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Exploring Esculetin's Protective Role: Countering Doxorubicin-Induced Oxidative Stress in Rat Heart

Eskuletin'in Koruyucu Rolünü Keşfetmek: Sıçan Kalbinde Doksorubisinin Neden Olduğu Oksidatif Stresle Mücadele

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ABSTRACT

Doxorubicin (DOX) and other anthracyclines are potent chemotherapy drugs used against cancer; however, their clinical application is linked to significant and potentially life-threatening cardiotoxicity. Despite extensive research over many years, the available treatment choices are still constrained. DOX is typically believed to primarily affect mitochondria, and the characteristic feature of DOX-induced cardiotoxicity is mitochondrial dysfunction. Esculetin is a coumarin derivative found in nature. It has anti-inflammatory, antioxidant, anti-diabetes and antibacterial properties. This study was designed to investigate the protective effect of esculetin against DOX-induced cardiotoxicity in Sprague-Dawley rats considering the mentioned properties. Cardiotoxicity was induced by administering DOX via intraperitoneal injection at every other day dosage of 5 mg/kg body weight for two consecutive weeks. Rats receiving DOX injections were simultaneously supplemented with esculetin at doses of 50 and 100 mg/kg body weight through intraperitoneal administration over the same period. The investigation, oxidative stress enzymes in heart tissue of rats employed biochemical and molecular methods. The heart tissues were evaluated for the enzyme activity and and expression levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). The results indicate a substantial decrease in mRNA expression of GPx and CAT in the DOX group compared to the control, but SOD did not show any significant change. The DOX group exhibited a substantial drop in particular specific enzyme activity, specifically SOD, compared to the control group. However, the activity of CAT and GPx enzymes remained unaltered. The current investigation posits that the deleterious impacts of DOX on cardiac tissue may be alleviated by esculetin through the regulation of oxidative stress.

Keywords: Cardiotoxicity, doxorubicin, esculetin, oxidative stress.

ÖZ

Doksorubisin (DOX) ve diğer antrasiklinler kansere karşı kullanılan güçlü kemoterapi ilaçlarıdır; ancak klinik uygulamaları önemli ve potansiyel olarak yaşamı tehdit eden kardiyotoksisite ile bağlantılıdır. Uzun yıllar süren kapsamlı araştırmalara rağmen, mevcut tedavi seçenekleri hala kısıtlıdır. DOX'un tipik olarak öncelikle mitokondriyi etkilediğine inanılır ve DOX kaynaklı kardiyotoksisitenin karakteristik özelliği mitokondriyal disfonksiyondur. Eskuletin, doğada bulunan bir kumarin türevidir. Anti-inflamatuar, antioksidan, anti-diyabet ve antibakteriyal özellikleri bulunmaktadır. Bu çalışma, eskuletinin bahsedilen özellikleri göz önünde bulundurularak Sprague-Dawley sıçanlarında DOX kaynaklı kardiyotoksisiteye karşı koruyucu etkisini araştırmak üzere tasarlandı. Bu çalışma, bilinen özellikleri göz önünde bulundurularak Sprague-Dawley sıçanlarında DOX kaynaklı kardiyotoksisiteye karşı eskuletin'in koruyucu etkilerini araştırmak üzere tasarlandı. Kardiyotoksisite, DOX'un intraperitonal enjeksiyon yoluyla iki günde bir 5 mg/kg vücut ağırlığı dozunda iki ardışık hafta boyunca uygulanmasıyla indüklendi. DOX enjeksiyonu yapılan sıçanlara aynı süre boyunca 50 ve 100 mg/kg vücut ağırlığı dozlarında intraperitonal uygulama yoluyla eş zamanlı olarak eskuletin takviyesi yapıldı. Sıçan kalp dokusundaki oksidatif stres enzimlerinin araştırılmasında biyokimyasal ve moleküler yöntemler kullanıldı. Kalp dokularında katalaz (CAT), glutatyon peroksidaz (GPx) ve süperoksit dismutaz (SOD) enzim aktiviteleri ve ekspresyon seviyeleri değerlendirildi. Sonuçlar, DOX grubunda GPx ve CAT'in mRNA ekspresyonunda kontrole kıyasla önemli bir düşüş olduğunu, ancak SOD'da önemli bir değişiklik göstermediğini ortaya koymaktadır. DOX grubu, kontrol grubuna kıyasla özellikle SOD spesifik enzim aktivitesinde önemli bir düşüş sergilemiştir. Ancak CAT ve GPx enzimlerinin aktivitesi değişmemiştir. Mevcut araştırma, DOX'un kalp dokusu üzerindeki zararlı etkilerinin, oksidatif stresin düzenlenmesi yoluyla eskuletin tarafından hafifletilebileceğini ortaya koymaktadır.

Anahtar Kelimeler: Doksorubisin, eskuletin, kardiyotoksisite, oksidatif stress.

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Introduction

Doxorubicin (DOX), belonging to the anthracycline family, serves as a powerful chemotherapeutic agent employed in the treatment of diverse solid tumors (including gastrointestinal, brain, breast and ovary) and hematologic malignancies (such as leukemia and lymphoma) (Minotti et al., 2004; Sohail et al., 2021). Despite its effectiveness, up to one-fourth of patients encounter DOX-induced cardiotoxicity, leading to limitations in its clinical application (Rawat et al., 2021). DOX-induced cardiotoxicity manifests clinically in different forms, including chronic phases, early, and acute. Acute cardiotoxicity manifests within minutes to a week after a single dose or course of therapy, characterized by electrocardiographic abnormalities, transient left ventricular dysfunction, and supraventricular arrhythmia. While acute toxicity typically resolves upon discontinuation of medication, it may be linked to cardiomyocyte injury, potentially advancing into chronic or early cardiotoxicity (Qiu et al., 2023).

While there has been extensive research on the mechanisms underlying doxorubicin-induced cardiotoxicity (DIC), the molecular pathogenesis of DIC remains not fully understood. Cardiomyocyte cell death is primarily attributed to three major sources of cell damage: (1) topoisomerase DNA II beta (TOP2β) poisoning causing double-strand breaks; (2) damage to mitochondria and (3) the overproduction of reactive oxygen species (ROS) leads to damage in proteins, lipids, and DNA. DOX induces a significant reduction in endogenous antioxidant levels, including glutathione and catalase, resulting in redox imbalances and increased oxidative stress (Kong et al., 2022). The myocardium's oxidative damage is probably associated with lower levels of antioxidant enzymes and in comparison to other tissues (Marques et al., 2015). Superoxide dismutase (SOD), serving as a natural scavenger for superoxide radicals in tissues, converts detrimental superoxide radicals into H^2O^2. Catalyzing the oxidation of substrates, peroxidases are located in the carrier's peroxisomes, with iron porphyrin serving as the prosthetic group using H^2O^2 as the electron acceptor, eliminating its toxicity and oxidizing various compounds (Palma et al., 2020). Playing a central role in the clearance of H2O2, glutathione peroxidase 1 (GPx1), a principal isoform of GPx, is present in both the cytosol and mitochondria. GPx1-deficient mice exhibited increased susceptibility to DOX-induced damage compared to wild type mice (Lei et al., 2023). Similarly, catalase (CAT) plays a crucial role in the biological defense system, facilitating the breakdown of H^2O^2 into water and molecular oxygen. This process helps eliminate H^2O^2 from the body, safeguarding cells against H2O2 poisoning (Nandi et al., 2019). Upon treatment with DOX, myocardial cells exhibit a diminished capacity for antioxidant enzymes, leading to the accumulation of superoxide anions and H^2O^2. This accumulation triggers oxidative stress, resulting in damage to cardiac myocytes (Shi et al., 2023).

Esculetin is a 6,7-dihydroxy coumarin derivative that occurs naturally in various medicinal plants, including *Fraxinus rhynchophylla, Artemisia capillaris, Euphorbia lathyrism, Aesculus hippocastanum and Citrus limonia*. It has anticancer, antidiabetes, antioxidant, antiapoptotic, antiapoptotic, neuroprotective, anti-inflammatory, antibacterial and cardiovascular protective effects and has the potential as a therapeutic drug in non-communicable diseases such as cancer, diabetes, obesity and neurological disorders. Additionally, it has been identified as an inhibitor of ROS production (Kadakol et al., 2016; Zhang et al., 2022).

Due to its bioavailable properties, there is an increasing demand for in vivo analyses and pharmacokinetic studies of esculetin. Therefore, in this study, we investigated the therapeutic potential of esculetin against DOX-induced side effects in rat heart via antioxidant systems.

Materials and Methods

Materials

The study utilized a sample of 48 male Sprague-Dawley rats weighing 180 ± 20 g, which were acquired from the Medical Experimental Application and Research Center at Atatürk University. The animals were maintained in controlled environments, following a 12-hour light/12-hour darkness photoperiod. The temperature was maintained at 22 ± 1 °C, while the relative humidity was maintained at 60%. Ad-libitum provision of water and nutrition was ensured. After a one-week period of acclimatization, the experimental animals were randomized to six groups in a random manner and were subjected to the following treatments:

Control group: Rats received intraperitoneal injections of normal saline for 14 days.

DOX group: Rats were intraperitoneally injected with DOX (5 mg/kg) every other day for 14 days.

E50 group: Rats received intraperitoneal injections of esculetin (50 mg/kg) for 14 days.

E100 group: Rats were injected intraperitoneally with esculetin (100 mg/kg) for 14 days.

DOX+E50 group: Rats were intraperitoneally injected with DOX, and esculetin was administered 1 hour before the DOX treatment, following the regimen of the DOX and E50 groups.

DOX+E100 group: Rats were injected intraperitoneally with DOX, and esculetin was administered 1 hour before the DOX treatment, following the regimen of the DOX and E100 groups.

After a 14-day period, all rat groups were euthanized under 1/5 xylazine and ketamine anesthesia at a dosage of 1 mg/kg. The heart tissues were expeditiously obtained, rapidly frozen in liquid nitrogen, and thereafter preserved at a temperature of -80°C until subsequent study. The experimental protocols followed the guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals and received approval from the Atatürk University Local Ethics Council for Animal Experiments and assigned Protocol Number: 2021/4–123.

Methods

RNA isolation and gene expression analysis

Heart tissue total RNA extraction utilized the EcoPURE total RNA kit (EcoTech) following the provided instructions. The Multiskan GO Microplate Spectrophotometer (Thermo Scientific) was employed to assess RNA purity and concentration. Subsequently, cDNA synthesis from total RNA was accomplished using the iScript cDNA synthesis kit (Bio-Rad) in accordance with the manufacturer's protocol. For gene expression analysis, the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) was employed, and Rotor-Gene Q (Qiagen) was used for the determination of gene expression levels. GAPDH served as a housekeeping gene for data normalization. The primer sequences utilized were detailed in a prior study (Yeşilkent and Ceylan, 2022). The $2^{-\Delta\Delta Ct}$ formula was applied to calculate the relative mRNA expression of the target genes (Karagac and Ceylan, 2023).

Tissue Homogenate Preparation

Heart tissues underwent homogenization in 50 mM phosphate buffer (pH:7.4), supplemented with 1 mM EDTA and 1 mM DTT, utilizing the TissueLyser LT device (Qiagen). Following homogenization, centrifugation at 10000 rpm and 4 °C for 30 minutes was carried out (Ceylan et al., 2019). The resultant supernatant was utilized for biochemical analyses. Protein content in the supernatants was determined using the Bradford method (1976).

Analysis of Antioxidant Enzyme Activities

SOD activity was assessed following the procedure recommended by Sun et al. (1988), with one unit of SOD defined as the quantity inhibiting 50% of nitroblue tetrazolium chloride reduction. CAT activity was determined spectrophotometrically according to the method outlined by Aebi (1984), and one unit of CAT was expressed as the enzyme amount required for hydrogen peroxide decomposition per minute. GPx activity was measured in accordance with the approach reported by Wendel et al. (1981), with one unit of GPx defined as the enzyme amount needed for NADPH oxidation per minute.

Statistical Analysis

The determination of the number of groups and the allocation of rats within each group were established through a thorough G-power analysis. G-power Software 3.1.9.7 (University of Dusseldorf, Germany) was employed for conducting the a priori power analysis, aiming to determine the minimum sample size required for the study. The analysis utilized F tests aligned with the study's design, which involved repeated measures ANOVA with both within and between factors analysis. The study comprised six groups with measurements taken at a single time point. The significance level (α) was set at 0.05, the minimum effect size was specified as 0.25, the correlation among repeated measures was set at 0.5, and the nonsphericity correction was fixed at 1. To achieve a power (1-β error probability) of 0.80, the calculated minimum sample size necessary for statistical significance was determined to be 36 subjects in total, resulting in an actual power of 81.0%.

The normality of numerical variables was assessed utilizing the Shapiro-Wilk test. Results indicated that all variables exhibited a normal distribution ($p > 0.05$). According to the results of this test, it has been determined that the mRNA parameters exhibit a normal distribution ($p>0.05$).

Each animal and sample were subjected to triple measurements. The statistical analysis involved doing one-way ANOVA and subsequently performing Tukey's post-hoc test using Prism software (GraphPad Software, San Diego, CA) to compare the findings. Significant differences are denoted by the following criteria: $p > 0.05$ (indicating lack of significance), *:$p < 0.05$ (indicating significance), **:$p < 0.01$, ***:$p < 0.001$ and ****:$p < 0.0001$

Results

When SOD specific enzyme activity results were analyzed, a significant decrease was observed in the E100 and DOX groups compared to the control group, while a significant increase was observed in the E50 group. E100 and DOX+E100 groups were analyzed among themselves, a significant increase was observed (Figure 1). In mRNA expression results, although there was a decrease in DOX group compared to the control, it was not statistically significant. There was a significant increase in the E100 and DOX+E50 groups compared to control (Table 1,2 and Figure 1).

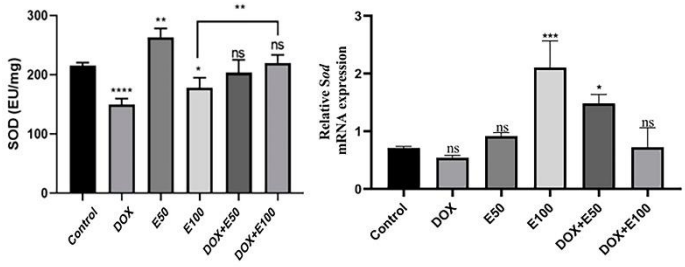

Figure 1. Analyses of the relative gene expression and specific activity for SOD from the heart tissues of rats treated with DOX and E. DOX = Doxorubicin (intraperitoneally 5 mg/kg); E = Esculetin (intraperitoneally 50 mg/kg and 100 mg/kg); Each bar represents the mean ± SEM. Asterisk (*) indicates statistically significant difference between the means (ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****:p < 0.0001)

Şekil 1. DOX ve E ile tedavi edilen ratların kalp dokularından SOD için göreceli gen ekspresyonu ve spesifik aktivite analizleri. DOX = Doksorubisin (intraperitoneal 5 mg/kg); E = Esculetin (intraperitoneal 50 mg/kg ve 100 mg/kg); Her çubuk ortalama ± SEM'i temsil etmektedir. Yıldız işareti (*) ortalamalar arasındaki istatistiksel olarak anlamlı farkı gösterir (ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 ve ****:p < 0.0001)

Table 1. One-way ANOVA test results of *Sod* qPCR data

Tablo 1. *Sod* qPCR verilerinin tek yönlü ANOVA testi sonuçları

Groups	N	Mean	Std. Error	F	p
Control[a]	8	,78	,009		
DOX[b]	8	,34	,007		
E50[c]	8	1,00	,009	310,957	,000*
E100[d]	8	1,20	,008		
DOX+E50[e]	8	,48	,040		
DOX+E100[f]	8	,79	,008		

*significant statistical differences were identified when comparing the groups based on DOX-E Cat (p≤ .000).

Table 2. Results of Tukey HSD test of *Sod* qPCR data

Tablo 2. *Sod* qPCR verilerinin Tukey HSD testi sonuçları

Tukey's multiple comparisons test	Significant
a vs. b	ns
a vs. c	ns
a vs. d	***
a vs. e	*
a vs. f	ns
b vs. c	ns
b vs. d	****
b vs. e	**
b vs. f	ns
c vs. d	***
c vs. e	ns
c vs. f	ns
d vs. e	ns
d vs. f	***
e vs. f	*

a: Control, b: DOX, c: E50, d:E100, e: DOX+E50 f: DOX+E100, ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****:p < 0.0001

When CAT specific enzyme activity results were analyzed, E50 group showed a significant increase while E100 group showed a significant decrease compared to the control (Figure 2). In mRNA expression results, there was a significant decrease in the DOX group compared to the control. This decrease was regulated in the DOX+E100 combination group. *Cat* gene expression increased in the E100 group (Table 3, 4, Figure 2).

When GPx1 specific enzyme activity results was analyzed, there was a significant decrease in in the E100 group compared to the control group. (Figure 3). There was no statistically significant change in DOX, E50, DOX+E50 and DOX+E100 groups compared to the control. E100 showed a significant decrease compared to E50. In mRNA expression results, the E50 and E100 treated group showed a statistically significant increase in gene expression compared to the control group. Nonetheless, a noteworthy reduction was noted in the group treated with DOX alone. In comparison to the control group, a marked decrease was evident in the group receiving a combination of E50 with DOX, whereas no alteration was observed in the group treated with E100 (Table 5, 6 and Figure 3).

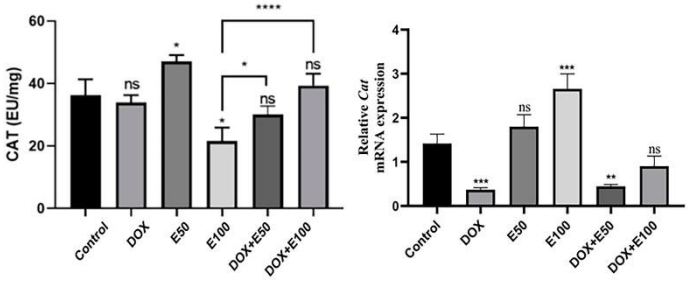

Figure 2. Analyses of the relative gene expression and specific activity for CAT from the heart tissues of rats treated with DOX and E. Each bar represents the mean ± SEM. Asterisk (*) indicates statistically significant difference between the means (ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****:p < 0.0001)

Şekil 2. DOX ve E ile muamele edilen ratların kalp dokularından CAT için göreceli gen ekspresyonu ve spesifik aktivite analizleri. Her çubuk ortalama ± SEM'i temsil etmektedir. Yıldız işareti (*) ortalamalar arasında istatistiksel olarak anlamlı farkı gösterir (ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 ve ****:p < 0.0001)

Table 3. One-way ANOVA test results of *Cat* qPCR data

Tablo 3. *Cat* qPCR verilerinin tek yönlü ANOVA testi sonuçları

Groups	N	Mean	Std. Error	F	p
Control[a]	8	1,42	,04		
DOX[b]	8	,37	,01		
E50[c]	8	1,76	,06		
E100[d]	8	2,66	,06	362,223	,000*
DOX+E50[e]	8	,44	,01		
DOX+E100[f]	8	,88	,04		

*significant statistical differences were identified when comparing the groups based on DOX-E Cat (p≤ .000)

Table 4. Results of Tukey HSD test of *Cat* qPCR data

Tablo 4. *Cat* qPCR verilerinin Tukey HSD testi sonuçları

Tukey's multiple comparisons test	Significant
a vs. b	***
a vs. c	ns
a vs. d	***
a vs. e	**
a vs. f	ns
b vs. c	****
b vs. d	****
b vs. e	ns
b vs. f	ns
c vs. d	**
c vs. e	****
c vs. f	**
d vs. e	****
d vs. f	****
e vs. f	ns

a: Control, b: DOX, c: E50, d:E100, e: DOX+E50 f: DOX+E100, ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****:p < 0.0001

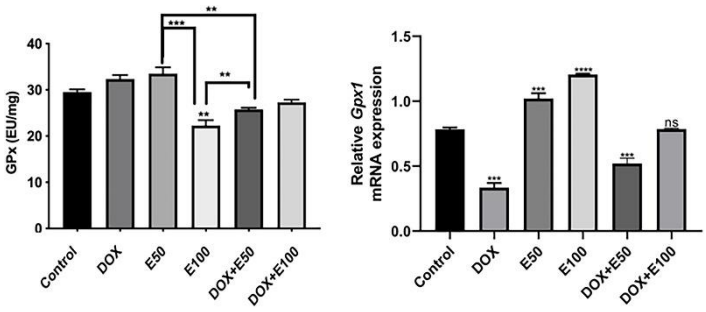

Figure 3. Analyses of the relative gene expression and specific activity for GPx from the heart tissues of rat treated with DOX and E. Each bar represents the mean ± SEM. Asterisk (*) indicates statistically significant difference between the means (ns: p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****p:< 0.0001)

Şekil 3. DOX ve E ile muamele edilen ratların kalp dokularından GPx için göreceli gen ekspresyonu ve spesifik aktivite analizleri. Her çubuk ortalama ± SEM'i temsil etmektedir. Yıldız işareti (*) ortalamalar arasında istatistiksel olarak anlamlı farkı gösterir (ns: p >0.05, * p:< 0.05, **:p < 0.01, ***:p < 0.001 ve ****:p < 0.0001)

Table 5. One-way ANOVA test results of *Gpx1* qPCR data

Tablo 5. *Gpx1* qPCR verilerinin tek yönlü ANOVA testi sonuçları

Groups	N	Mean	Std. Error	F	p
Control[a]	8	,70	,00		
DOX[b]	8	,54	,01		
E50[c]	8	,90	,01		
E100[d]	8	2,17	,09	147,831	,000*
DOX+E50[e]	8	1,46	,03		
DOX+E100[f]	8	,74	,06		

*significant statistical differences were identified when comparing the groups based on DOX-E Gpx1 (p≤ .000).

Table 6. Results of Tukey HSD test of *Gpx1* qPCR data

Tablo 6. *Gpx1* qPCR verilerinin Tukey HSD testi sonuçları

Tukey's multiple comparisons test	Significant
a vs. b	****
a vs. c	***
a vs. d	****
a vs. e	***
a vs. f	ns
b vs. c	****
b vs. d	****
b vs. e	**
b vs. f	****
c vs. d	**
c vs. e	****
c vs. f	***
d vs. e	****
d vs. f	****
e vs. f	***

a: Control, b: DOX, c: E50, d:E100, e: DOX+E50 f: DOX+E100, ns:p >0.05, *:p < 0.05, **:p < 0.01, ***:p < 0.001 and ****: p < 0.0001

Discussion

The investigation into the mechanisms of DIC is ongoing, and there is an urgent need for adjuvant therapies that can prevent or alleviate DIC (Jiang et al., 2021). The main mechanisms believed to contribute to DOX-induced cardiotoxicity include a compromised antioxidant defense system and increased production of ROS. Mitochondria, identified as the primary sites of ROS generation, represent crucial targets of DOX (Yang et al., 2020). The myocardium, in contrast to other tissues, exhibits higher susceptibility to oxidative stress, potentially attributed to lower levels of antioxidant enzymes (De Geest and Mishra, 2021). Hence, investigating approaches to natural antioxidant system and safeguard mitochondria is an attractive way to mitigate DIC.

Natural products emerge as promising candidates for novel drug development, and in this study, esculetin was recognized as a protective agent against cardiotoxicity, partially by activating the fundamental antioxidant signaling pathway. Esculetin (6,7-dihydroxycoumarin) displays diverse pharmacological effects, encompassing anti-oxidative properties, the inhibition of anti-atherogenic activity and human leukemia cell growth (Yun et al., 2011). Additionally, it has shown efficacy in restraining tumor growth in cell cultures and a mouse tumor xenograft model in living organisms (Lee et al., 2013).

In this study, we investigated the impact of esculetin on DOX-induced cardiotoxicity and explored the underlying mechanisms of the synergistic cardiotoxic effects associated with combined esculetin and DOX treatment. Mitochondria plays a central role as the primary target organelle in DOX-induced toxicity within cardiomyocytes (Shi et al., 2018). Notably, DOX exhibits a higher concentration within mitochondria compared to its simultaneous serum concentration, based on clinical data (Tadokoro et al., 2023). DOX utilizes enzymatic-related mechanisms in the mitochondrial respiratory chain, leading to the accumulation of ROS, which is a primary factor in DOX-induced cardiotoxicity (Jiang et al., 2022). Childs et al. (2002) found that when DOX triggers the generation of ROS in mitochondria, it causes the mitochondrial membrane potential to decrease and the release of cytochrome C.

Heart cells, known for their diminished antioxidant enzyme activity, are recognized as a principal focus of oxidative stress induced by DOX (Shi et al. 2023). In our study, exposing the heart to DOX resulted in heightened ROS production and inhibited the activities of CAT, SOD, and GPx, all of which play crucial roles in antioxidant defense. SOD and CAT levels in rat heart tissue experienced a remarkable decrease upon DOX treatment, but the application of esculetin significantly raised these enzyme levels. To conclude, esculetin played a crucial role in reducing DOX-induced oxidative stress in cardiac tissue.

Considering their mechanisms, antioxidant supplements could play a crucial role in cardio-oncology signaling. Flavonoids emerge as a potentially effective category of herbal medications to mitigate DOX cardiotoxicity, given their iron-chelating properties, antioxidant effects, carbonyl reductase-inhibitory attributes and inhibition of lipid peroxidation (Abdelghffar et al., 2021).

Studies in the literature have explored the impact of phenolic compounds on both the induction and cardiotoxicity associated with DOX. Quercetin, a flavonoid glycoside, has been studied for its preventive impacts on heart-related disorders and cardiac damage. Several studies have documented its protective role against DOX-induced cardiomyopathy, attributing the expression and the subsequent improvement in antioxidant defense, including SOD and CAT (Abdelghffar et al., 2021). Another flavonoid glycoside, apigenin, has been reported to reduce blood levels of lactate dehydrogenase and creatine kinase myocardial band in a myocardial ischemia rat model. Its protective effects are associated with the inhibition of NF-κB activation, leading to the suppression of pro-inflammatory cytokines (Ojha et al., 2016). Furthermore, the delivery of apigenin to adult rat cardiomyocytes resulted in enhanced cell survival when exposed to DOX. This effect was attributed to many mechanisms, including the mitigation of lipid peroxidation, quenching of ROS, and avoidance of myocyte necrosis (Psotová et al., 2004). Berberine pretreatment exhibited significant enhancements in GPx, and CAT, SOD activities, while concurrently reducing MDA levels. Furthermore, it ameliorated both electrocardiogram patterns and histopathological alterations in the myocardium of DOX-treated rats (Wu et al., 2019). The research conducted by Dai and colleagues (2018) delves into the potential of octreotide (OCT) in counteracting DOX-induced cardiac toxicity in rats. The cardiac homogenate of the OCT group revealed elevated activities of CAT, SOD, and GSH compared to the DOX group. Notably, a substantial reduction in MDA activity and ROS levels was observed in the OCT group in comparison with the DOX group ($p < 0.05$).

Conclusion

In summary, herbal products present valuable opportunities for drug discovery in preventing and treating DIC. Our study, for the first time, showcased the cardioprotective effects of esculetin against DIC. Esculetin demonstrated the ability to protect mitochondria and alleviate oxidative stress through the antioxidant signaling pathway. Esculetin shows potential in reducing the harmful effects of DOX on the heart and might be used as an additional medication in cancer treatment.

Etik Komite Onayı: Etik kurul onayı Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan (2021/4–123) alınmıştır.
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