FABAD J. Pharm. Sci., 49, 1, 181-196, 2024
Doi: 10.55262/fabadeczacilik.1361591
RESEARCH ARTICLE

Protective Role of *Pistacia palaestina* Boiss. Fruit and Leaf Extracts in Isoproterenol-Induced Cardiac Ischemia

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Protective Role of Pistacia palaestina Boiss. Fruit and Leaf Extracts in Isoproterenol-Induced Cardiac Ischemia

SUMMARY

Myocardial infarction (MI) is one of the leading causes of death worldwide. This study aimed to investigate the protective effects of Pistacia palaestina Boiss (PP) leaf and fruit extracts, which are thought to have antioxidant and anti-inflammatory activity, in isoproterenol (ISO)-induced MI. 80 Sprague-Dawley rats were divided into ten groups. The control group was given 0.9% isotonic sodium chloride saline. PP leaf and fruit extracts at doses of 250 mg/kg and 500 mg/kg were administered by oral gavage for 21 days. ISO 100 mg/kg subcutaneously was administered to the MI and MI-treatment groups on the 17th and 18th days of the experiment. Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities were measured in heart tissue. In serum, troponin T, CK-MB; pro-inflammatory cytokine necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β,) and IL-6; anti-inflammatory IL-10 levels were determined by the ELISA method. Heart tissue was examined by hematoxylin-eosin staining. While lipid peroxidation indicator TBARS activity increased in the MI group, antioxidant enzyme activities and GSH levels decreased. While troponin T, CK-MB, TNF-α, IL-1β, and IL-6 levels increased, anti-inflammatory IL-10 levels decreased. Low and high dose PP leaf and fruit extracts significantly decreased TBARS, troponin T, CK-MB, TNF-α, IL-1β, and IL-6 levels improved antioxidant enzyme activity, and GSH and IL-10 levels. PP ameliorated cardiac biomarkers and histopathological changes in ISO-induced MI by suppressing oxidative stress and inflammation. PP extracts may play an important cardioprotective role in the treatment of MI through their antioxidant and anti-inflammatory effects.

Key Words: *Pistacia palaestina boiss, myocardial infarction, isoproterenol, antioxidant, anti-inflammatory activity, cardiac markers.*

Pistacia palaestina Boiss. Meyve ve Yaprak Ekstrelerinin İzoproterenol Kaynaklı Kardiyak İskemide Koruyucu Rolü

ÖZ

Miyokard enfarktüsü (MI) dünya çapında önde gelen ölüm nedenlerinden biridir. Bu çalışmada antioksidan ve antiinflamatuar aktiviteye sahip olduğu düşünülen Pistacia palaestina Boiss (PP) yaprak ve meyve ekstrelerinin izoproterenol (ISO)-indüklü MI'da koruyucu etkilerinin araştırılması amaçlanmıştır. 80 adet Sprague-Dawley sıçan 10 gruba ayrıldı. Kontrol grubuna %0,9 izotonik sodyum klorür verildi. 250 mg/kg ve 500 mg/kg dozlarında PP yaprak ve meyve ekstreleri 21 gün süreyle gavaj yoluyla uygulandı. MI ve MI-tedavi gruplarına deneyin 17. ve 18. günlerinde ISO 100 mg/kg subkutan olarak uygulandı. Kalp dokusunda tiyobarbitürik asit reaktif maddeleri (TBARS) ve glutatyon (GSH) düzeyleri, katalaz (CAT), süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) aktiviteleri ölçüldü. Serumda troponin T, CK-MB; proinflamatuar sitokin nekroz faktörü-alfa (TNF-α), interlökin-1β (IL-1β) ve IL-6; antiinflamatuar IL-10 düzeyleri ELISA yöntemiyle belirlendi. Kalp dokusu hematoksilen-eozin boyama ile incelendi. MI grubunda lipid peroksidasyon göstergesi TBARS aktivitesi artarken, antioksidan enzim aktiviteleri ve GSH seviyeleri azaldı. Troponin T, CK-MB, TNF-α, IL-1β ve IL-6 düzeyleri artarken antiinflamatuar IL-10 düzeyleri azaldı. Düşük ve yüksek doz PP yaprak ve meyve ekstreleri TBARS, troponin T, CK-MB, TNF-α, IL-1β, IL-6 düzeylerini önemli ölçüde azaltmış, antioksidan enzim aktivitelerini, GSH ve IL-10 düzeylerini iyileştirmiştir. PP, oksidatif stres ve inflamasyonu baskılayarak ISO-indüklü MI'daki kardiyak biyobelirteçleri ve histopatolojik değişiklikleri düzeltmiştir. PP ekstreleri antioksidan ve antiinflamatuar etkileriyle MI tedavisinde önemli bir kardiyoprotektif rol oynayabilir.

Anahtar Kelimeler: *Pistacia palaestina Boiss, miyokard enfarktüsü, izoproterenol, antioksidan, antiinflamatuar aktivite, kardiak belirteçler.*

Received: 17.09.2023
Revised: 26.01.2024
Accepted: 29.01.2024

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INTRODUCTION

In 2020, 928.741 people in the United States and approximately 19.05 million people worldwide died from cardiovascular disease (Tsao et al. 2023). Myocardial infarction (MI), one of the most common cardiovascular diseases, is characterized by insufficient blood circulation feeding the heart and causes irreversible acute necrosis (Mechanic et al. 2022). Although there are many surgical and pharmacological treatment methods for MI and ischemic heart diseases, these methods are insufficient in reversing the damage to the heart and improving the changing biochemical parameters. Developing new molecules that reduce damage to the heart and have fewer side effects is of vital importance for reducing MI complications and mortality.

Subcutaneous administration of isoproterenol (ISO), a synthetic catecholamine, and β-adrenergic receptor agonist, has been reported to induce infarction-like myocardial lesions (El-Gohary & Allam, 2017). The ISO-induced cardiac ischemia model is often used to investigate new treatment modalities against MI, myocardial ischemia, and cardiac fibrosis in experimental animals. ISO causes progressive cardiotoxicity at low doses, whereas it causes acute cardiac damage at high doses (Allawadhi et al. 2018). ISO causes mitochondrial damage and increased oxidative stress, resulting in changes in cardiac biochemical parameters that lead to cardiac damage (Nwokocha et al. 2017, Allawadhi et al. 2018). Therefore, products of natural origin with antioxidant properties can reduce the damage by protecting against cardiac damage caused by MI (Feng et al. 2019).

Recent studies have suggested that phytotherapeutics are effective in the prevention and treatment of cardiovascular diseases (Deng et al. 2015; Allawadhi et al. 2018; Feng et al. 2019). The genus *Pistacia* has recently attracted attention in the field of phytotherapy as an important source of phenolic compounds, terpenoids, monoterpenes, flavonoids, alkaloids, saponins, fatty acids, and sterols. It has been reported that different parts of these plants, such as the leaves, trunk, bark, arborvitae, and fruit, have different uses. Its medicinal uses are mentioned in ancient pharmacopoeias, Ayurveda, traditional Chinese medicine, Iranian folk medicine, and other similar sources. As a result of the increased interest in the use of *Pistacia* species, studies have been conducted to evaluate various properties, such as antioxidant, antimicrobial, antiviral, anticholinesterase, anti-inflammatory, antinociceptive, antidiabetic, antitumor, antihyperlipidemic, antiatherosclerotic, and hepatoprotective properties (Tomaino et al. 2010; Hosseinzadeh et al. 2012; Rauf et al. 2017). It has been reported that the seeds of the *Pistacia vera* L. plant are used as cardiotonic and exhilarating (Sobhani et al. 2017). In the literature review about *Pistacia palaestina* Boiss (PP), another species of this genus, research has suggested that the amounts of essential oils vary depending on the region where the plant grows. The essential oil obtained from the leaves of the plant collected from Osmaniye was analyzed by gas chromatography-mass spectrometry (GC-MS), and it was determined that it contained α-pinene (19.9%) as the major compound. In addition, the antibacterial and insecticidal activities of essential oils have been reported (Ulukanli et al. 2014). The contents of the essential oils obtained separately from the leaves, thuja, raw and ripe fruits of the plant collected in Jordan, and the major compounds were α-pinene in the leaf (61.3%), α-pinene in the thuja (49.4%), and (E)-ocimene (41.3%) in the raw fruit and (E)-ocimene (33.8%) were detected in ripe fruit (Flamini et al. 2004). In another study conducted in Lebanon, 29 components were found in the essential oil obtained from the fruits of the plant, and the main components with the highest ratios were sabinene (17.08%) and limonene (8.56%). The antiviral activity of the plant evaluated in vitro was found to be low (Loizzo et al. 2008). It has been reported that the essential oil obtained from the seeds of the plant collected in Lebanon inhibits the proliferation of K562 human leukemia cells (Lampronti et al. 2005). In the human hepatocyte cell line (HepG2), PP extracts

showed cytotoxic activity by reducing cell viability (Saad et al. 2006). Although content analysis has been performed for PP leaves and fruits, their biological activities are not yet fully known. Studies on its biological activity are limited. However, it is known that in traditional medicine, ripe fruits of PP are consumed as food by adding them to the medicinal herb mixture called zahter (Flamini et al. 2004). PP likely has a powerful antioxidant and anti-inflammatory activity due to the high amounts of α-pinene, (E)-ocimene, sabinene, and limonene components found in its fruits and leaves.

This study aimed to determine the cardioprotective role of low and high doses of leaf and fruit extracts of the PP in ISO-induced MI in rats. To determine the antioxidant capacity of the extracts, thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities were measured in heart tissue. Levels of the pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), and anti-inflammatory cytokine IL-10 were evaluated in the blood serum. Additionally, the creatine kinase-myocardial band (CK-MB) and troponin T levels, which are biomarkers of cardiac ischemia, were determined. Histopathological examination of heart tissues was performed using hematoxylin-eosin staining.

MATERIALS AND METHOD

Preparation of Plant Extract

The leaves and fruits of the PP plant, which were collected in the Pütürge district of Malatya province in the Eastern Anatolia Region, from the end of August to early September 2020, were dried in the shade. Diagnosis of PP plant İnönü University Faculty of Pharmacy, Department of Pharmaceutical Botany, the lecturer was made by Prof. Dr. Turan Arabacı. PP samples were kept in the herbarium of İnönü University Faculty of Pharmacy with the number Sİ1032. Dried leaves and fruits were carefully separated and weighed. 530 g of leaves and 420 g of fruit were weighed and ground in a grinder and macerated with a sufficient amount of 80% ethanol (Sigma-Aldrich, Cas no: 64-7-5, Germany) at room temperature for 24 hours. The extract was filtered using filter paper. The filtrate was collected, and 80% ethanol was added to the plant again, and macerated again for 24 h. The filtrates collected in this way, 7 repetitions in total, were concentrated under low pressure in the rotavapor (Laborota 4011-digital, Heidolph, Germany) at temperatures not exceeding 45°C and dried with a lyophilizer. The extract amounts and yields are presented in Table 1.

Table 1. Amount of PP leaves and fruits used for extraction, amount of extraction obtained, and yield.

PP	Amount (g)	Obtained extract (g)	Yield (%)
Leaf	530	86.13	16.25%
Fruit	420	78.36	18.65%

Establishment of the Cardiac Ischemia Model

Eighty Sprague-Dawley rats with an average weight of 250-300 g were used in this study. Rats were obtained from the İnönü University Experimental Animals Production and Research Center. This study was carried out with the approval of the İnönü University Animal Experiments Local Ethics Committee dated 25.02.2020 and numbered 2020/3-4. Rats were divided into ten groups; control, MI, MI+PP leaf (250 mg/kg), MI+PP leaf (500 mg/kg), MI+PP fruit (250 mg/kg), MI+PP fruit (500 mg/kg), PP leaf (250 mg/kg), PP leaf (500 mg/kg), PP fruit (250 mg/kg), PP fruit (500 mg/kg). The control group was given 0.9% isotonic sodium chloride (NaCl) saline by gavage for 21 days. The control group was injected subcutaneously (sc) with saline on the 17th and 18th days of the experiment. ISO (100 mg/kg, Sigma-Aldrich, Cas no: 51-30-9, China) was dissolved in 0.9% NaCl and injected sc into the right thigh of the rat for two consecutive days, 24 hours apart, to induce MI (Boarescu

et al. 2019). 250 mg/kg and 500 mg/kg doses of PP leaf and fruit extracts were dissolved in 0.1% CMC (carboxy methyl cellulose) and administered by gastric gavage for 21 days. The rats were sacrificed at the end of the 21st day. The heart tissue was carefully removed, and divided vertically into two pieces, and the left atrium and ventricle were stored at -80°C for biochemical examination. The right atrium and ventricle were fixed with 10% formalin.

Biochemical Examination of Heart Tissue

Heart tissue samples were stored at -80°C until biochemical enzyme analysis. Before the analysis, the heart tissues were washed with physiological saline, weighed, diluted 1:10, and homogenized using a Teflon glass homogenizer in 20 mM Tris-HCl (pH 7.4) buffer. Some homogenates were separated and used for TBARS and CAT activity measurements. The remaining homogenates were centrifuged at 3.500 rpm for 30 min for GSH, SOD, and GPx analyses in a refrigerated centrifuge (NF 800R, Nüve, Türkiye), and supernatants were collected.

Determination of TBARS Levels

The levels of thiobarbituric acid reactive substance (TBARS), a lipid peroxidation marker, were determined using the Yagi method in homogenized heart tissue (Yagi, 1998). Tissue homogenate was precipitated with 10% trichloroacetic acid (TCA, Sigma-Aldrich, Cas no: 76-03-9, Germany) (pH: 3.5) and kept in a hot water bath (Memmert, Germany) for 15 minutes. The mixture was then cooled and centrifuged at 3000 rpm for 10 min. The remaining supernatant was carefully removed. The supernatant was incubated with thiobarbituric acid (TBA, Merck, L55063680 731, Germany) in a 95°C water bath for 50 min and cooled. The absorbance of the pink complex formed as a result of the reaction of malondialdehyde with TBA was measured at 532 nm using a spectrophotometer (T60 UV/VIS, Enotek, UK).

Determination of GSH Levels

10% TCA (0.2 M, pH: 8.9) was added to the heart tissue homogenate and tissue was precipitated for 10 min at 3500 rpm. The resulting supernatants were added with tris-ethylenediamine tetraacetic acid (EDTA, Sigma-Aldrich, Cas no: 6381-92-6, USA) and 0.01 M 5,5'-dithiobis (2-nitrobenzoic acid) (1:9) buffer solution was added. By adding 0.01 mol/L DTNB (5-5'-dithiobis – nitro benzoic acid) to the mixture, it is reduced by sulfhydryl compounds and forms a yellow complex, which is a disulfide compound. The absorbance of this yellow compound was measured at 412 nm in a spectrophotometer (Sedlak & Lindsay, 1968).

Determination of SOD Activity

The superoxide radical formed during the conversion of xanthine to uric acid by xanthine oxidase reacts with nitroblue tetrazolium (NBT) in the environment to form purple-colored formazan. In the presence of superoxide dismutase in the environment, because the superoxide radical formed is converted to hydrogen peroxide, NBT reduction and, therefore, formazan formation will decrease. The reduction of formazan formation was determined spectrophotometrically by measuring at 560 nm. Enzyme activity that inhibited NBT reduction by 50% was considered SOD activity (Sun et al. 1968).

Determination of CAT Activity

Homogenates of heart tissue were subjected to sanitation for 3×10 seconds in a cold environment. Hydrogen peroxide was gradually added to the pH=7 buffer solution prepared with $NaH_2PO_42H_2O$ and $NaH_2PO_412H_2O$. Buffer solution was added to the samples at a ratio of 300:1, and the decrease in absorbance as a result of the breakdown of hydrogen peroxide by catalase was measured at a wavelength of 240 nm. This decrease in absorbance is directly proportional to enzyme activity (Aebi, 1974).

Determination of GPx Activity

To determine the GPx activity, a buffer solution consisting of NaH_2, NaH_2PO, and EDTA was prepared. Reduced glutathione, NADPH, and sodium

azide were dissolved separately in a buffer solution. GSH reductase dissolves ammonium sulfate solution. These separately prepared solutions were added to the sample and incubated for 30 min. Hydrogen peroxide was added to the mixture, and the decrease in absorbance was immediately measured in a spectrophotometer at 340 nm for 2 min. The decrease in absorbance was directly proportional to glutathione peroxidase activity (Paglia & Valentine 1967).

Determination of Total Protein Amount

The amount of total protein in the heart tissue was measured according to the method determined by Lowry et al. (Lowry et al. 1951) Reagent A, consisting of $CuSO_4$ and anhydrous sodium citrate, and reagent B, consisting of Na_2CO_3 and NaOH, was mixed at a ratio of 1:50 and added to the sample. Thus, alkaline copper (Cu^{+2}) forms a complex with peptide bonds, and every 7 or 8 amino acid residues are bonded to 1 atom of Cu. When Folin-Cieolteu reagent was added to the reaction, a purple-blue color was formed, and the color change of these samples was measured with a spectrophotometer at 700 nm. Parameters measured in heart tissue homogenates were normalized according to the amount of protein.

ELISA Analysis

Four mL of blood taken from all experimental groups was centrifuged at 2500 g for 10 minutes at room temperature, separated into serum, and stored at -80°C until analysis. Troponin T (E0311Ra), CK-MB (E0311Ra), TNF-α (E0764Ra), IL-1β (E0107Ra), IL-6 (E0135Ra), and IL-10 (E0108Ra) levels were determined at 450 nm by using commercially available ELISA kits (Bioassay Technology Laboratory, China) and ELISA plate reader (Biotek Instruments Inc., USA).

Histopathological Analysis

Heart tissues were fixed in 10% formalin. Tissues undergoing follow-up procedures were enclosed in paraffin blocks. Sections of 5 μm thickness were taken from the prepared blocks with a microtome knife system (Leica RM 2245), and the hematoxylin-eosin staining method was applied to the sections. Tissues were examined for necrosis, mononuclear cell infiltration, hemorrhage, and vascular occlusion using a Leica DFC 280 light microscope and Leica Q Win Image Analysis System (Leica Microsystems Imaging Solutions, Cambridge, UK). The histopathological damage score was calculated according to the findings obtained.

Statistical Analysis

SPSS 12.0 (SPSS Inc.; Chicago, IL, USA) was used for the analyses. The Kruskal-Wallis test was used for intergroup comparisons for histopathological examinations. The difference between the groups was evaluated with the Bonferr Mann-Whitney U test. One-way ANOVA followed by Tukey's post-hoc test was used to statistically evaluate the biochemical parameters and ELISA results. $p<0.05$ was considered significant. Data are shown as mean±SEM.

RESULTS and DISCUSSION

Biochemical Results

CAT, SOD, and GPx activities and TBARS, GSH levels of PP plant leaf and fruit extracts in rat heart tissue are given in Table 2. TBARS levels, which indicate the amount of lipid peroxidation, were statistically significantly higher in the MI group compared to all other groups. However, it was determined that PP treatment significantly reduced the increase in TBARS caused by ISO. GSH levels, which are antioxidant defense system elements, and CAT, SOD, and GPx activities were significantly decreased in the MI group. Leaf and fruit extracts of PP ameliorated the decrease in antioxidant enzyme activity. It was observed that fruit extract treatment at a dose of 500 mg/kg decreased TBARS levels and increased antioxidant enzyme levels more than other treatment groups, but this was not statistically significant.

Table 2. TBARS, GSH levels, and CAT SOD, GPx activities in rats (Mean±SEM, n=8).

Groups	TBARS (nmol/g tissue)	GSH (nmol/mL)	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Control	11.97±0.73	94.75±1.96	0.032±0.0017	40.66±1.70	269.1±4.8
MI	20.48±1.12*	58.84±2.98*	0.018±0.0005*	22.52±1.75*	153.6±2.7*
MI+PP leaf 250 mg/kg	15.99±0.61$^{*,\#}$	78.94±3.00$^{*,\#}$	0.024±0.0012$^{*,\#}$	31.28±0.82$^{*,\#}$	193.9±8.2$^{*,\#}$
MI+PP leaf 500 mg/kg	15.76±0.67$^{*,\#}$	71.44±4.20*	0.025±0.0010$^{*,\#}$	31.04±0.85$^{*,\#}$	198.3±6.0$^{*,\#}$
PP leaf 250 mg/kg	10.66±0.90$^{\#}$	95.25±2.27$^{\#}$	0.034±0.0019$^{\#}$	39.33±1.52$^{\#}$	270.7±7.3$^{\#}$
PP leaf 500 mg/kg	11.24±0.99$^{\#}$	94.44±4.08$^{\#}$	0.032±0.0010$^{\#}$	40.01±1.18$^{\#}$	272.1±2.8$^{\#}$
MI+PP fruit 250 mg/kg	15.54±0.65$^{*,\#}$	78.08±2.18$^{*,\#}$	0.025±0.0005*	31.20±1.05$^{*,\#}$	198.3±5.9$^{*,\#}$
MI+PP fruit 500 mg/kg	14.17±0.64$^{*,\#}$	78.20±4.40$^{*,\#}$	0.026±0.0010*	32.40±0.070$^{*,\#}$	200.8±3.7$^{*,\#}$
PP fruit 250 mg/kg	10.88±0.33$^{\#}$	94.67±2.75$^{\#}$	0.030±0.0005$^{\#}$	40.73±0.75$^{\#}$	274.7±4.0$^{\#}$
PP fruit 500 mg/kg	11.05±0.17$^{\#}$	98.92±2.44$^{\#}$	0.034±0.0015$^{\#}$	42.77±1.26$^{\#}$	270.1±2.9$^{\#}$

*: Significant compared to the control group (*: $p<0.05$), $^{\#}$: Significant compared to the MI group ($^{\#}$: $p<0.05$). (CAT: catalase, GPx: glutathione peroxidase, GSH: glutathione, SOD: superoxide dismutase, TBARS: thiobarbituric acid reagents, PP: *Pistacia palaestina* Boiss)

The effect of PP leaf and fruit extracts on serum troponin T, CK-MB, TNF-α, IL-1β, IL-6, and IL-10 levels is given in Figure 1. According to the results, the levels of cardiac serum markers troponin T, CK-MB, and pro-inflammatory cytokine TNF-α, IL-1β, and IL-6 increased in the MI group with the administration of ISO. On the other hand, anti-inflammatory IL-10 levels decreased. This increase in pro-inflammatory cytokine levels caused by ISO in MI groups was statistically decreased in both doses (250 mg/kg and 500 mg/kg) in the treatment groups (MI+PP leaf and MI+PP fruit). It was observed that the decrease in troponin T, CK-MB, and proinflammatory cytokine levels was more pronounced in the treatment with 500mg/kg fruit extracts. The cytokine levels of the groups given PP alone (Groups 7, 8, 9, and 10) were similar to the control group in the fruit and leaf groups for both doses.

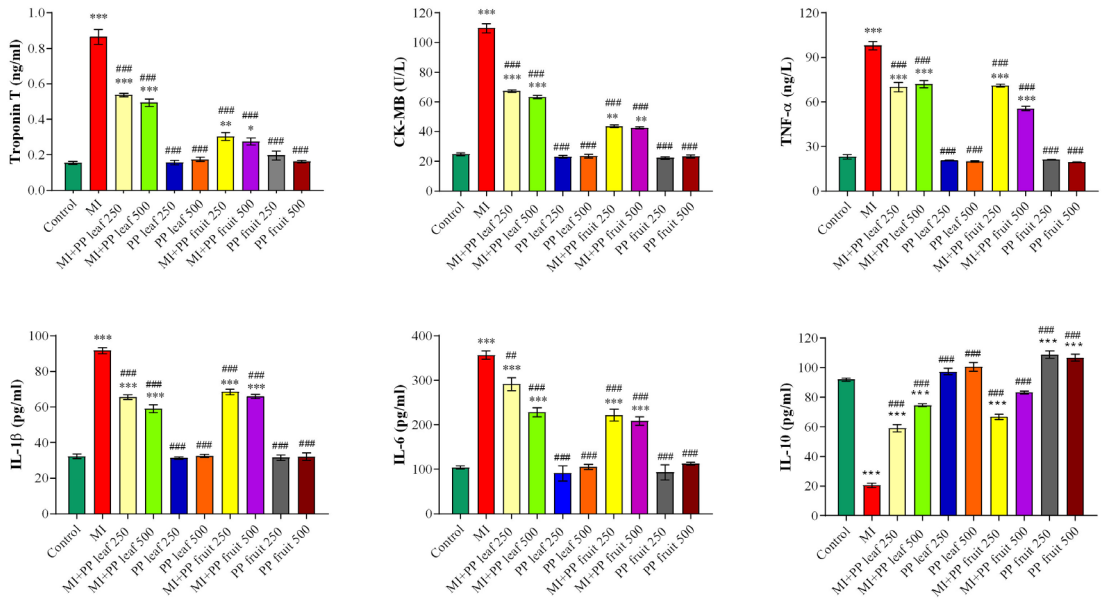

Figure 1. All values are presented as mean±SEM (n=8). *Pistacia palaestina* Boiss (PP) leaves and fruits (250 mg/kg and 500 mg/kg) in isoproterenol-induced myocardial infarction (MI); Effect on troponin T, creatine kinase-myocardial band (CK-MB), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL- 10) levels. Values with different superscripts in the same column are statistically significantly different ($p<0.05$).

: Significant compared to the control group (: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)

#: Significant compared to the MI group (##: $p<0.01$, ###: $p<0.001$)

Histopathological Results

In the control group (Figure 2A, 2B), heart tissue was observed in normal histological appearance. Myofibril loss (Figure 3A, 3C, 3D), vascular congestion (white arrows) (Figure 3B, 3D), cell infiltration (Figure 3D), hemorrhage (black stars) (Figure 3C, 3E), necrosis thick black arrows) (Figure 3E), cells with eosinophilic cytoplasm, pycnotic nuclei (Figure 3C, 3F), and vacuolization (thin black arrows) (Figure 3F) were observed in the MI group. A small amount of hemorrhage and cell infiltration was observed in the heart muscle fibers in the MI+PP fruit 250 mg/kg group (Figure 4A, 4B) and the MI+PP fruit 500 mg/kg group (Figure 4C, 4D). A small amount of cell infiltration was observed in the cardiac muscle fibers in the PP fruit 250 mg/kg group (Figure 5A, 5B), and a small amount of hemorrhage in the heart muscle fibers in the PP fruit 500 mg/kg group (Figure 5C, 5D). In the MI+PP leaf, 250 mg/kg group (Figure 6A, 6B) and in the MI+PP leaf 500 mg/kg group (Figure 6C, 6D), hemorrhage in the heart muscle fibers and moderate cell infiltration compared to the MI group were observed. A small amount of hemorrhage and cell infiltration was observed in the cardiac muscle fibers in the PP leaf 250 mg/kg group (Figure 7A, 7B), whereas a small amount of vascular congestion was observed in the cardiac muscle fibers in the PP leaf 500 mg/kg group (Figure 7C, 7D).

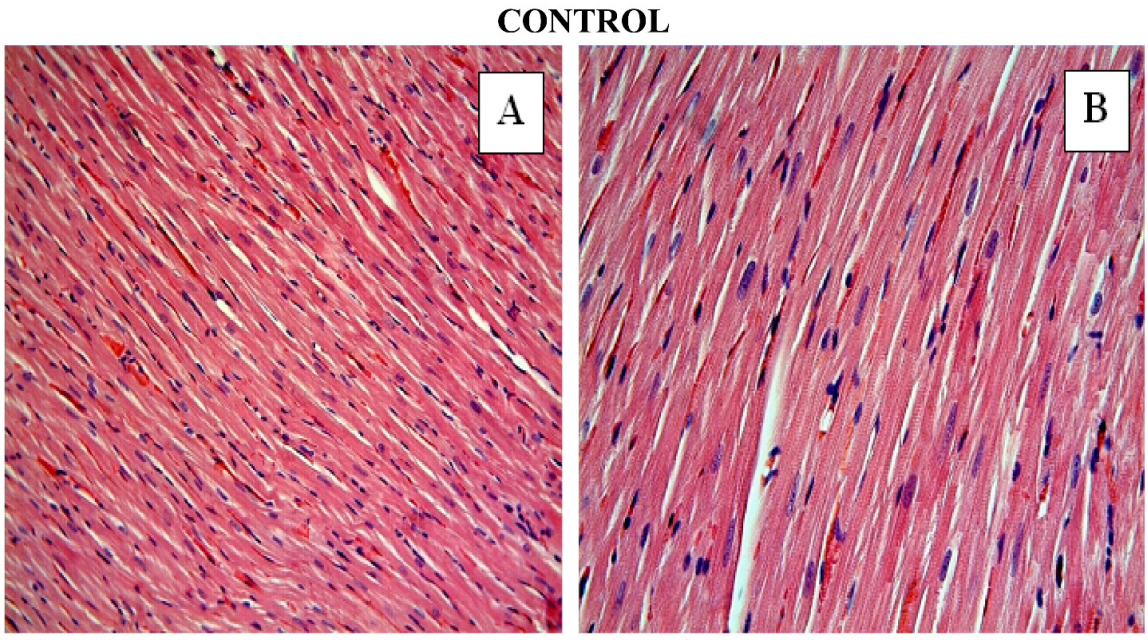

Figure 2. Heart tissue in the control (A, B) group showed a normal histological appearance. A: H-E; X20, B: H-E; X40.

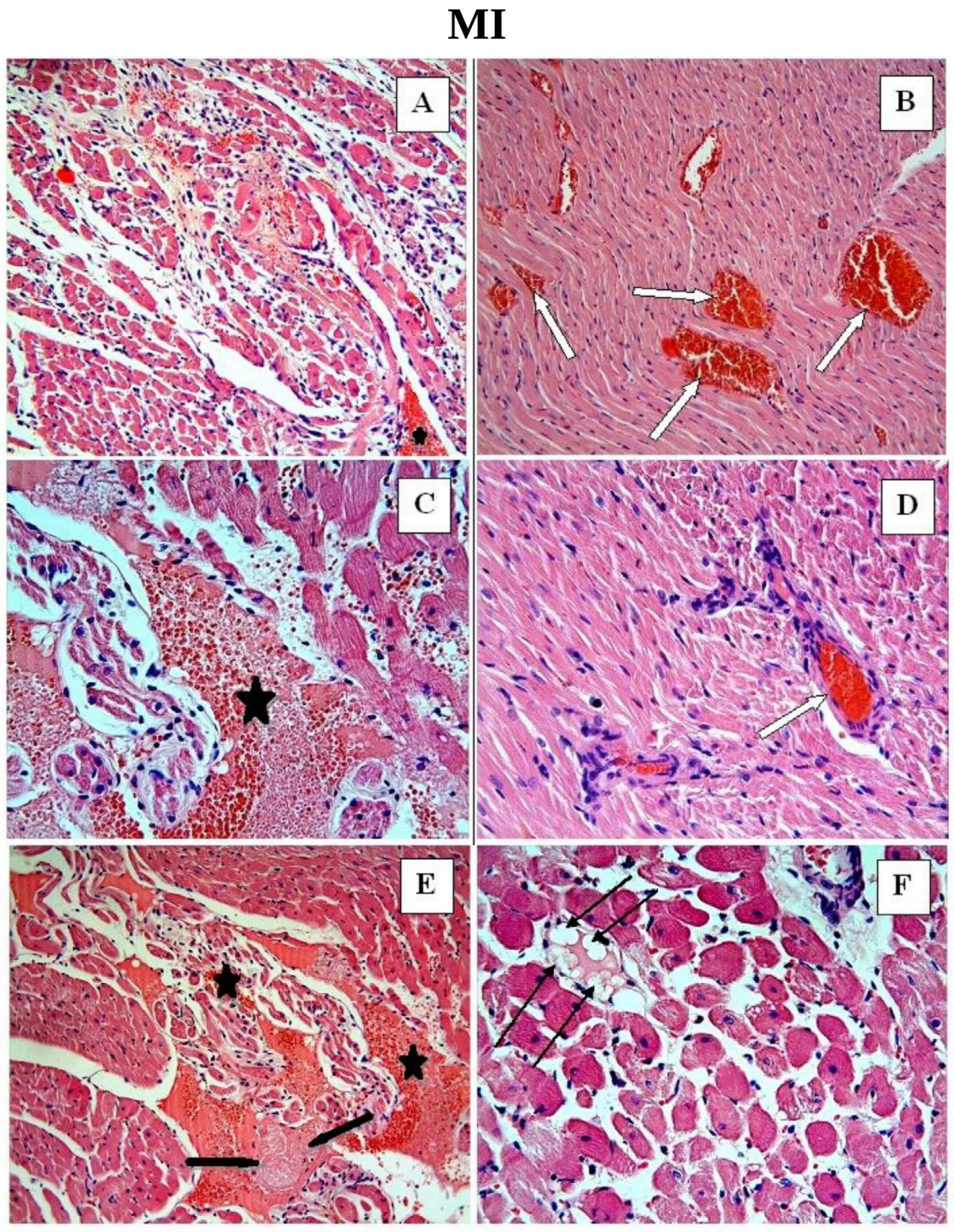

Figure 3. Myofibril loss (A, C, D), vascular congestion (white arrows) (B, D), mononuclear cell infiltration (D), hemorrhage (black stars) (C, E), necrosis (thick black arrows) (E), cells with eosinophilic cytoplasm and pycnotic nuclei (C, F), vacuolization (thin black arrows) (F) were observed in MI group. A, B, E: H-E; X20, C, D, F: H-E; X40. (MI: Myocardial infarction)

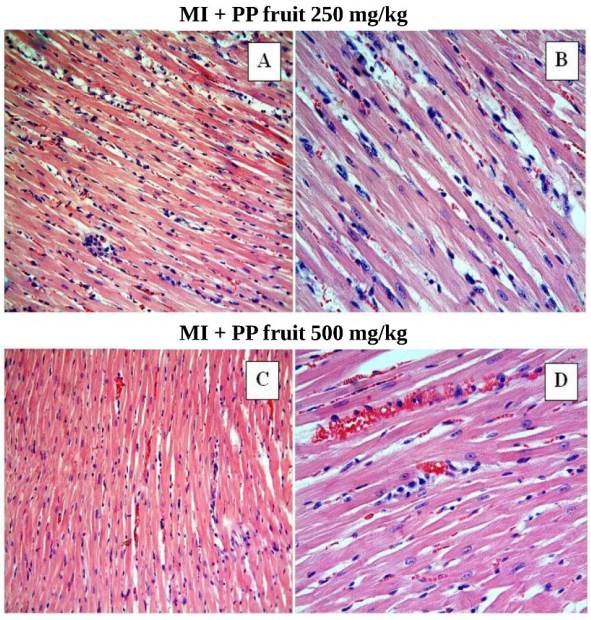

Figure 4. A small amount of hemorrhage and cell infiltration was observed in the heart muscle fibers in the MI+PP fruit 250 mg/kg group (A, B) and the MI+PP fruit 500 mg/kg group (C, D). A, C: H-E; X20, B, D: H-E; X40. (PP: *Pistacia palaestina* Boiss)

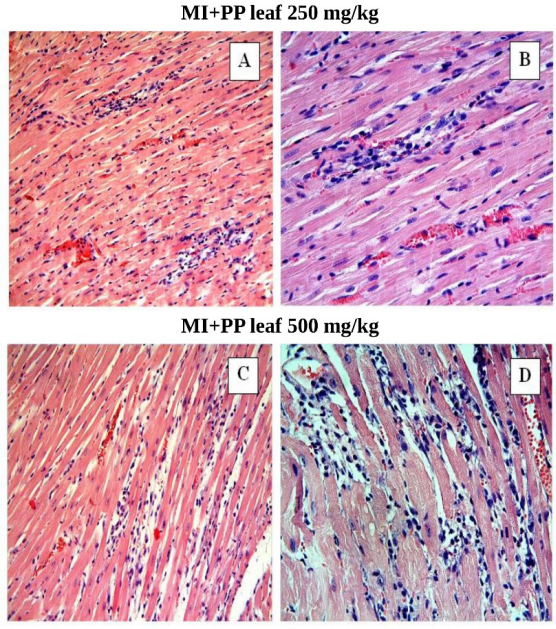

Figure 6. In the MI+PP leaf 250 mg/kg group (A, B) and the MI+PP leaf 500 mg/kg group (C, D), hemorrhage in the heart muscle fibers and moderate cell infiltration were observed compared to the MI group. A, C: H-E; X20, B, D: H-E; X40.

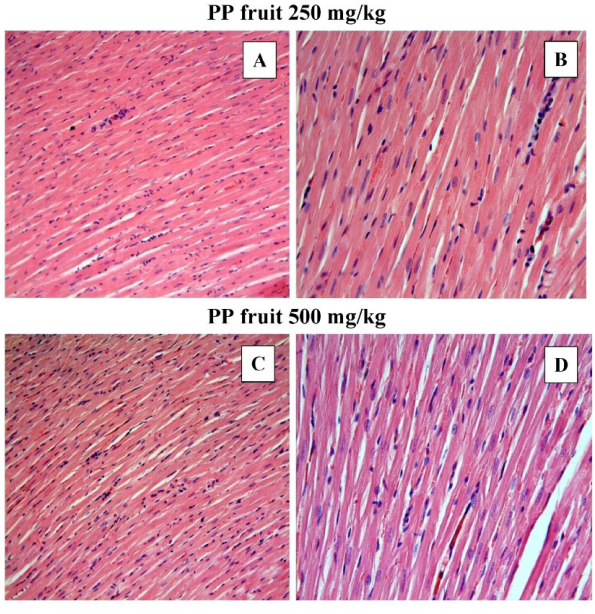

Figure 5. A small amount of cell infiltration was observed in the cardiac muscle fibers in the PP fruit 250 mg/kg group (A, B), and a small amount of hemorrhage in the cardiac muscle fibers in the PP fruit 500 mg/kg group (C, D). A, C: H-E; X20, B, D: H-E; X40.

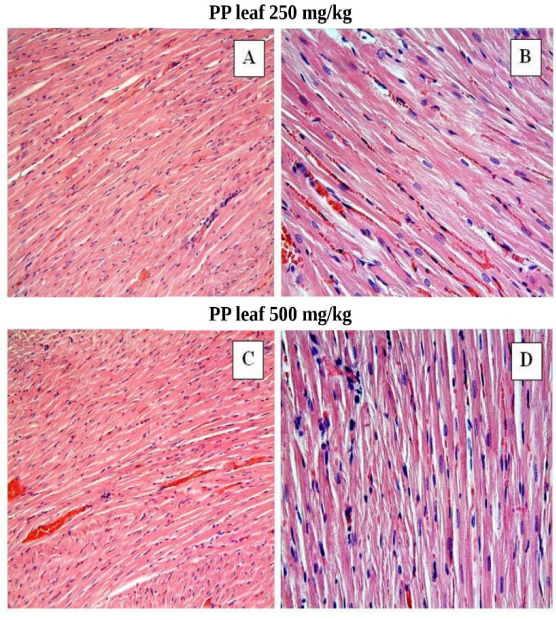

Figure 7. A small amount of hemorrhage and cell infiltration in the cardiac muscle fibers was observed in the PP leaf 250 mg/kg group (A, B), and a small amount of vascular congestion in the cardiac muscle fibers in the PP leaf 500 mg/kg group (C, D). A, C: H-E; X20, B, D: H-E; X40.

Myocardial infarction causes permanent and progressive cardiac damage. After MI, oxidative stress, inflammatory responses, and mitochondrial dysfunction increase, and apoptotic pathways are disrupted, resulting in necrosis and cell death (Schirone et al. 2022). Previous studies have reported that oxidative stress, inflammatory pathways, and mitochondrial dysfunction increase, and histopathological changes occur in cardiomyocytes during ISO-induced MI (Allawadhi et al. 2018; Feng et al. 2019; Xing et al. 2022). Oxidative stress, which increases with the production of free radicals and disruption of the scavenging balance through antioxidant mechanisms, further promotes myocardial hypoxia reactions with the secretion of proinflammatory mediators. This causes increased cardiac damage (Giordano, 2005). Therefore, we investigated the protective role of the fruit and leaf extracts of PP, which have antioxidant and anti-inflammatory activities, in ISO-induced MI.

In this study, we determined that the application of ISO caused an increase in TBARS levels in heart tissue. ISO increases lipid peroxidation, free radicals, and ROS production in heart tissue. In addition, it inhibits antioxidant defense mechanisms, and as a result of this imbalance, oxidative stress increases in cardiomyocytes and tissue damage occurs (Long et al. 2012; Zaafan et al. 2013). As suggested by previous studies, it is thought that ISO may cause cardiovascular damage by inducing oxidative stress in the heart (Goyal et al. 2015; Ganapathy et al. 2020; Zhang et al. 2021; Althunibat et al. 2022). Our study results are consistent with and support previous studies. Zhang et al. reported that ISO reduces GSH levels and CAT, SOD, and GPx activities (Zhang et al. 2021). Ganapathy et al. showed in their study that the *Thraatchathi chooranam* plant, which has antioxidant properties, reduces the increased TBARS levels and increases the levels of antioxidant defense system elements (GSH, CAT, SOD, GPx) in ISO-induced MI (Ganapathy et al. 2020). In the literature, many studies show that natural products with antioxidant properties increase antioxidant enzyme activities in ISO-induced MI (El-Gohary & Allam, 2017; Allawadhi et al. 2018; Huang et al. 2018; Ardjmand et al. 2019; Feng et al. 2019; Althunibat et al. 2022). Similarly, in our study, PP fruit and leaf extracts reduced ISO-induced oxidative stress by decreasing TBARS levels and increasing GSH levels, CAT, SOD, and GPx activity. In this study, the antioxidant properties of fruit and leaf extracts were similar. This effect is attributed to the high antioxidant capacity of *P. palaestina*. Therefore, PP may be an effective protective agent against cardiac damage caused by MI.

Troponin, a protein specific to skeletal and cardiac muscle fibers, is a highly sensitive and specific indicator of myocardial injury. In the event of a cardiac attack, it passes from the muscle tissue to the systemic circulation due to cardiac degeneration. With cardiovascular damage, the level of troponin in the blood rises (Tiwari et al. 2012). The CK-MB isoenzyme, which is especially located in cardiac muscle cells, is a highly sensitive marker with increased blood levels in major heart diseases and in patients diagnosed with acute MI (Karimkhani et al. 2021). In this study, troponin T and CK-MB levels increased in the ISO-induced MI group, consistent with previous findings. Troponin T and CK-MB levels were significantly decreased in all treatments with PP extracts. In another study, antioxidant treatment after ISO-induced MI improved oxidative stress parameters but slightly reduced serum troponin T levels and did not sufficiently reduce CK-MB levels (Ardjmand et al. 2019). As observed in our study, the use of PP, a natural antioxidant, before and after MI improved cardiac parameters more effectively. In addition, we suggest that high-dose fruit extracts may be more effective in treatment, since high-dose fruit extract treatment reduces troponin T and CK-MB levels more significantly.

Cytokines are produced by various cells, have a polypeptide structure, and are intercellular communication tools. It regulates the immune and inflammatory events of cells, including growth, healing, inflammation, and systemic response to injury. TNF-α,

IL-1β, and IL-6 are the main proinflammatory cytokines. IL-10, which is one of the anti-inflammatory cytokines, is a factor that inhibits cytokine synthesis (Huang et al. 2018; Ahmed & Ivashkiv, 2000). TNF-α is a cell signaling protein involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. TNF-α levels were also increased in regions without infarction area after chronic MI. However, it has been suggested that inflammatory cytokine levels decrease and survival rate increases after MI in TNF-α knockout mice in cardiac tissue (Sun et al. 2004). These results may suggest that TNF-α inhibition may reduce cardiac damage caused by MI. It has been reported that IL-1β and IL-6 have both harmful and protective effects during MI (Fuchs et al. 2003; Jong et al. 2016; Schirone et al. 2022). In a stress period, the inflammatory response is necessary for the cell survival. However, highly increased inflammation may exacerbate MI injury. Therefore, it is recommended not to suppress the inflammation excessively, especially in the acute phase of MI. However, reducing the levels of these cytokines, whose role has been proven in cardiac damage, is seen as an effective strategy (Schirone et al. 2022).

Previous studies have suggested that the levels of inflammatory factors, including IL-1β, IL-6, and TNF-α, are increased in the ISO-induced MI model (Huang et al. 2018; Ganapathy et al. 2020; Mohamed et al. 2021; Schirone et al. 2022; Xing et al. 2022; Cinar et al. 2022). In this study, while TNF-α, IL-1, and IL-6 levels in pro-inflammatory cytokines increased in the ISO-induced MI group, they decreased significantly with PP treatment. While the anti-inflammatory IL-10 level decreased in the MI group, it increased in the treatment groups. Significant reduction in proinflammatory cytokines and upregulation of anti-inflammatory IL-10 in the treatment groups were remarkable.

In this study, it was shown that ISO causes severe myofibril loss, vascular occlusion, hemorrhage, necrosis, and infiltration of heart muscle cells. The pathological findings decreased, and partial improvement was observed in the groups treated with both doses of PP fruit and leaves. Although no histopathological changes were observed in the control group, a small amount of cell infiltration and bleeding was observed in the groups in which only PP extract was applied. Huang et al. showed that ISO induces inflammatory cell infiltration and bleeding in hematoxylin-eosin staining of heart tissue (Huang et al. 2018). When another study group examined the histological structures of myocardial cells under a light microscope, they found that the myocardiocytes of rats in the ISO-induced MI group were severely damaged. In addition, they detected necrotic myofibril structures, mononuclear cell infiltration, edema, and vascular occlusion (Karimkhani et al. 2021). Several recent studies have suggested that antioxidant and anti-inflammatory compounds may reduce cardiac damage caused by ISO (Abbas, 2016; Kalkan et al. 2018; Ganapathy et al. 2020). Based on our study results, we can suggest that PP administration may reduce immunologically induced cardiovascular damage by reducing inflammatory cytokines and increasing antioxidant enzyme activity.

In this study, the cardioprotective roles of PP leaf and fruit extracts, a member of the *Pistacia* genus with antioxidant and anti-inflammatory properties grown in Malatya, were evaluated in an ISO-induced MI rat model. In all treatment groups, the lipid peroxidation indicator TBARS levels; the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6; the cardiac damage indicator troponin T; and CK-MB levels decreased. Low- and high-dose extracts of PP increased anti-inflammatory IL-10 levels and antioxidant enzyme activities. Improved antioxidant mechanisms and decreased inflammation also reduce histopathological changes, indicating tissue damage. PP leaf and fruit extracts may play an important cardioprotective role in treating MI with their antioxidant and anti-inflammatory effects on heart tissue. With this study, it was suggested for the first time that PP extracts have antioxidant, anti-inflammatory, and cardioprotective effects against cardiac damage. Our study results showed that PP extracts can be considered as a natu-

ral and new treatment alternative for cardiovascular diseases. However, its effectiveness is insufficient for treatment. In addition to its effectiveness, comprehensive research on its safety, optimum dose, and cellular mechanism of action is required.

CONCLUSION

For the first time, this study found that PP leaf and fruit extracts reduced oxidative stress and inflammatory response in cardiac tissue, improved histopathological changes, and ameliorated ISO-induced MI. However, more comprehensive and advanced mechanistic studies are needed regarding the protective role of this plant in heart diseases.

ACKNOWLEDGMENT

This study was supported by IUBAP (İnönü University Scientific Research Project Unit) numbered TSA-2020-2146. All authors would like to thank Prof. Dr. Turan Arabacı, who identified *Pistacia palaestina* Boiss. plant material.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

M.S.İ., literature search, research concepts, design, experimental studies; H.Y., literature search, experimental studies, data acquisition, design; N.B.T., experimental studies, manuscript writing, research concepts, design; A.T., experimental studies, data analysis; D.A.Ö., data analysis, manuscript writing, literature search, experimental studies; S.Ü., data analysis, conducting the research, interpreting the data, compiling and revising the article

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