



# The protective effects of glycyrrhizin on doxorubicin-induced cardiotoxicity in rats

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## Abstract

**Aim:** Doxorubicin (DOX) is a type of chemotherapy drug frequently used to treat different malignancies. However, one of the most serious adverse effects of DOX usage is the potential of cardiotoxicity. Cardioprotective medications may be used to reduce cardiac damage because of DOX therapy. Glycyrrhizin (GL) is found in high amounts in the roots of the ‘Licorice’ plant from the Glycyrrhiza species. Due to its possible effects on blood pressure (BP) and cardiovascular health, GL has attracted attention concerning the heart. Oxidative stress and inflammatory process have been shown to be responsible for DOX-induced cardiotoxicity (DIC). For this reason, in consequence of its possible pharmacological benefits, such as antiinflammatory and antioxidant GL has been researched in this study. Here in, we aimed to investigate the protective effects of GL on DIC.

**Materials and Methods:** In this study, thirty-two male *Wistar albino* adult male rats were used. Four groups of rats were assigned at randomly: Control, DOX, GL+DOX, and GL groups. DOX was given 20 mg/kg intraperitoneally (i.p.) and 100 mg/kg GL was administered orally (p.o.) once a day for 14 days. Electrocardiography (ECG) and BP records of the rats were obtained. In addition, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels in the tissue were measured. Histopathological analyses were performed on the myocardium and descending aorta.

**Results:** In the DOX group, mean and diastolic BP were higher than in the control group ( $p < 0.05$ ). In the GL+DOX group, diastolic BP was lower than in the DOX group ( $p < 0.05$ ). Pathological ECG changes such as ST segment changes and T negativity were observed in DOX-treated groups. MDA, SOD, CAT, and GSH levels studied in heart tissue were similar in all groups ( $p > 0.05$ ). GSH level in descending aorta was significantly lower in the GL+DOX group compared to the other groups ( $p < 0.05$ ). In the DOX group, degenerated cardiomyocyte density, interstitial edema, and severity of congestion-hemorrhage were statistically significantly increased compared to the control group ( $p < 0.05$ ). On the other hand, degenerated cardiomyocyte density was found to be significantly decreased in the GL+DOX group compared to the DOX group ( $p < 0.05$ ). In the DOX group, thinning of elastic lamellae and loss of myofibrils in muscle cells were observed in the descending aorta. Therefore, the histopathological alterations identified in the DOX group exhibited a significant statistical improvement in the GL+DOX group ( $p < 0.05$ ).

**Conclusion:** Based on the study’s findings, GL can regulate high BP caused by DOX and also alleviate the toxic effects of DOX on both the myocardium and descending aorta.

## ARTICLE INFO

### Keywords:

Cardiotoxicity  
Doxorubicin  
Glycyrrhizin  
Oxidative stress  
Rat

Received: Aug 24, 2023

Accepted: Oct 13, 2023

Available Online: 25.10.2023

DOI:

[10.5455/annalsmedres.2023.08.219](https://doi.org/10.5455/annalsmedres.2023.08.219)



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## Introduction

Cardiovascular disease and cancer are the two main causes of morbidity and mortality worldwide [1]. Cardiac injury

resulting from cardiotoxic effects of cancer treatments is a frequent cause of mortality post-treatment. Administering cardioprotective therapies earlier may allow chemotherapy to continue without interruption [1,2].

Doxorubicin (DOX) is a potent chemotherapy drug that

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is used to treat solid tumors, such as malignancies of the ovaries, breast, and gastrointestinal tract [2]. Cardiotoxicity induced by DOX is a fundamental aspect of cancer treatment. Patients undergoing anthracycline therapy may experience both irreversible adverse effects such as cardiomyopathy and heart failure, as well as reversible side effects such as changes in blood pressure (BP), pericarditis, and myocarditis [3, 4].

DOX-induced cardiotoxicity (DIC) has undergone significant examination in various animal models, particularly rats, in order to understand its mechanisms and potential interventions [5]. DIC is produced by a wide range of causes and routes. The pathophysiology of DIC is heavily influenced by inflammatory cytokines, oxidative stress, mitochondrial damage, intracellular  $\text{Ca}^{+2}$  excess, iron-free radical generation, DNA damage, and myocyte membrane injuries [2]. Several studies have suggested implementing preventative therapies to reduce the severity or frequency of DIC. Antiinflammatory or antioxidant drugs are used as additional treatment to reduce DIC [2].

Licorice root contains a naturally occurring substance known as glycyrrhizin (GL), which has been linked to potential effects on the cardiovascular system. While some research indicates the possibility that GL may have specific cardiac effects, it's critical to highlight that further research is required in this area. GL is a prodrug that is sequentially converted in the intestines [6]. GL has a broad spectrum of pharmacological actions, including antiviral, antiulcer, and antiinflammatory properties [7-9]. Recently several investigations have been performed to treat or prevent DIC. Treatment of cancer patients may be interrupted due to DOX cardiotoxicity. Therefore, there is a need for treatments that can effectively prevent cardiotoxicity. The present study was planned to determine the role of GL in preventing DIC.

## Materials and Methods

According to the results of the theoretical power analysis, at the 5% significance level, when the power of the test is 80% the effect size is 0.75 and the alternative hypothesis ( $H_1$ ) is the sample size that needs to be reached in order to find a statistically significant difference between the groups using the independent samples ANOVA should be at least 24 in total. The primary outcome variables were oxidative variables (MDA nmol/g wet tissue, GSH nmol/g wet tissue, SOD U/g protein, CAT K/g protein) in this study.

For this study, a total of 32 male *Wistar albino* rats aged 9–12 weeks weighing 350-400 g were obtained from Inonu University Laboratory Animals Researcher Center. Ethics committee approval of the study was obtained from the animal ethics committee of Inonu University Faculty of Medicine (protocol number: 2016/A-43). They were kept in polycarbonate-based cages under standard laboratory conditions ( $22\pm 2$  °C, 60% humidity environment, 12-hour light/12-hour dark cycle). Standard pellet feed of 8 millimeters (mm) was used for feeding. The rats were given a usual chow pellet diet and had unrestricted access to water and pelleted feed. The rats were simple randomly assigned to four groups using Random Allocation Rule method as follows: Control Group: Vehicle solution was applied in

the rats. DOX Group: A single dose of 20 mg/kg DOX was administered intraperitoneally (i.p). GL+DOX Group: 100 mg/kg GL was administered orally (p.o.) once a day for 14 days. On the 12<sup>th</sup> day of the study, 20 mg/kg DOX was administered i.p. as a single dose. GL Group: 100 mg/kg GL was administered p.o. once a day for 14 days.

GL was dissolved in 1 mL of distilled water and the control group received the same volume of distilled water by the same route. The dose of DOX was adjusted according to the literature [10]. Different doses have been used in studies with GL. In this study, a final concentration of 100 mg/mL GL was obtained with distilled water [11,12].

After the experiment, 75 mg/kg of ketamine hydrochloride (Park-Davis, Eczacıbasi, İstanbul, Türkiye) and 8 mg/kg of xylazine (i.p.) (Rompun, Bayer Drug, Leverkusen, Germany) were combined to anesthetize all rats. The left carotid arteries of the rats were cannulated to measure the systolic, diastolic, and mean BP. Heart rate (HR) was calculated from the electrocardiography (ECG) recording. The Biopac MP-100 Data Acquisition system computer recording program (Biopac Systems, Inc., Santa Barbara, CA) was used to record the BP, HR, and ECG for 3 minutes. One expert physician who was blind to the condition of the animals reviewed the ECG data. Diverse arrhythmias, alterations in the ST segment, and T waves were assessed. PR, QRS, and QT intervals were calculated. Cardiovascular tissue samples were taken for histopathological and biochemical analysis. The inferior vena cava was used to collect blood samples, and the heart and aortic tissues were taken out for histological examination.

### Biochemical analysis

The technique proposed by Mihara and Uchiyama was used to measure the malondialdehyde (MDA) level in tissue homogenates, and the findings were reported as nanomoles of MDA per gram of wet tissue (nmol/g wet tissue) [13]. Ellman's method was used to measure glutathione (GSH) levels [14]. When reduced GSH was combined with 5,5-dithiobis-2-nitrobenzoic acid, spectrophotometric measurement was carried out at a wavelength of 410 nm. The findings were shown as nmol/g wet tissue. The Sun et al. approach was used to evaluate copper-zinc superoxide dismutase (SOD) activity in cardiac tissues [15]. U/g protein was used to represent the results. Catalase (CAT) activity was found using the Luck technique, which involved reading the samples spectrophotometrically at 240 nm every 15 s over the course of 90 s [16]. In tissues, enzyme activity was shown to be the first-order reaction kinetics rate constant as K/g protein.

### Protein determination

Protein concentration was assigned as described by the Biuret Method and a calibration curve was constructed using bovine serum albumin [17]. The protein concentration values were used to calculate biochemical analyses.

### Histopathological analysis

The heart and vascular tissues were fixed in 10% formaldehyde after the experiment. Tissue was followed and paraffin blocks were prepared. Then, sections of 4-5  $\mu\text{m}$  thickness were taken. Hematoxylin-eosin (H-E) staining was

used to identify the general histopathological structure of the sections. Heart sections were assessed for congestion-hemorrhage, interstitial edema, and cardiomyocyte degeneration (myofibril loss, dense eosinophilic cytoplasm, pyknotic nucleus). Ten randomly selected areas were examined and the areas were according to the degree of histopathological changes; 0: no change, 1: mild, 2: moderate, 3: severe change.

In the evaluation of the aorta; for each sample, the whole area was examined in 2 separate sections. Histopathological changes in the vessel wall (myofibril loss in muscle cells, thinning and rupture of elastic lamellae) were scored as 0: no change, 1: mild, 2: moderate, and 3: severe change. In addition, tunica intima-media thickness was measured by randomly selecting ten areas from each section. Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) and a Leica DFC-280 research microscope were used for the analyses.

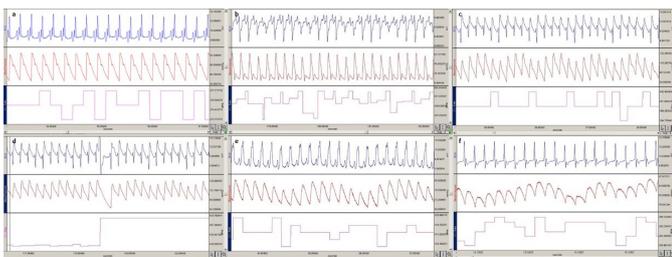
### Statistical analysis

In the current study, while the quantitative variables in the dataset were presented as median (minimum-maximum), and the qualitative variables were presented as frequency (percent). The Kruskal-Wallis H test, one of the non-parametric tests, was used to determine if there was a statistical difference between the groups in terms of independent variables. The pairwise comparisons were conducted with the Conover test after the Kruskal-Wallis H test. The statistical significance level was accepted as  $p \leq 0.05$ . Three different software developed by İnönü University Faculty of Medicine Department of Biostatistics and Medical Informatics were used in statistical analyses. These were respectively: *WSSPAS: Web-Based Sample Size and Power Analysis Software* for power analysis, *RAS: Random Allocation Software* for randomization, and *Kruskal-Wallis* software for the Kruskal-Wallis H test [18,19,20].

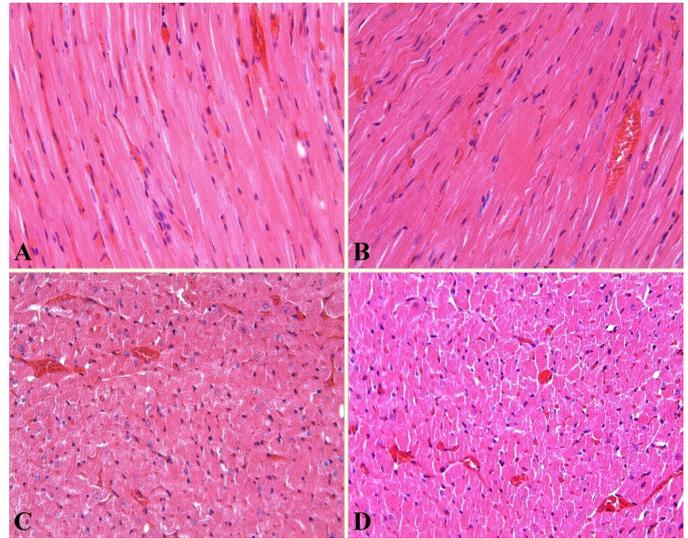
## Results

### Hemodynamic parameters and ECG results

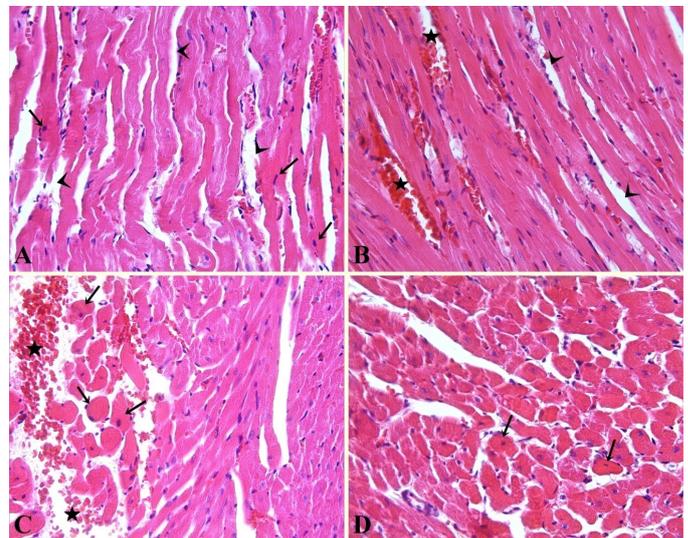
When the groups were compared, there was no significant difference in the level of HR ( $p > 0.05$ ). In the ECG evaluations, there was no significant changes were observed in terms of PR, QRS, and QT distances ( $p > 0.05$ ). Mean and diastolic BP were significantly higher in the DOX group than in the control group ( $p < 0.05$ ). Diastolic BP



**Figure 1.** Samples of ECG changes seen in the experimental groups. a: Normal ECG, b: ST depression, c: ST elevation, d: Arrhythmia (ventricular extrasystole), e: Bundle branch block, f: T negativity.



**Figure 2.** Histological appearance of myocardial tissue in the control (A and C) and GL (B and D) groups H-E x40.



**Figure 3.** Interstitial edema (arrowheads), degenerated cardiomyocytes (arrows) and congestion-hemorrhage (stars) are observed in the DOX group (A and C). In the GL+DOX group (B and D), a decrease in interstitial edema and degenerated cardiomyocyte density is remarkable. H-E x40.

was significantly increased in the DOX group compared to the GL+DOX group ( $p < 0.05$ ) (Table 1). No pathological ECG changes were observed in the control group. In the DOX group, two rats had ST depression, two rats had T negativity, and two rats had block. In the group given GL+DOX, arrhythmia was observed in one rat, T negativity in two rats, and block in one rat. In the group given only GL, one rat had T negativity and three rats had block (Table 2) (Figure 1).

### Biochemical results

Based on the results of the cardiac tissue biochemistry analysis, the MDA level showed a minor increase in the

**Table 1.** ECG and hemodynamic parameters.

Variables	Groups				p
	Control (n=8)	DOX (n=8)	GL+DOX (n=8)	GL (n=8)	
HR (beats/min)	291 (175-390)	347.5(290-410)	325.5 (172-384)	278.5 (230-434)	0.0879
SBP (mmHg)	71 <sup>ab</sup> (37-122)	88.5 <sup>a</sup> (51-112)	98.5 <sup>a</sup> (23-163)	56.5 <sup>b</sup> (41-73)	<b>0.0345</b>
DBP (mmHg)	53 <sup>a</sup> (18-73)	77.5 <sup>b</sup> (46-97)	56 <sup>a</sup> (17-78)	45 <sub>a</sub> (26-59)	<b>0.0117</b>
Mean BP (mmHg)	64 <sup>a</sup> (29-95)	83 <sup>b</sup> (49-105)	78.5 <sup>ab</sup> (21-98)	51.5 <sup>a</sup> (37-62)	<b>0.0224</b>
PR (ms)	44 (36-58)	46 (38-52)	42 (36-58)	39 (28-48)	0.2257
QRS (ms)	70 (64-78)	71 (56-88)	82 (52-106)	85 (66-106)	0.2205
QT (ms)	102 (96-118)	105 (96-118)	106 (96-176)	118 (104-132)	0.0692

There is a statistically significant difference in group categories that do not contain the same letter.

ECG: Electrocardiography; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BP: Blood pressure; DOX: Doxorubicin.

**Table 2.** ECG changes during the experimental period.

Groups	Variables			
	Arrhythmia	ST depression	T negativity	Bundle branch block
Control (n=8)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
DOX (n=8)	0 (0%)	2 (100%)	2 (40%)	2 (33.33%)
GL+DOX (n=8)	1 (100%)	0 (0%)	2 (40%)	1 (16.67%)
GL (n=8)	0 (0%)	0 (0%)	1 (20%)	3 (50%)
Total (n=32)	<b>1 (100%)</b>	<b>1 (100%)</b>	<b>5 (100%)</b>	<b>6 (100%)</b>

ECG: Electrocardiography, DOX: Doxorubicin, GL: Glycyrrhizin.

**Table 3.** MDA, GSH, CAT and SOD levels in the cardiac tissue.

Variables	Groups				p
	Control (n=8)	DOX (n=8)	GL+DOX (n=8)	GL (n=8)	
MDA nmol/gwt	90.44 (78.88-111.52)	101.32 (76.84-129.2)	84.32 (68-107.44)	93.84 (82.96-174.76)	0.1123
GSH nmol/gwt	3918.21 (3030.94-4780.02)	4091.17 (2983.02-4756.06)	3641.92 (2887.18-5223.28)	3492.17 (2893.17-4654.23)	0.1961
SOD U/g protein	256.47 (137.09-337.81)	253.52 (217.51-317.24)	248.51 (129-287.52)	220.1 (187.78-261.76)	0.0971
CAT K/g protein	265.6 (133.48-383.07)	291.92 (254.48-446.19)	349.38 (201.11-469)	292.59 (162.09-356.03)	0.1002

Malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD), Catalase (CAT), Control (C), Doxorubicin (DOX), Glycyrrhizin (GL), gram wet tissue (gwt).

**Table 4.** GSH and SOD levels in the descending aorta.

Variables	Groups				p
	Control (n=8)	DOX (n=8)	GL+DOX (n=8)	GL (n=8)	
GSH nmol/gwt	1974.45 <sup>a</sup> (1114.14-3150.74)	2114.47 <sup>a</sup> (1593.34-2995)	1419.63 <sup>b</sup> (964.39-1940.76)	1791.01 <sup>a</sup> (1335.77-2839.26)	<b>0.007</b>
SOD U/g protein	279.96 (92.41-499.15)	273.23 (108.07-376.35)	230.25 (59.02-404.38)	291.47 (164.34-411.54)	0.8337

There is a statistically significant difference in group categories that do not contain the same letter.

Glutathione (GSH), superoxide dismutase (SOD), Control (C), Doxorubicin (DOX), Glycyrrhizin (GL), gram wet tissue (gwt).

**Table 5.** Histopathologic score results on myocardial tissue.

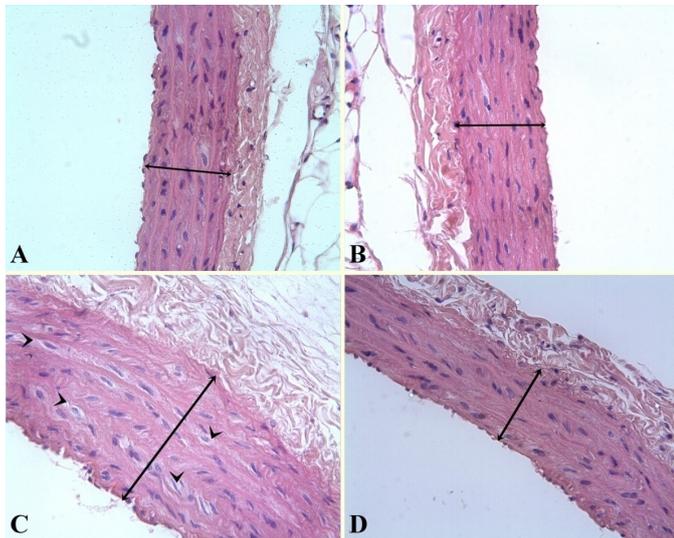
Variables	Groups				p
	Control (n=8)	DOX (n=8)	GL+DOX (n=8)	GL (n=8)	
Congestion-Hemorrhage	0.0 <sup>a</sup> (0.0-2.0)	1.0 <sup>b</sup> (0.0-3.0)	1.0 <sup>b</sup> (0.0-3.0)	0.0 <sup>a</sup> (0.0-3.0)	<b>&lt;0.001</b>
Interstitial edema	0.0 <sup>a</sup> (0.0-1.0)	0.0 <sup>b</sup> (0.0-3.0)	0.0 <sup>b</sup> (0.0-3.0)	0.0 <sup>b</sup> (0.0-3.0)	<b>&lt;0.001</b>
Degenerated cardiomyocyte	0.0 <sup>a</sup> (0.0-2.0)	1.0 <sup>b</sup> (0.0-3.0)	1.0 <sup>c</sup> (0.0-3.0)	0.0 <sup>c</sup> (0.0-3.0)	<b>&lt;0.001</b>

The variable are expressed as median (min-max). There is a statistically significant difference in group categories that do not contain the same letter.

**Table 6.** Histopathologic score results in the descending aorta.

Variables	Groups				p
	Control (n=8)	DOX (n=8)	GL+DOX (n=8)	GL (n=8)	
Degenerative changes	0.0 <sup>a</sup> (0.0-2.0)	1.0 <sup>b</sup> (0.0-3.0)	0.0 <sup>c</sup> (0.0-3.0)	0.0 <sup>c</sup> (0.0-2.0)	<0.001
Tunica intima-media thickness	91.6 <sup>a</sup> (63.73 – 176.53)	105.31 <sup>b</sup> (56.33 – 164.30)	94.53 <sup>a</sup> (59.63 – 172.21)	97.30 <sup>a</sup> (68.52 – 158.10)	<0.001

The variables are expressed as median (min-max). There is a statistically significant difference in group categories that do not contain the same letter.



**Figure 4.** Normal histological appearance of the vessel wall is observed in the control (A) and GL (B) groups. In the DOX group (C), a significant increase in tunica intima-media thickness and loss of myofibrils in muscle cells (arrowheads) are remarkable. It is observed that the vessel wall in the GL+DOX group is similar to the control group. Double-headed arrows indicate tunica intima-media thickness. H-Ex40.

DOX group compared to the other groups, but it was not statistically significant ( $p>0.05$ ). Furthermore, there was no variation between the groups in regards to GSH and SOD levels ( $p>0.05$ ). Although the CAT level increased slightly in the GL+DOX group, there was no statistically significant difference discovered ( $p>0.05$ ) (Table 3).

In the descending aorta, the GSH level was significantly decreased in the GL+DOX group compared to all other groups ( $p<0.05$ ). SOD levels were similar in all vessel groups ( $p>0.05$ ) (Table 4).

### Histopathological results

#### Heart

Histopathological analysis showed no significant differences in myocardial tissue between the control and GL groups, as seen in Figures 2A, B, C, and D. However, the DOX group exhibited a significant increase in degenerated cardiomyocyte density, interstitial edema, and congestion-hemorrhage severity compared to the control group ( $p<0.05$ ) (Figures 3A and C). In contrast, a significant reduction in degenerated cardiomyocyte density

was observed in the GL+DOX group compared to the DOX group ( $p<0.05$ ). Furthermore, interstitial edema decreased in the GL+DOX group, although the difference was not statistically significant when compared with the DOX group (Figures 3B and D) ( $p>0.05$ ). The results of the histopathological score for myocardial tissue are provided in Table 5.

#### Descending aorta

In the sections of the control and GL groups, the vessel wall was observed in normal histopathological appearance (Figures 4A and B). In the DOX group, thinning of the elastic lamellae and loss of myofibrils in the muscle cells were observed (Figure 4C). On the other hand, the histopathological changes observed in the DOX group were found to be statistically significantly decreased in the GL+DOX group (Figure 4D) ( $p<0.05$ ). The tunica intima-media thickness of the groups was  $96.10\pm 17.25\ \mu\text{m}$  in the control group,  $99.08\pm 16.86\ \mu\text{m}$  in the GL group,  $106.22\pm 19.87\ \mu\text{m}$  in the DOX group, and  $97.08\pm 19.06\ \mu\text{m}$  in the GL+DOX group. Statistically significant increases in tunica intima-media thickness were observed in the DOX group when compared to the control group ( $p<0.05$ ). The GL+DOX group displayed a statistically significant reduction in tunica intima-media thickness when compared to the DOX group ( $p<0.05$ ). Histopathological score results of the vessel wall and tunica intima-media thicknesses are given in Table 6.

### Discussion

In the present study, DOX treatment induced a rise in mean and diastolic BP. Conversely, the inclusion of GL in DOX therapy resulted in a decrease in diastolic BP. Pathological ECG alterations were detected in the groups receiving DOX. Histopathologically, distinct evidence of cardiac damage was observed in the DOX group. Furthermore, the addition of GL to DOX therapy considerably enhanced cardiomyocyte degeneration.

Cardiomyocytes are more susceptible to cardiotoxicity than other tissues due to mitochondrial damage [21]. DOX treatment can cause an increase in inflammation and free radicals, which can then lead to DNA damage and cell destruction. The consequences of this procedure include cardiac cell death and cardiomyopathy. Additionally, oxidative stress and the development of reactive oxygen species (ROS) are significant factors in DIC. DOX may lead to an increase in ROS and a decrease in the levels of antioxidants such as SOD, GSH, and CAT. Cardiomyocyte death, apoptosis, oxidative stress, and lipid peroxidation can lead

to cellular damage, heart failure, and ultimately, apoptosis [21].

GL is found in high amounts in the roots of the 'Licorice' plant from the glycyrrhiza species. It is a compound that is widely used as a sweetener. The studies revealed the anti-inflammatory, immune regulatory, antioxidant, antitumor, antiviral, and hepatoprotective properties of GL [22].

BP is one of the GL's cardiac effects that have been extensively researched. BP has been linked to excessive GL consumption, notably through licorice-containing products. This is because the substance can throw off the balance between sodium and potassium levels, causing fluid retention and potentially elevating BP. Again, GL's metabolites can cause changes in cortisol metabolism [23]. However, the effect of GL on BP is controversial. In preeclampsia rats, GL significantly decreased systolic and diastolic BP, and 24-hour proteinuria [24]. In this study, it was observed that the increase in BP seen in the DOX group was significantly reduced in the GL+DOX group. Yang et al. reported that GL attenuated the development of pulmonary hypertension and pulmonary vascular remodeling in rats. Therefore, GL may have a protective effect in both systemic and pulmonary hypertension. However, further research is necessary [25].

The concentration of antioxidants, namely GSH peroxidase, CAT and SOD, is lower in myocardial tissue than in other tissues. Consequently, the myocardium is vulnerable to oxidative damage [26]. Despite the description of oxidative stress in DIC, its underlying mechanisms are complex. The redox reaction in myocardial cells is multifaceted and variable. Additionally, not all cardiomyocytes are necrotic in DIC, and it is essential to explore the survival mechanisms of cardiomyocytes [27].

When examining the biochemical analysis in this study, no significant differences were found between the groups. MDA, an end product of peroxidation of polyunsaturated fatty acids, is frequently used to assess lipid peroxidation and oxidative stress levels [28]. The reaction of antioxidants with free radicals neutralizes them. There are two endogenous antioxidant defense mechanisms, enzymatic and non-enzymatic. Endogenous enzymes, including GSH reductase, CAT, GSH peroxidase, and SOD, make up the enzymatic antioxidant defence system. Meanwhile, GSH represents the non-enzymatic defensive system [29,30]. Results of this study demonstrate that while MDA levels exhibit no change in both cardiac and vascular tissues, the group GL+DOX had significantly lower GSH levels than the other groups. There were no significant differences observed for SOD and CAT activities between the vascular and cardiac tissues, suggesting that GL may not impact the antioxidant status of these tissues from a biochemical analysis perspective.

One possible mechanism of cardiotoxicity caused by DOX is inflammation. This study provides histopathological evidence that cardiotoxicity develops in the DOX group, with significantly increased degenerative cardiomyocyte density, interstitial edema, and congestion-hemorrhage severity [31]. Chronic inflammation is known to be involved in different cardiovascular diseases [32]. GL may have a positive impact on cardiac tissue by effectively reducing inflammation. In the group that was pre-treated with GL, the

density of degenerate cardiomyocytes decreased in comparison to the DOX group. Additionally, GL treatment prior to DOX application led to a decrease in interstitial edema. In line with the myocardial findings, the DOX group exhibited a detrimental effect on the vascular structure. Our observations indicated reduced thickness of elastic lamellae and loss of myofibril in muscle cells in the DOX group. However, in the GL+DOX group, we observed an improvement in histopathological changes compared to the DOX group.

A recent study demonstrated that GL suppresses the Akt/mTOR autophagy signaling pathway in H9c2 cells treated with DOX. This study also suggests that increased autophagy can help reduce DIC, which sheds new light on the mechanisms underlying GL's cardioprotective effects. Additionally, the study proposed that GL could serve as an effective preventive therapy for DIC [33]. Glycyrrhiza glabra, which contains GL, has demonstrated protective effects on the heart against the detrimental impact of DOX therapy. This positive outcome has been attributed to its ability to decrease oxidative stress, regulate lipid homeostasis and cardiac metabolism [34]. In addition, another study has indicated that *Glycyrrhiza uralensis* can alleviate the increase of DOX-induced apoptosis [35]. After noting the occurrence of an apoptotic response in cancer cells, GL emerged as a favourable therapeutic agent for the treatment of cancer [36]. Additional research is needed to confirm and refine our experimental results before clinical administration.

Limited funding in the present study necessitates certain considerations. To demonstrate cardiotoxicity, it is advisable to assess troponin and natriuretic peptide levels with rat-specific kits. Additionally, evaluating ventricular functions can be achieved through the implementation of transthoracic echocardiography on rats.

## Conclusion

Pre-treatment of GL can be administered to prevent DOX's hypertensive side effects, especially in the regulation of blood pressure. The use of GL as a protective agent may prevent DIC histopathologically. However, the effectiveness of this approach is still under investigation. Additional research is necessary to determine the mechanisms and therapeutic consequences of GL's effect on DIC.

## Conflict of interest

There is no conflict of interest.

## Financial disclosure

The Scientific and Technological Research Council of Türkiye (TÜBİTAK) supported this project. (Application number: 1919B011601307).

## Ethical approval

The approval of the ethics committee was obtained from the ethics committee of animal experiments of Inonu University (Protocol number: 2016/A-43).

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