86	64.2 ± 34.37	.602
Creatinine (mg/dL)	0.11	0.5	0.4 ± 0.17	0.4	0.4	0.4 ± 0	.232
e-GFR (mg/dL)	112	132	122.8 ± 8.2	132	132	132 ± 0	.180
ALT (U/L)	20	58	29.6 ± 15.96	19	28	23.8 ± 3.49	1.000
CK-MB (U/L)	0.01	0.03	0.02 ± 0.01	0.01	0.04	0.02 ± 0.01	.661
Troponin-I (pg/mL)	23.31	193.32	149.52 ± 72.69	8.05	200.4	138.89 ± 75.93	.602
Pro-BNP (ng/L)	56	65.8	60.74 ± 3.7	57.55	64	61.06 ± 3.05	.917

[a]Mann–Whitney U-test. $P < 0.05$, $P < 0.001$. Group III (DOX + *P.v.L.* 50 mg) male (n = 6), group III (DOX + *P.v.L.* 50 mg) female (n = 6).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinase; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; *P.v.L.*, *Pistacia vera* L.; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

Table 2D. Group IV (DOX + *P.v.L.* 100 mg) Statistical Analysis by Gender

	Group IV (DOX + *P.v.L.* 100 mg) Male			Group IV (DOX + *P.v.L.* 100 mg) Female			
	Min	Max	SD	Min	Max	SD	*P*[a]
8-OHdG (ng/mL)	0.54	2.3	1.5 ± 0.65	0.71	0.95	0.81 ± 0.12	.116
Caspase 3 (ng/L)	1.32	1.81	1.52 ± 0.18	1.2	1.54	1.36 ± 0.15	.175
Caspase 7 (ng/L)	0.19	1.38	0.84 ± 0.45	0.88	1.44	1.08 ± 0.25	.600
TAS (mmol Trolox equivalent/L)	1.44	1.91	1.75 ± 0.18	1.61	1.88	1.7 ± 0.11	.346
TOS (μmol H_2O_2 equivalent/L)	12.3	16.3	14.48 ± 1.66	10.4	15.3	12.72 ± 2.38	.161
OSI (AU)	0.69	1.06	0.84 ± 0.15	0.62	0.92	0.75 ± 0.12	.293
Urea (mg/dL)	70.62	211.86	164.78 ± 54.5	27.82	150.4	85.29 ± 60.3	.280
Creatinine (mg/dL)	0.12	0.6	0.36 ± 0.2	0.2	0.5	0.36 ± 0.11	.915
e-GFR (mg/dL)	112	175	131.8 ± 25.18	112	175	134.2 ± 24.34	.746
ALT (U/L)	13	21	14.8 ± 3.49	25	58	36.2 ± 13.63	.812
CK-MB (U/L)	0.01	0.05	0.03 ± 0.01	0.01	0.05	0.02 ± 0.02	.514
Troponin-I (pg/mL)	19.44	40.11	29.92 ± 7.47	9.21	37.98	28.48 ± 11.34	.754
Pro-BNP (ng/L)	51.01	65.2	59.41 ± 6.06	53.5	63.45	59.12 ± 3.97	.754

[a]Mann–Whitney U-test. $P < 0.05$, $P < 0.001$. Group IV (DOX + *P.v.L.* 100 mg) male (n = 6), group IV (DOX + *P.v.L.* 100 mg) female (n = 6).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; *P.v.L.*, *Pistacia vera* L.; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

distributed data, and the Mann–Whitney *U*-test was used for comparisons between 2 non-normally distributed independent groups. In the comparison of 2 or more independent groups, one-way analysis of variance multiple comparison tests were used for normally distributed data, and the Kruskal–Wallis test and all the pairwise multiple comparison tests were used for non-normally distributed data. The data were presented as mean ± standard deviation for numerical variables and as numbers and percentages for categorical variables. Statistical significance was accepted as $P < .05$, and the Statistical Package for Social Sciences Windows version 24.0 software package was used for analysis.

RESULTS

No statistically significant results were obtained in the evaluation of rats according to their genders. *Pistacia vera* L. female and male rats were evaluated and compared separately. The data obtained are presented in Tables 2A to 2E. Statistical analyses of the determined groups are shown in Table 3. The 8-OHdG, caspase 3, caspase 7, TAS, TOS, OSI, urea, ALT, CK-MB, troponin-I, and

Table 2E. Group V (*P.v.L.* 100 mg) Statistical Analysis by Gender

	Group V (*P.v.L.* 100 mg) Male			Group V (*P.v.L.* 100 mg) Female			
	Min	Max	SD	Min	Max	SD	*P*[a]
8-OHdG (ng/mL)	0.84	1.14	0.98 ± 0.1	0.84	1.14	0.98 ± 0.1	.522
Caspase 3 (ng/L)	0.16	1.15	0.72 ± 0.33	0.16	1.15	0.72 ± 0.33	.226
Caspase 7 (ng/L)	0.34	0.67	0.48 ± 0.13	0.34	0.67	0.48 ± 0.13	.335
TAS (mmol Trolox equivalent/L)	1.62	2.1	1.92 ± 0.16	1.62	2.1	1.92 ± 0.16	.626
TOS (μmol H_2O_2 equivalent/L)	9.69	11.97	10.39 ± 0.87	9.69	11.97	10.39 ± 0.87	.300
OSI (AU)	0.47	0.64	0.55 ± 0.06	0.47	0.64	0.55 ± 0.06	.296
Urea (mg/dL)	29.96	42.8	34.01 ± 4.66	29.96	42.8	34.01 ± 4.66	.101
Creatinine (mg/dL)	0.3	0.5	0.4 ± 0.06	0.3	0.5	0.4 ± 0.06	1.000
e-GFR (mg/dL)	120	148	132.67 ± 8.91	120	148	132.67 ± 8.91	1.000
ALT (U/L)	45	56	50.5 ± 3.78	45	56	50.5 ± 3.78	.149
CK-MB (U/L)	0.01	0.03	0.02 ± 0.01	0.01	0.03	0.02 ± 0.01	.473
Troponin-I (pg/mL)	9.67	35.55	22.85 ± 9	9.67	35.55	22.85 ± 9	.631
Pro-BNP (ng/L)	47.2	62.1	55.46 ± 5.78	47.2	62.1	55.46 ± 5.78	.337

[a]Mann–Whitney U-test. $P < 0.05$, $P < 0.001$. Group V (*P.v.L.* 100 mg) male (n = 6), group V (*P.v.L.* 100 mg) female (n = 6).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; *P.v.L.*, *Pistacia vera* L.; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

Table 3. Statistical Analysis of More Than 2 Groups

	Group I (Sham)			Group II (DOX)			Group III (DOX + *P.v.L.* 50 mg)			Group IV (DOX + *P.v.L.* 100 mg)			Group V (*P.v.L.* 100 mg)			
	Min	Max	SD	Min	Max	SD	Min	Max	SD	Min	Max	SD	Min	Max	SD	*P*[a]
8-OHdG (ng/mL)	0.23	0.69	0.5 ± 0.17	1.17	2.14	1.58 ± 0.36	1.1	1.6	1.27 ± 0.18	0.54	2.3	1.16 ± 0.57	0.84	1.3	1.02 ± 0.15	**.001****
Caspase 3 (ng/L)	0.66	1.32	1.14 ± 0.22	1.24	2.95	2.37 ± 0.5	1.75	2.24	1.99 ± 0.15	1.2	1.81	1.44 ± 0.18	0.16	1.41	0.83 ± 0.31	**.001****
Caspase 7 (ng/L)	0.51	0.97	0.78 ± 0.13	1.74	2.24	1.98 ± 0.19	1.16	2.01	1.49 ± 0.26	0.19	1.44	0.96 ± 0.36	0.12	0.77	0.43 ± 0.19	**.001****
TAS (mmol Trolox equivalent/L)	1.43	2.03	1.67 ± 0.18	0.66	1.1	0.88 ± 0.15	0.9	2.21	1.41 ± 0.47	1.44	1.91	1.72 ± 0.14	1.62	2.34	1.96 ± 0.19	**.001****
TOS (μmol H_2O_2 equivalent/L)	10.2	15.6	12.84 ± 1.78	15.3	19.09	16.97 ± 1.48	12.1	16.5	14.64 ± 1.63	10.4	16.3	13.6 ± 2.15	9.69	14.2	11.31 ± 1.47	**.001****
OSI (AU)	0.65	0.97	0.78 ± 0.11	1.58	2.64	1.97 ± 0.32	0.73	1.59	1.11 ± 0.28	0.62	1.06	0.79 ± 0.14	0.47	0.8	0.58 ± 0.1	**.001****
Urea (mg/dL)	23.54	42.8	34.75 ± 6.2	14.74	51.36	27.57 ± 11.75	29.96	104.86	59.66 ± 27.7	27.82	211.86	125.04 ± 68.49	29.96	55.64	39.47 ± 7.47	**.001****
Creatinine (mg/dL)	0.3	0.6	0.42 ± 0.1	0.3	0.6	0.45 ± 0.12	0.11	0.5	0.4 ± 0.11	0.12	0.6	0.36 ± 0.15	0.3	0.5	0.4 ± 0.06	.566
e-GFR (mg/dL)	112	148	131.2 ± 13.31	112	148	132.5 ± 14.41	112	132	127.4 ± 7.31	112	175	133 ± 23.38	120	148	132.67 ± 8.5	.901
ALT (U/L)	34	78	49 ± 12.17	16	35	27.13 ± 5.94	19	58	26.7 ± 11.31	13	58	25.5 ± 14.67	40	56	48.5 ± 4.87	**.001****
CK-MB (ng/ml)	0.01	0.04	0.02 ± 0.01	0.01	0.08	0.04 ± 0.02	0.01	0.04	0.02 ± 0.01	0.01	0.05	0.03 ± 0.01	0.01	0.04	0.02 ± 0.01	**.014***
Troponin-I (pg/ml)	7.6	58.7	17.08 ± 14.99	7.93	27.55	18.48 ± 7.41	8.05	200.4	144.2 ± 70.3	9.21	40.11	29.2 ± 9.08	5.61	37.92	21.77 ± 11.18	**.001****
Pro-BNP (ng/L)	40	60.3	50.35 ± 7.34	59	69.9	65.12 ± 3.94	56	65.8	60.9 ± 3.2	51.01	65.2	59.27 ± 4.83	47.2	62.1	57.12 ± 4.69	**.001****

[a]ANOVA test. *$P < .05$, **$P < .001$ Sham (n=12), DOX (n=11), DOX+P.v.L. 50 mg extract (n=11), DOX+P.v.L. 100 mg extract (n=12), P.v.L. 100 mg extract (n=12)8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; *P.v.L.*, *Pistacia vera* L.; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

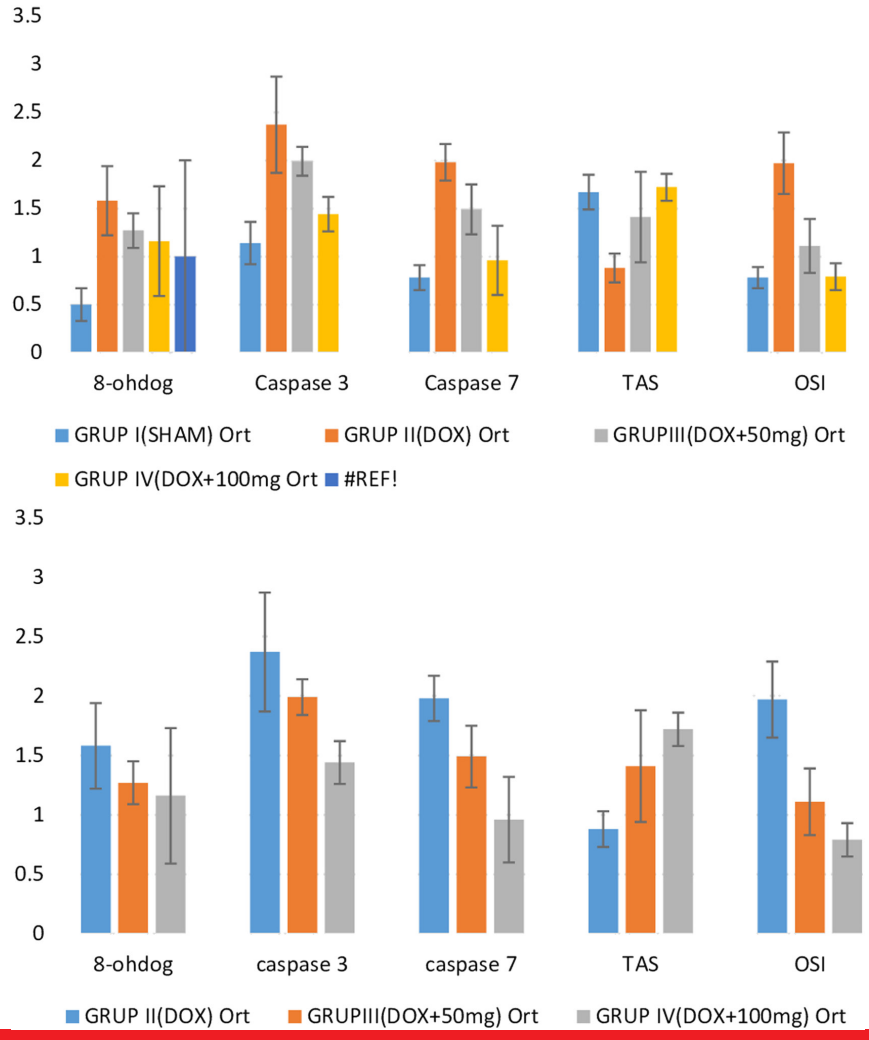

Figure 2. 8-OHdG, caspase 3, caspase 7, TAS, OSI and TOS levels. 8-OHdG, 8-hydroxy-deoxyguanosine; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.

Table 4. Statistical Analysis of DOX, DOX + *P.v.L.* 50 mg, and DOX+ *P.v.L.* 100 mg Groups

	Group II (DOX)			Group III (DOX + *P.v.L.* 50 mg)			Group IV (DOX + *P.v.L.* 100 mg)			
	Min	**Max**	**SD**	**Min**	**Max**	**SD**	**Min**	**Max**	**SD**	***P*[a]**
8-OHdG (ng/mL)	1.17	2.14	1.58 ± 0.36	1.1	1.6	1.27 ± 0.18	0.54	2.3	1.16 ± 0.57	**.010***
Caspase 3 (ng/L)	1.24	2.95	2.37 ± 0.5	1.75	2.24	1.99 ± 0.15	1.2	1.81	1.44 ± 0.18	**.001****
Caspase 7 (ng/L)	1.74	2.24	1.98 ± 0.19	1.16	2.01	1.49 ± 0.26	0.19	1.44	0.96 ± 0.36	**.001****
TAS (mmol Trolox equivalent/L)	0.66	1.1	0.88 ± 0.15	0.9	2.21	1.41 ± 0.47	1.44	1.91	1.72 ± 0.14	**.001****
TOS (μmol H_2O_2 equivalent/L)	15.3	19.09	16.97 ± 1.48	12.1	16.5	14.64 ± 1.63	10.4	16.3	13.6 ± 2.15	**.001****
OSI (AU)	1.58	2.64	1.97 ± 0.32	0.73	1.59	1.11 ± 0.28	0.62	1.06	0.79 ± 0.14	**.001****
Urea (mg/dL)	14.74	51.36	27.57 ± 11.75	29.96	104.86	59.66 ± 27.7	27.82	211.86	125.04 ± 68.49	**.001****
Creatinine (mg/dL)	0.3	0.6	0.45 ± 0.12	0.11	0.5	0.4 ± 0.11	0.12	0.6	0.36 ± 0.15	.459
e-GFR (mg/dL)	112	148	132.5 ± 14.41	112	132	127.4 ± 7.31	112	175	133 ± 23.38	.799
ALT (U/L)	16	35	27.13 ± 5.94	19	58	26.7 ± 11.31	13	58	25.5 ± 14.67	**.001****
CK-MB (ng/mL)	0.01	0.08	0.04 ± 0.02	0.01	0.04	0.02 ± 0.01	0.01	0.05	0.03 ± 0.01	**.012***
Troponin-I (pg/mL)	7.93	27.55	18.48 ± 7.41	8.05	200.4	144.2 ± 70.3	9.21	40.11	29.2 ± 9.08	**.001****
Pro-BNP (ng/L)	59	69.9	65.12 ± 3.94	56	65.8	60.9 ± 3.2	51.01	65.2	59.27 ± 4.83	**.002***

[a]ANOVA test. **P* < .05, ***P* < .001. Group II (DOX), group III (DOX + *P.v.L.* 50 mg extract) (n = 12), group IV (DOX + *P.v.L.* 100 mg extract) (n = 12).
8-OHdG, 8-hydroxy-deoxyguanosine; ALT, alanine aminotransferase; ANOVA, analysis of variance; AU, arbitrary units; CK-MB, creatine kinaz; e-GFR, estimated glomerular filtration rate; Max, maximum; Min, minimum; OSI, oxidative stress index; *P.v.L.*, *Pistacia vera* L.; Pro-BNP, pro-brain natriuretic peptide; SD, standard deviation; TAS, total antioxidant status; TOS, total oxidant status.

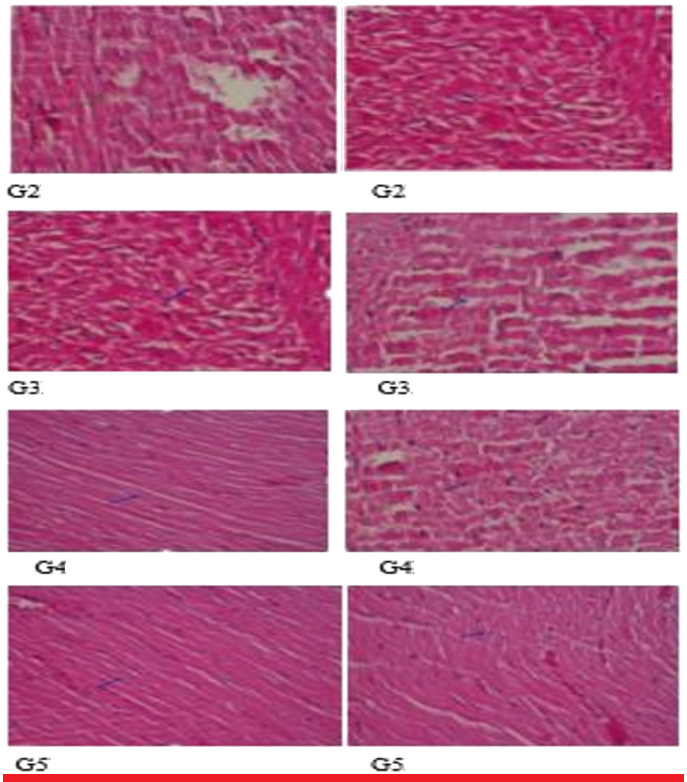

Figure 3. Histopathological examination of heart tissues.

Pro-BNP values differed statistically significantly between the groups ($P < .05$, $P < .001$) (Figure 2). Group II had higher 8-OHdG ($P = .001$), caspase 3 ($P = .001$), caspase 7 ($P = .001$), TOS ($P = .001$), OSI ($P = .001$), and Pro-BNP ($P = .001$) values than the other groups. Total antioxidant status value, on the other hand, was lower in group II. A decrease was observed in 8-OHdG, caspase 3, caspase 7, TOS, OSI, and Pro-BNP values in the treatment groups (group III and group IV). Total antioxidant status ($P = .001$) values increased in the treatment groups ($P < .05$, $P < .001$) and was the highest in group V. The comparison of group II and treatment groups (group III and group IV) in Table 4 showed that 8-OHdG ($P = .001$), caspase 3 ($P = .001$), caspase 7 ($P = .001$), TOS ($P = .001$), OSI ($P = .001$), and Pro-BNP ($P = .001$) values were decreased in group III and group IV. Total antioxidant status ($P = .001$) values increased in the treatment groups. The comparison of the treatment groups among themselves revealed a greater decrease in 8-OHdG ($P = .001$), caspase 3 ($P = .001$), caspase 7 ($P = .001$), TOS ($P = .001$), OSI ($P = .001$), and Pro-BNP ($P = .001$) values in group IV compared to group III. Among the groups, the highest TAS ($P = .001$) value was detected in group IV (Figure 2).

Histopathological Examination of Cardiac Tissue

The histopathological examination of the cardiac muscles of 24 rats belonging to group I and group V revealed that the regular structure was preserved in the cardiac muscle of all rats. In group II, areas of focal necrosis were observed. The damage rate was rated moderate. In histopathological comparison of cardiac sections taken from group III with group II, slight regression was observed in focal necrosis areas. The damage was rated as moderate. Compared to group II, cardiac sections taken from group III had slight regression in areas of focal necrosis. The damage was rated moderate. Areas of focal necrosis were even fewer in the cardiac sections of group IV than in group III. The damage was rated mild (Figure 3). In the histopathological sections of the heart muscle, the areas of necrosis per tissue surface area were calculated for each pathological sample (mm^2). The median value of necrosis rate, which was 7.33% in group II rats, was reduced to 6.45% in the first treatment group (group III) and further to 5.3% in the second treatment group (group IV) (Table 5) (Figure 4).

DISCUSSION

Despite being a clinically beneficial anthracycline in chemotherapy due to its broad spectrum and effectiveness, the extreme toxicity of DOX possesses a disadvantage.[21] In their DOX-induced cardiotoxicity study, Demir et al[18] evaluated

Table 5. DOX, Treatment 1 (DOX + *P.v.L.* 50 mg), and Treatment 2 (DOX + *P.v.L.* 100 mg) Groups Necrosis Areas

	DOX Group			Treatment 1 (DOX + *P.v.L.* 50 mg)			Treatment 2 (DOX + *P.v.L.* 100 mg)		
mm^2	Area	Necrosis	%	Area	Necrosis	%	Area	Necrosis	%
1	4.60	0.34	7.39	4.40	0.30	6.81	4.02	0.28	6.96
2	4.83	0.28	5.78	4.50	0.27	6.0	4.00	0.25	6.25
3	5.29	0.26	7.56	5.25	0.25	4.76	4.50	0.20	4.44
4	5.06	0.40	7.9	4.90	0.38	7.75	4.02	0.27	6.71
5	5.52	0.39	7.06	4.83	0.35	7.24	4.10	0.29	7.07
6	5.10	0.30	5.88	5.00	0.27	5.4	4.00	0.20	5.0
7	5.06	0.25	4.94	4.70	0.24	5.1	4.30	0.17	3.95
8	5.01	0.37	7.38	4.95	0.35	7.07	4.20	0.14	3.33
9	4.90	0.50	10.2	4.80	0.40	8.33	4.60	0.24	5.21
10	4.70	0.30	10.6	4.50	0.28	6.22	4.06	0.20	4.92
11	4.98	0.32	6.02	4.75	0.30	6.31	4.00	0.19	4.75
12							4.05	0.20	4.93
Median	5.01	0.37	7.33	4.78	0.30	6.45	4.15	0.22	5.3

DOX, doxorubicin; *P.v.L.*, *Pistacia vera* L.

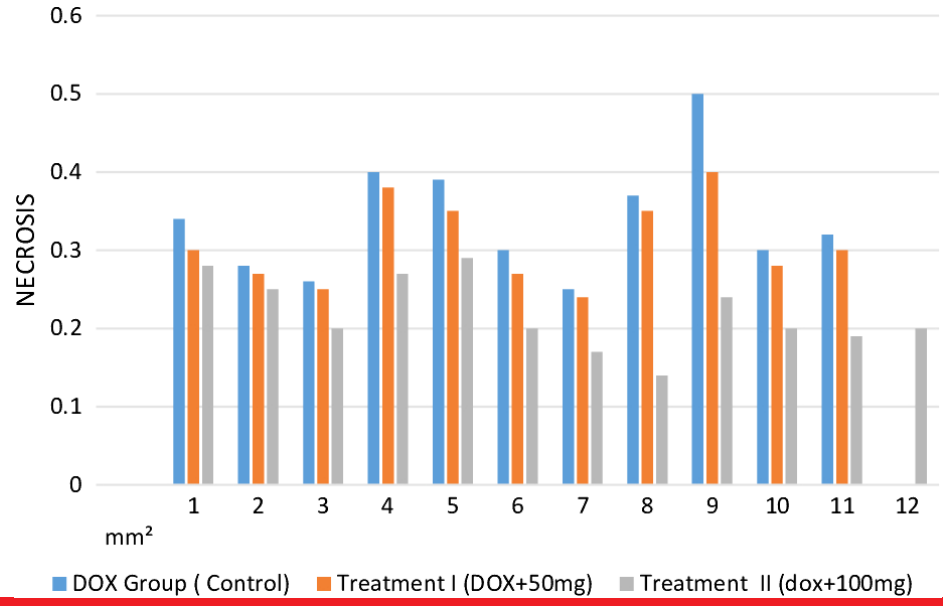

Figure 4. Necrosis areas comparison [DOX, treatment 1 (DOX + *P.v.L.* 50 mg), treatment 2 (DOX + *P.v.L.* 100 mg) groups]. DOX, doxorubicin; *P.v.L.*, *Pistacia vera* L.

the antioxidant system and lipid peroxidation after the last DOX injection and concluded that DOX reduced heart tissue plasma glutathione peroxidase and increased lipid peroxidation products in the chronic process. Dindaş et al[22] created cardiac damage with DOX in their study on mice. In recent years, biological markers have been used together with echocardiography to determine anthracycline-induced cardiotoxicity. Pro-brain natriuretic peptide and troponin-I are 2 of these markers.[23] In a 3-year follow-up of ejection fraction, Berger et al[24] identified the BNP level as the only independent predictor of sudden death. Cordeanu et al[25] found in their study that 34% of the patients had elevated troponin-I, and these patients had a 4-fold increased risk of death compared to healthy patients. In a study on patients receiving anthracycline treatment, Öztarhan et al[26] showed an increase in troponin-I levels with increasing cumulative anthracycline doses. In our study, pro-BNP and troponin-I levels were increased due to DOX toxicity in group II rats, indicating that cardiac myocytes were severely affected, and the cardiac damage mechanism was induced as expected.

The most widely measured, well-established damage biomarker today is 8-OHdG.[27] A decrease in 8-OHdG levels has been shown with antioxidant interventions in experimental animal models.[28,29] One study has shown that the measurement of 8-OHdG in plasma rather than tissues of diabetic rats is a more beneficial marker in revealing oxidative deoxyribonucleic acid (DNA) damage.[30] Di Minno et al[31,32] reported a relationship between heart damage and 8-OHDG levels. In our study, 8-OHdG levels that increased in rats with DOX-induced cardiomyocyte damage declined significantly in the treatment group (group IV), suggesting that the *P.v.L.* extract could effectively prevent DNA damage by suppressing oxidative stress.

Apoptosis, which maintains tissue homeostasis, is a normal cell death under both physiological and pathological conditions, including oxidative damage.[33] Apoptosis requires energy and is accomplished without cellular damage or inflammation. DNA damage is eliminated by apoptosis.[34] Apoptosis can be classified as normal and abnormal. While normal apoptosis is considered very important in the cell cycle, abnormal apoptosis causes neurodegenerative diseases, ischemic damage, and many types of cancer.[35] Excess apoptosis is one of the main causes of various pathological processes.[36] In heart failure, stimulation of apoptosis, necrosis, and autophagy causes excessive death of myocytes.[37,38] Studies in human heart tissue have determined that apoptosis is associated with events such as cardiomyopathy (idiopathic dilated, ischemic, and hypertrophic cardiomyopathy) and arrhythmogenic right ventricular dysplasia.[39,40] Caspases, which have an essential function in apoptosis, are aspartate-specific proteolytic enzymes with cysteine residues in their active sites. Caspase-3, one of the most significant indicators of apoptosis, is activated by caspase-9.[41,42] Caspase 3/7 activation is the most widely identified molecular marker for apoptotic programmed cell death.[43] Fanton et al[44] showed in rabbits that noretandrolone activated apoptosis by increasing caspase-3 activity in cardiac tissue. Recent studies have shown that consuming natural antioxidant-rich plant foods can prevent many diseases, especially CVD. Antioxidant phenolic compounds in the structure of these foods are thought to prevent various diseases.[45] Carino-Cortes et al[46] stated that Naringin, along with the anticancer agent daunorubicin, prevented oxidative stress-induced DNA damage in hepatocytes and cardiomyocytes. Similarly, Dong et al[47] reported that quercetin, a powerful antioxidant, suppressed DOX-induced cardiotoxicity. Aristatile et al[48] showed that carvacrol, which has antioxidant properties, protected the cell against lipid peroxidation, oxidative stress, and DNA damage. Another study found that the outer shell of the pistachio contained high antioxidants.[49] Koyuncu et al[50] showed that the pistachio green hull extract, *P.v.L.*, could induce

apoptosis through multiple signaling mechanisms by causing oxidative stress in colon cancer cells and hence can be used in cancer treatment.

In parallel with Pro-BNP, troponin-I, and 8-OHdG levels, caspase 3 and caspase 7 levels were elevated in group II rats, indicating that cardiac damage mechanism was successfully induced with DOX, and cardiomyocyte damage was achieved. More importantly, caspase levels and other damage parameters decreased in treatment group rats (group III and group IV). Despite anthracycline DOX-induced severe cardiomyocyte damage, *P.v.L.* extract effectively reduced both abnormal apoptosis mechanisms and oxidative stress-induced DNA damage. In addition, antioxidant markers TOS and OSI increased due to DOX in group II rats, while TAS, a marker of antioxidant capacity, decreased. In treatment groups (group III, group IV, and group V), TOS and OSI values decreased, while TAS increased. These parameters showed oxidative damage and antioxidant capacity in parallel with other Pro-BNP, troponin-I, and 8-OHdG levels. These effects of *P.v.L.* extract can be attributed to the anthocyanins and flavonoids it contains. At this point, it is necessary to mention one of the significant limitations of the study. This limitation is the presence of different active ingredients in the extract. These active ingredients were not separated during the study and were given collectively in extract form. Therefore, the specific compound responsible for this anti-apoptotic and DNA damage-reducing effect will remain the objective of future studies.

CONCLUSION

In this study, Pro-BNP, troponin-I, 8-OHdG, caspase 3, caspase 7, TOS, and OSI values showed significant regression in the groups treated with *P.v.L.* hull extract. *Pistacia vera* L. hull extract effectively reduces oxidative stress, oxidative stress-induced DNA damage, and abnormal apoptosis mechanisms in cardiomyocytes. In the future, our knowledge on this subject will increase with sub-studies to be made with *P.v.L.* hull extract.

Ethics Committee Approval: It was carried out with the approval of Harran University Animal Experiments Local Ethics Committee with decision no. 17.12.2020-E.49516, session number 2021/008/07.

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