Phoenix Dactylifera L. Tree Fruit Exerts Cardioprotective Effect Against Doxorubicin-Induced Heart Damage in Rats via Inhibition of Oxidative Stress

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ABSTRACT

Introduction: Phoenix Dactylifera L (PDL) is a fruit containing a rich source of nutrients and bioactive molecules. Doxorubicin is a widely used agent, especially in the treatment of solid cancers. However, cardiotoxicity is one of its most challenging side effects. The present study aimed to investigate the preventive effect of PDL extract against doxorubicin-induced cardiotoxicity.

Patients and Methods: A total of 24 albino Wistar rats were divided into four equal groups. Phoenix Dactylifera L (PDLG) and Phoenix Dactylifera L + doxorubicin (PDXG) groups were strictly fed PDL for two weeks. The control group (CG) and the doxorubicin group (DOXG) were fed a standard diet. During this time, 5 mg/kg of doxorubicin was injected intraperitoneally to DOXG and PDXG once a day.

Results: Administration of doxorubicin to the DOXG significantly increased tissue oxidative stress parameters and caused the cardiac biomarker troponin-I (TP-I) to be released into the circulation; on the contrary, the levels of potent antioxidants such as total glutathione, superoxide dismutase, and catalase significantly decreased in DOXG compared to the other three groups. However, feeding purely with PDL decreased oxidative stress parameters and TP-I levels in PDXG animals, despite exposure to doxorubicin. Additionally, an excessive decrease of tissue antioxidants was prevented when compared to the DOXG. Histopathological damage signs, such as necrosis and hemorrhage, were severe in the DOXG. However, in the PDXG animals, feeding with PDL provided the integrity of the heart tissue structure.

Conclusion: PDL was able to improve the cardiotoxic consequences of doxorubicin biochemically and histopathologically, possibly due to its antioxidant properties.

Key Words: Doxorubicin; oxidative stress; cardiotoxicity; Phoenix Dactylifera L; rat

Phoenix Dactylifera L. Ağacı Meyvesi Sıçanlarda Doksorubisinin Neden Olduğu Kalp Hasarına Karşı Oksidatif Stres İnhibisyonu Yoluyla Kardiyoprotektif Etki Göstermektedir

ÖZET

Giriş: Phoenix Dactylifera L (PDL), birçok besin kaynağı içeren biyoaktif moleküller yönünden zengin bir meyvedir. Doksorubisin özellikle solid kanser türlerinin tedavisinde yaygın olarak kullanılan bir ajandır. Bununla birlikte, kardiyotoksisite en çok sorun oluşturan yan etkilerinden biridir. Bu çalışma, doksorubisin kaynaklı kardiyotoksisiteye karşı PDL ekstraktının önleyici etkisini araştırmayı amaçladı.

Hastalar ve Yöntem: Toplam 24 albino Wistar erkek sıçan dört gruba ayrıldı. Phoenix Dactylifera L (PDLG) ve Phoenix Dactylifera L + doksorubisin (PDXG) grupları iki hafta boyunca sadece PDL ile beslendi. Kontrol grubu (CG) ve doksorubisin grubu (DOXG) standart diyetle beslendi. Bu süre zarfında doksorubisin grubuna (DOXG) ve PDXG'ye günde bir kez 5 mg/kg doksorubisin intraperitoneal olarak enjekte edildi.

Bulgular: DOXG'ye doksorubisin uygulanması doku oksidatif stres parametrelerini önemli ölçüde artırdı ve kardiyak biyobelirteç olan troponin-I'nın (TP-I) dolaşıma salınmasına neden oldu. Total glutatyon, süperoksit dismutaz ve katalaz gibi güçlü antioksidanların seviyelerinin ise diğer üç gruba göre önemli ölçüde azalmasına neden oldu. Sadece PDL ile beslenen PDXG'deki hayvanlarda, doksorubisine maruz kalmalarına rağmen, oksidatif stres parametreleri ve TP-I seviyeleri belirgin şekilde azaldı. Ayrıca, DOXG'ye göre, doku antioksidanların aşırı azalması önlendi. DOXG'de nekroz ve kanama gibi histopatolojik hasar bulguları şiddetliydi. Ancak PDXG'deki hayvanlarda PDL ile beslenme kalp doku yapısının bütünlüğünün korunmasında etkili oldu.

Sonuç: PDL, muhtemelen antioksidan özelliklerinden dolayı doksorubisinin kardiyotoksik etkilerini biyokimyasal ve histopatolojik olarak iyileştirebildi.

Anahtar Kelimeler: Doksorubisin; oksidatif stres; kardiyotoksisite; Phoenix Dactylifera L; sıçan

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INTRODUCTION

Doxorubicin is an anthracycline-derived cytotoxic agent that is widely used for the treatment of many systemic neoplasms and solid tumors. The use of anthracyclines markedly improved the survival of many cancer patients. However, the side effects seen in many tissues due to doxorubicin treatment are challenging. Cardiotoxicity is one of the most important side effects and may require dose reduction or discontinuation of the drug[1]. Cardiotoxic side effects due to doxorubicin were detected for the first time in children presenting with signs of heart failure[2]. Clinical observations have shown that the possibility of cardiotoxic side effects such as myocarditis, arrhythmia, and heart failure usually arises with high doses of doxorubicin, and the risk increases with prolonged treatment duration[3].

Literature reviews show that free oxygen radicals have an important role in the emergence of cardiotoxicity caused by doxorubicin[4]. Since cardiomyocytes are characterized by low intrinsic antioxidant content such as glutathione (GSH) and superoxide dismutase (SOD), the myocardial tissue is relatively more susceptible to the destructive effects of free radicals[1].

The commonly described mechanism of doxorubicin-related side effects is the transformation of doxorubicin to primary quinone, leading to the formation of highly destructive reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2)[5]. Some other mechanisms attributable to doxorubicin-induced cardiotoxicity are increased intracellular iron levels and associated reduction of antioxidants such as catalase (CAT), increased rate of apoptosis, acceleration of cardiac fibrosis via induced proinflammatory cytokines, energy imbalance, and development of congestive heart failure through inhibition of cardiac fatty acid oxidation. Doxorubicin-induced oxidative stress ultimately increases intracellular calcium and accelerates lipid peroxidation, damaging the cell membrane and other cellular components, e.g., deoxyribonucleic acid (DNA)[6,7].

Superoxide dismutase, CAT, and GSH are first-line antioxidants protecting cell membranes and DNA against the detrimental effects of ROS. Previous studies have shown that exposure to doxorubicin increases oxidative damage parameters such as malondialdehyde (MDA) and decreases antioxidant parameters such as GSH[8]. Studies have also shown that antioxidants can be a useful treatment option in chemotherapy-induced cardiotoxicity[9].

Phoenix Dactylifera L. tree fruit (PDL) is an antioxidant plant belonging to the palmaceae family, widely consumed in the Middle East as fresh and dried. PDL contains vitamins, trace elements, amino acids, oils, and bioactive molecules such as polyphenols and flavonoids, with potent antioxidant properties[10]. Experimentally, PDL has shown protective effects on many organs such as the heart, liver, kidney, and nervous system with prominent antioxidant activity[11]. PDL also provided evidence for its antioxidant property in laboratory conditions by removing superoxide and hydroxyl radicals[12]. In the present study, we aimed to establish the therapeutic potential of PDL against doxorubicin-induced heart damage.

PATIENTS and METHODS

Animals

A total of 24 albino Wistar male rats weighing an average of 220-230 grams were used for the experiment. All rats were obtained from Atatürk University Medical Experimental Application and Research Center. Animals were housed under appropriate conditions in the laboratory environment at an average room temperature (22°C) with alternating 12 hr light/dark cycles. The local Animal Experimentation Ethics Committee approved the protocols and procedures (Date: 29.01.2016, Decision no: 1/22).

Chemicals

For the experiment, doxorubicin was obtained from Saba (Türkiye), and thiopental sodium from I. E. Ulagay (Türkiye) and Phoenix Dactylifera L from Biota (Türkiye).

Experimental Groups

A total of 24 albino Wistar male rats were divided into four groups, six in each: Doxorubicin (DOXG), Phoenix Dactylifera L + doxorubicin (PDXG), Phoenix Dactylifera L (PDLG) and control (CG) groups.

Experimental Procedures

PDXG and PDLG were strictly fed PDL for two weeks to determine the effect of the fruit. These groups did not have access to any food other than PDL and water. The amount of PDL consumed per day was determined as an average of 20 grams per animal. DOXG and CG animals were fed a standard diet that did not contain PDL. The animals had access to food and water without amount restriction all day and night. The animals were observed daily for any changes in feeding behavior and general activities. PDL, standard diet, and water were carefully replaced daily. During the experiment, 5 mg/kg of doxorubicin was injected intraperitoneally to DOXG and PDXG once a day. Distilled water was injected into PDLG and CG animals in the same way. At the end of this period, blood samples were drawn by cardiac puncture and collected into tubes with EDTA, then the animals were sacrificed with a high dose of anesthesia (50 mg/kg thiopental sodium) and their hearts were removed. Blood samples and heart tissues were used for biochemical analysis and histopathological examination. The results obtained from DOXG, PDXG, PDLG, and CG were compared with each other.

Biochemical Analysis

Preparation of samples

The tissue homogenates taken from the left ventricle were centrifuged at 10.000 rpm for 20 min at 4°C, and the supernatants were extracted to analyze MDA, GSH, CAT, and SOD.

MDA Analysis

MDA analysis is performed by using thiobarbituric acid and was measured spectrophotometrically as defined by Ohkawa et al[13].

tGSH Analysis

tGSH analysis was performed according to Bradley et al. The analysis mechanism was defined as using 5.5'-dithiobis 2-nitrobenzoic acid[14].

SOD Analysis

SOD Measurements were performed according to Sun et al., the described method was adding nitroblue tetrazolium[15].

CAT Analysis

CAT activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute at 25°C and pH 7.8. The decomposition of H_2O_2 in the presence of CAT was measured at 240 nm[16].

DNA Oxidation and Tropo Analysis

DNA oxidation analysis was performed according to the description of Shigenaga et al. 8-OHdG and deoxyguanine (dG) levels were measured in HPLC with HPLC-UV and HPLC-ECD electrochemical detectors at various wavelengths. 8-OHdG /105 dG was accepted as an indicator of DNA damage[17,18].

Troponin I (TP- I) Analysis

TP-I levels were measured in the VIDAS Troponin I Ultra kit by utilizing the ELFA (Enzyme-Linked Fluorescent Assay) technique.

Histopathological Examination

Necropsies of the rats were made and the heart tissues from the left ventricle were fixed in a 10% neutral formalin solution. Tissues were taken into paraffin blocks after routine alcohol-xylol follow-up procedures. Five μ sections taken on slides with poly-lysine were stained with hematoxylin-eosin, and six random areas were determined to be absent (-), mild (+), moderate (++), and severe (+++) in terms of necrosis, hemorrhage, mononuclear cell infiltration and edema evaluated under the light microscope.

Statistical Analysis

Descriptive statistics were generated for biochemical analysis in each group. The results obtained from the experiments were expressed as "mean value ± standard deviation" (x ± SD). Outlier analysis was performed using the Tukey test. Differences between groups were compared by one-way analysis of variance (ANOVA). All statistical analyses were performed using "SPSS Statistics Version 18" statistical software and p values< 0.05 were considered significant.

RESULTS

Results of MDA, 8-OHGua, and TP-I Analyses

MDA levels were significantly increased in the heart tissue of the group treated with doxorubicin alone (DOXG), compared to the PDLG, PDXG, and CG (p< 0.0001) (Figure 1A). An excessive increase in MDA levels was prevented in the doxorubicin plus PDL-administered PDXG group when compared to the DOXG (p< 0.001). There was no statistical difference between PDXG and CG (p> 0.05). Additionally, the level of MDA was found to be significantly lower in PDLG than in the CG (p< 0.05) (Figure 1A). The level of 8-OHGua and TP-I significantly increased in the DOXG compared to PDLG, PDXG, and CG (p< 0.0001). Despite exposure to doxorubicin, feeding with PDL significantly reduced the 8-OHGua tissue levels and also prevented the excessive leakage of TP-I into the circulation in the heart tissues of the PDXG, when compared to DOXG (p< 0.0001). PDXG TP-I and 8-OHGua levels were found to be similar to PDLG and CG (p> 0.05) (Figures 1B, 1C).

Results of tGSH, SOD, and CAT Analyses

In the heart tissues of the doxorubicin (DOXG) group, the levels of all three antioxidants (tGSH, SOD, and CAT) have decreased significantly (p< 0.001) compared to PDXG, PDLG, and CG. Feeding with PDL prevented the excessive decrease of antioxidant levels in PDXG compared to DOXG (p< 0.001) and brought the values closer to CG and PDLG (p> 0.05) (Figures 1D, 1E, 1F).

Histopathological Findings

Statistically significant differences were found between the groups (p< 0.05) (Table 1). The myocardium of the CG and PDLG rats had a normal histological appearance. Histopathological findings, such as necrosis and hemorrhage, were severe in the DOXG. However, in the PDXG animals, the aforementioned findings were significantly milder. Additionally, mononuclear cell infiltration and edema were severe in the DOXG but mild in the PDXG (Table 1, Figures 2,3).

DISCUSSION

The present study was conducted to investigate the effect of PDL on doxorubicin-induced cardiotoxicity in Wistar rats both biochemically and histopathologically. The study results showed that high-dose doxorubicin exposure changes heart tissue oxidant/antioxidant balance in favor of oxidants.

Table 1. Histopathological findings of the rat heart tissues

Groups	Necrosis	Hemorrhage	Mononuclear Cell Infiltration	Edema
CG	(-)[a]	(-)[a]	(-)[a]	(-)[a]
PDLG	(-)[a]	(-)[a]	(-)[a]	(-)[a]
DOXG	(+++)[d]	(+++)[d]	(+++)[d]	(+++)[d]
PDXG	(+)[b]	(+)[b]	(+)[b]	(++)[c]

Doxorubicin (DOXG), Phoenix Dactylifera L + doxorubicin (PDXG), Phoenix Dactylifera L (PDLG) and control (CG) groups.
[a,b,c] Shows the difference between groups.
a: Absent (-), b: Mild (+), c: Moderate (++), d: Severe (+++).

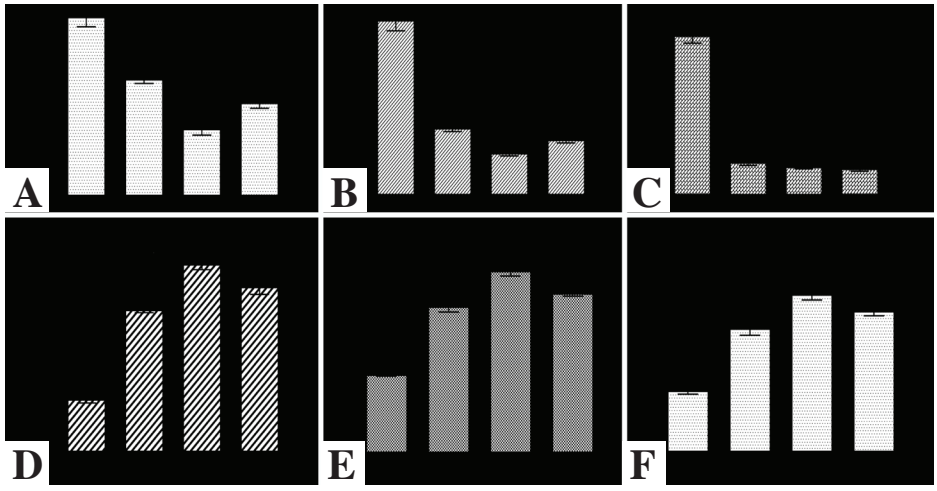

Figure 1. Heart tissue biochemical parameter changes and blood TP-I levels of DOXG, PDXG, PDLG, and CG groups. **A;** MDA heart tissue levels of all groups. *= p< 0.0001 according to PDXG, PDLG, and CG. **= p< 0.05 according to CG. **B;** 8-OHGua heart tissue levels of all groups. *= p< 0.0001 according to PDXG, PDLG, and CG. **C;** TP-I heart tissue levels of all groups. *= p< 0.0001 according to PDXG, PDLG, and CG. **D;** tGSH heart tissue levels of all groups. *= p< 0.001 according to PDXG, PDLG, and CG. **E;** CAT heart tissue levels of all groups. *= p< 0.001 according to PDXG, PDLG, and CG. **F;** SOD heart tissue levels of all groups. *= p< 0.001 according to PDXG, PDLG, and CG.

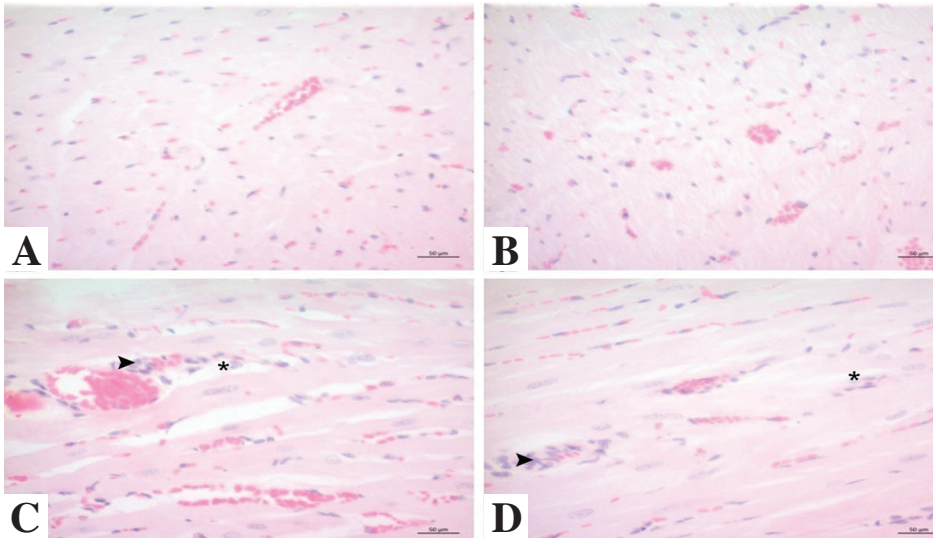

Figure 2. Heart tissue damage findings after PDL and doxorubicin administration **A;** CG and **B;** PDLG groups with normal histological appearance. **C;** DOXG group with severely necrotic-degenerative myocytes (arrowhead) and hemorrhage (asterisk). **D;** PDXG group with mild necrotic-degenerative myocytes (arrowhead) and hemorrhage (asterisk). Myocardium, H-E.

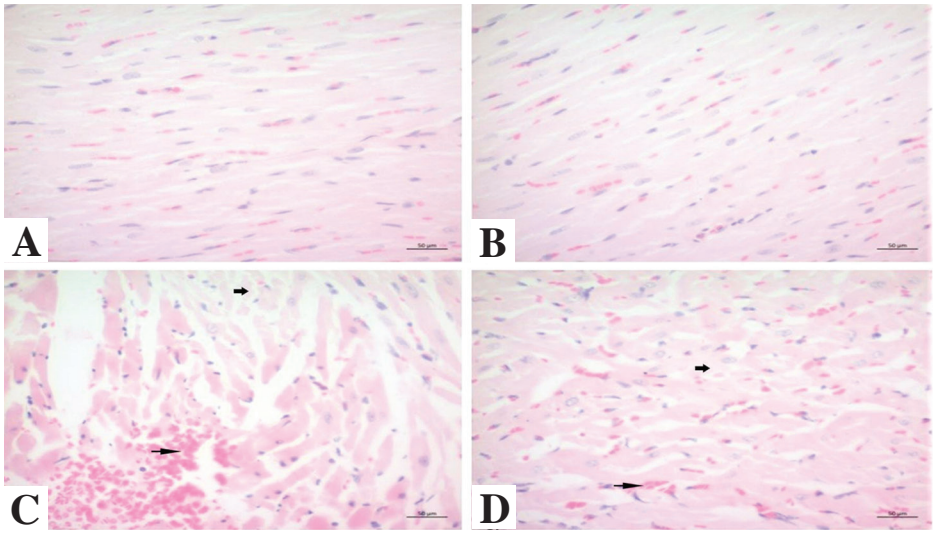

Figure 3. Heart tissue damage findings after PDL and doxorubicin administration **A;** CG and **B;** PDLG groups with normal histological appearance. **C;** DOXG group with severe mononuclear cell infiltrates (thick arrow) and edema (thin arrow). **D;** PDXG group with mild mononuclear cell infiltrates (thick arrow) and moderate edema (thin arrow) Myocardium, H-E.

Feeding with PDL was able to ameliorate heart tissue damage caused by doxorubicin. Phoenix Dactylifera L is a fruit containing a rich source of antioxidant nutrients and bioactive molecules that have previously shown therapeutic efficacy[10]. Current study experimental results showed that long-term exposure to doxorubicin significantly increases MDA levels in heart tissue. Reactive oxygen species led to the peroxidation of cell membrane fatty acids, resulting in the formation of cytotoxic end products such as MDA. As is known, lipid peroxidation and DNA destruction is a chemical reaction that is initiated by ROS and involves the oxidation of unsaturated fatty acids in the cell membrane and core[19]. According to the present study results, significantly increased MDA tissue levels in the DOXG and decreased levels in the PDXG suggest that PDL inhibits oxidative stress. Interestingly, MDA levels were significantly lower in the group fed strictly PDL (PDLG), compared to CG fed the standard rat diet. The present study demonstrated that feeding with PDL may improve the oxidative condition in favor of antioxidants, even in healthy animals. This result is consistent with a study by Habib and İbrahim who showed that feeding healthy rats with PDL seeds reduced oxidative stress significantly both in serum and liver tissues[20]. Phoenix Dactylifera L may have played an important role in preventing lipid peroxidation due to its abundant antioxidant content, such as δ-tocotrienol and ferulic acid. These bioactive molecules have been found to reduce inflammation, protect the heart structure from damage, and improve cardiac function in diet-induced obese and hypertensive rats[21].

Superoxide dismutase and catalase are endogenous antioxidant enzymes that protect the cell membrane against lipid peroxidation and DNA damage. Superoxide dismutase is an important enzyme that deactivates superoxide (O_2-) radicals by converting them into the less reactive H_2O_2. Catalase reacts with H_2O_2 and ultimately forms water and molecular oxygen. Prolonged exposure to oxidative stress results in decreased tissue antioxidant levels while efforts are made to remove superoxide radicals[22].

Glutathione maintains cell integrity in a reduced state, serving as an electron donor for certain antioxidant enzymes. Additionally, reduced GSH may give electrons to H_2O_2 and effectively reduce the amount of this harmful reactive molecule in the heart tissue[23]. Meanwhile, the production of ROS over the detoxifying capacity lowers GSH levels and causes damage to tissues[24]. In the present study, GSH, SOD, and CAT levels significantly decreased in the DOXG, probably due to ROS overproduction. However, detecting all three antioxidants close to CG in the PDXG suggests that PDL has an antioxidant protective effect on doxorubicin-induced cardiotoxicity. Phoenix Dactylifera L. also contains selenium, a cofactor for GSH, which is known to have an essential role in supporting heart function and maintaining heart muscle metabolism[25].

As with lipids, excess ROS products react with nucleic acids and cause severe damage to DNA. The reaction of H_2O_2 with iron-copper (Fe-Cu) ions causes the formation of hydroxyl radicals and ultimately results in the accumulation of 8-OHGua, a DNA oxidative damage product[26,27]. Previous studies showed that PDL is a potent scavenger of ROS, such as hydroxyl radical. Additionally, it has a protective effect against DNA damage by inhibiting iron-induced lipid peroxidation[12,28]. Doxorubicin stimulates the inducible nitric

oxide synthase enzyme, increasing nitric oxide (NO) production, which is linked to congestive heart failure[29]. The interaction between NO and H_2O_2 generates peroxynitrite anion (ONOO-), known to cause DNA damage[30]. In the present study, levels of 8-OHGua were significantly higher in the DOXG than in the PDXG. However, robust reduction of 8-OHGua levels in the PDXG points out to cardioprotective, antioxidant effects of PDL against oxidative damage.

Troponin-I is highly sensitive for myocardial injury; therefore, it is widely used to diagnose myocardial infarction. Studies have reported that oxidative stress caused by ROS leads to myocardial cell membrane damage resulting in TP-I release into the circulation as in myocardial infarction[31]. In a present study, feeding with PDL prevented the excessive rise of the TP-I blood serum levels in the PDXG compared to the DOXG; therefore, it probably preserved membrane integrity due to the limitation of leakage of this biomarker into circulation. The rich lutein and p-coumaric acid content in PDL may have contributed to lowering the level of circulating cardiac biomarkers and maintaining cell membrane integrity[21].

Previous studies showed that at the histological level, doxorubicin-induced ROS overproduction is related to diffuse fibrinoid necrosis of the arteriole walls, myofibrillary loss, intracellular vacuolization, widespread infiltration of leukocytes in rats[6]. Similarly, the current histopathological examination showed that doxorubicin administration caused severe damage signs such as degenerative myocytes, mononuclear cell infiltrates, hemorrhage, and edema. However, PDXG tissue examination showed that damage signs such as PNL infiltration, edema, and congestion were significantly reduced compared to the DOXG, despite exposure to high doses of doxorubicin. PDXG histopathological signs found close to CG point out that PDL preserved the structural integrity of the heart tissue.

CONCLUSION

Prolonged exposure to doxorubicin caused oxidative stress in rat heart tissue and blood serum biochemically and histopathologically. However, PDL was able to improve the toxic consequences of doxorubicin, possibly due to its potent antioxidant activity. More research is needed to determine which specific nutrient or bioactive molecule in PDL acts as a cardioprotective agent during doxorubicin-induced oxidative stress.

Ethics Committee Approval: The study was approved by Recep Tayyip Erdoğan University Medical Experimental Application and Research Center Local Animal Experimentation Ethics Committee (Decision no: 11, Date: 26.10.2017).

Informed Consent: This is retrospective study, we could not obtain written informed consent from the participants.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept/Design - RC, HS; Analysis/Interpretation - AİÇ, MSC; Data Collection - CD, AİÇ; Writing - RC, HS; Critical Revision - MT, HS; Statistical Analysis - RC, CD, MT; Final Approval - RC, MSC; Overall Responsibility - RC.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

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