

Effects of CAPE on biochemical, histopathological and cardiac parameters in doxorubicin induced cardiotoxicity

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ABSTRACT

OBJECTIVE: In this study, the protective effect of Caffeic acid phenethyl ester (CAPE) against doxorubicin (DOX)-induced cardiotoxicity was investigated by evaluating oxidative stress parameters, ECG changes, *matrix metalloproteinase 2 (MMP-2)* gene expression, troponin I level and histopathology in Wistar Albino rats.

METHODS: Forty rats were divided into 4 groups (n=10) including control (saline (vehicle for DOX) and 2.5% ethanol (vehicle for caffeic acid phenethyl ester), CAPE only (10 µmol/kg bw), DOX only (10 mg/kg bw) and CAPE+DOX groups. Molecular, biochemical and histopathological analyses were performed on blood and heart tissues.

RESULTS: No alterations were observed in oxidative stress parameters and *MMP-2* gene expression of DOX and CAPE+DOX groups compared to control. Troponin I levels were higher in DOX and CAPE+DOX groups than in the control. Variable ECG changes were observed in the experimental groups such as increased systolic blood pressure, decreased QRS and QT interval in DOX group compared to the control without any ameliorative effect of CAPE. The presence of dense degenerative cardiomyocytes in the myocardium of the DOX group was noted. DOX caused damage to cardiomyocytes. It was observed that CAPE showed a significant decrease in histopathological changes and histopathological scoring in the CAPE+DOX group compared to DOX group.

CONCLUSION: CAPE treatment ameliorated histopathological changes induced by DOX while other parameters including oxidative stress, *MMP-2* gene expression, Troponin I and ECG studied in our study were not altered remarkably.

Keywords: CAPE; cardiotoxicity; doxorubicin; rat.

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Doxorubicin (DOX), an antineoplastic agent used in cancer treatment, is obtained from the bacterium *Streptomyces peucetius*. This antineoplastic agent exhibits remarkable efficacy in the treatment of various malig-

nancies, encompassing leukemia, lymphomas, breast carcinoma, uterine carcinoma, pulmonary carcinoma, soft tissue sarcomas, and solid neoplasms [1–4]. Anthracycline derivatives such as DOX, epirubicin, idaru-



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bicin, daunorubicin, and mitoxantrone are employed in clinical practice, with DOX being the most extensively utilized. Although the cytotoxic effects of DOX are different, it mostly causes cardiotoxicity and hepatotoxicity [2, 5]. Despite their highly beneficial effects against cancer, anthracyclines have been reported to show severe cardiotoxicity in clinical use. Anthracyclines cause both acute and chronic cardiotoxicity, which is an important problem limiting the use of these drugs. Acute cardiotoxicity also presents with rhythm problems, hypotension and impaired cardiac function. DOX-associated chronic cardiotoxicity has been shown to lead to progressive left ventricular dysfunction and, in some cases, can result in fatal congestive heart failure [6, 7]. Reports indicate that patients administered DOX and its derivatives may suffer from cardiac complications for many years post-chemotherapy [8]. The use of DOX causes loss of contractility, heart rate (HR) and blood pressure differences. It has also been shown that DOX cardiotoxicity causes vacuolization, sarcomere damage, microtubule damage, loss of myofibrils, mitochondrial damage, and disruption of myocyte structure [9, 10]. Research suggests that DOX-induced cardiotoxicity may stem from a range of mechanisms, including endoplasmic reticulum-mediated apoptosis, damage to DNA/RNA, disruption of calcium homeostasis and autophagy, oxidative stress, and lipid peroxidation [11–13]. The hypothesis that doxorubicin is a mitochondrial toxin has been postulated on the basis that the drug induces cardiac dysfunction and cell death by damaging the mitochondrial membrane. To survive and contract, cardiomyocytes need large numbers of healthy and functional mitochondria that can generate adequate ATP. Overproduction of DOX-induced free radicals is predicted to cause various molecular damage, leading to cardiomyocyte toxicity and cell death [14, 15]. Due to free radicals causing doxorubicin-induced toxicity, the use of various antioxidants and substances with antioxidant properties on DOX toxicity has been studied previously [12, 16, 17].

Matrix metalloproteinases (MMPs) are endopeptidases that facilitate the degradation of structural components of the intercellular matrix, including laminin, collagen, and proteoglycan [18]. MMP activation, particularly MMP-2 activation, has been reported to be an acute marker of DOX-induced cardiotoxicity. Structural dysregulation of the cardiac extracellular space and increased expression of MMP-2 and MMP-9 have been described in doxorubicin cardiotoxicity [19–21]. Therefore, they can be used as degeneration markers in heart tissue.

Highlight key points

- Doxorubicin administration did not cause any change in some oxidative stress parameters and MMP-2 gene expression.
- Variable ECG changes such as increased systolic blood pressure and decreased QRS and QT intervals were seen with doxorubicin administration and this was not affected by CAPE.
- CAPE administration with doxorubicin reduced doxorubicin-induced histopathological changes and scoring.

Caffeic acid phenethyl ester (CAPE) is a potent phenolic compound with anti-inflammatory, antioxidant, and antineoplastic properties, derived from plant extracts gathered by bees [22–24]. The high cell permeability of CAPE is attributable to its phenolic nature and the presence of an ester bond, which facilitates its entry into the cell. Thereafter, it is subject to breakdown by intracellular esterases, resulting in the release of effective caffeic acid. CAPE has various potent therapeutic activities by inducing apoptosis and is currently utilized in the treatment of different disorders [25].

CAPE has been shown to reduce the toxicity of chemotherapy by suppressing free radicals [26] and inhibiting NF- κ B activation [27]. CAPE has been demonstrated to inhibit the 5-lipoxygenase-mediated oxygenation of linoleic acid and arachidonic acid [23]. It has been reported that CAPE reduces DOX-induced damage to the heart muscle by regulating the dysfunction in the endoplasmic reticulum and by helping proteins to fold correctly [28]. Karaboga [29] demonstrated that CAPE prevents tissue damage by reducing NF- κ B and inducible nitric oxide synthase inflammatory mediators. It has been demonstrated that the biochemical and histological alterations in tissues generated by cisplatin's oxidative and nitrosative damage are partially prevented by CAPE [30].

In this study, the mechanisms of the possible protective effects of CAPE in the prevention of cardiotoxicity induced by doxorubicin were evaluated. These mechanisms were investigated by studying MMP-2 gene expression, changes in Troponin I, electrocardiography (ECG) alterations, oxidative stress parameters, tissue damage, and histopathology.

MATERIALS AND METHODS

The present study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, as well as the institutional guidelines for the use of an-

TABLE 1. Primer sequences and expected product size for *MMP-2* and *β-actin*

Genes	Primer sequences	NCBI reference sequence	PCR product size (bp)
<i>β-Actin</i>	F: 5'-CTGGCTCCTAGCACCATGA-3' R: 5'-TAGAGCCACCAATCCACACA-3'	NM_031144.3	76
<i>MMP-2</i>	F: 5'-CACCACCGAGGATTATGACC-3' R: 5'-CACCCACAGTGGACATAGCA-3'	NM_031054.2	71

β-Actin: Beta Actin; *MMP-2*: Matrix metalloproteinase-2; F: Forward, R: Reverse; NCBI: The National Center for Biotechnology Information; PCR: Polymerase Chain Reaction; bp: base pair

imals in research. This commitment to animal welfare is evidenced by the approval of the research protocol by the Inonu University Animal Experiments Local Ethics Committee on 06/01/2022 (approval no: 2022/1-2). Rats were kept in at 21°C, 55–60% humidity, on a 12 h light/12 h dark cycle and divided into 4 groups randomly (n=10). Saline (vehicle for DOX) and 2.5% ethanol (vehicle for caffeic acid phenethyl ester (CAPE, Lot. No: 1016880, Bachem-Switzerland) were administered to the control group, while CAPE at 10 μmol/kg body weight (bw) was applied to the CAPE group intraperitoneally (ip) for 10 days. Doxorubicin (Adriamycin-Deva Holding A.Ş.-Turkey) at 10 mg/kg bw was given ip to DOX group for the last 3 days (a total of 30 mg/kg). In CAPE+ DOX group, CAPE at 10 μmol/kg body weight was given ip for 10 days, and DOX at 10 mg/kg bw was applied for the last 3 days of the study. Rats were assessed for mean arterial pressure (MAP) and heart rate (HR) on the final day of the experiment while under anesthesia induced by 1.2 g/kg of urethane (Ethyl carbamate, CAS: 51-79-6). Under anesthesia, ECG signal activity was monitored for a duration of at least one minute using disposable electrodes on the rat's thorax. Furthermore, high-precision measures of duration and variations in pulse rate, PR, QRS, and QT interval variability across groups were also examined.

Tissue and Sample Collection

The rats were sacrificed by exsanguination under xylazine/ketamine anesthesia. Serum samples were saved at -80 °C for biochemical analyses. The hearts were collected and bisected longitudinally to obtain the atrial and ventral cardia. One half was chopped into small pieces and placed in RNA saving solution and then saved at -80 °C for molecular analyses or freshly used for biochemical analyses. The remaining half was preserved for histological analysis in 10% neutral buffered formalin.

mRNA Expressions of *MMP-2*

Total RNA was extracted from heart tissue using the “EasyPure® RNA Kit” (Trans, Lot no: L41223). Subsequently, cDNA synthesis was carried out using the “Evoscript Universal cDNA Master Kit” (Roche, Ref no: 07912439001). RT-qPCR analysis was subsequently performed on a real-time PCR machine (Roche) using the “Fast Start Essential DNA Probes Master Kit (Ref no: 06402682001)” and the “Real Time Ready Assay “*MMP-2* (Config no: 100143836.)” and “*β-Actin* (Config no: 100129896.)” (Table 1). The PCR protocol followed these conditions: initial denaturation at 95°C for 10 min, followed by 55 cycles consisting of 10 s of denaturation at 95°C and 30 s of annealing at 60°C, and concluding with a final extension at 72°C for 1 s. The 2^{-ΔΔCt} technique was used to calculate mRNA expressions [31].

Heart Biochemical Analyses

Tissue samples were homogenized, and the subsequent homogenates were used to measure the levels of Glutathione (GSH), Malondialdehyde (MDA), Catalase (CAT), and Copper Zinc Superoxide Dismutase (CuZn-SOD). GSH levels were measured using Ellman's method (1961), with results expressed as nmol/g wet tissue (gwt) [32]. MDA levels were quantified at 535 nm/520 nm, then expressed as nmol/gwt [33]. CAT activity in heart tissue samples was measured at 240 nm and expressed as K/g protein [34]. CuZn-SOD activity was detected using the Sun et al. [35] method and read spectrophotometrically at 560 nm. Results were expressed as U/g protein.

Quantification of Troponin in Serum

Concentrations of Troponin-I were then quantified using the Elabscience troponin-I type-3 ELISA assay

TABLE 2. Group comparison findings on biochemical analyses for the heart

Parameters*	Groups**				p
	CAPE (n=10)	CAPE+DOX (n=10)	DOX (n=10)	Control (n=10)	
MDA nmol/gwt	46.79 ^a (8.35)	46.92 ^a (4.46)	50.23 ^a (2.10)	47.68 ^a (3.69)	0.435
GSH nmol/gwt	1456.87 ^a (192.93)	1320.37 ^{ab} (148.31)	1294.12 ^{ab} (568.31)	1197 ^b (326.81)	0.045
CuZn SOD U/g protein	652.52 ^a (116.52)	616.47 ^a (58.88)	645.99 ^a (40.18)	649.21 ^a (102.35)	0.929
CAT K/g protein	37.66 ^a (4.91)	35.06 ^a (11.28)	33.95 ^a (9.36)	29.82 ^a (7.69)	0.218
MMP-2 gene expression	0.002 ^a (0.023)	0.002 ^a (0.068)	0.004 ^a (0.005)	0.016 ^a (0.026)	0.425
Troponin I pg/ml	275.23 ^a (102.23)	524.462 ^b (248.75)	461.577 ^b (175)	217.538 ^a (106.442)	0.011

*: Data are summarized as median (interquartile range); **: There is a statistically significant difference in group categories that do not contain the same letter in each line. CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin; MDA: Malondialdehyde; gwt: Gram wet tissue; GSH: Glutathione; CuZn SOD: Copper Zinc Superoxide Dismutase; CAT: Catalase; MMP-2: Matrix Metalloproteinase-2.

(E-EL-R1253, lot no. KL17H68T9583). The reaction yielded a yellow-colored product. Its proportionality to the Troponin-I present in the sample was then determined. The 96-well plate was read at OD 450 nm at room temperature. Results were expressed as pg of cTnI per milliliter of total protein.

Histopathological Analysis

The heart samples were fixed in 10% formalin, dehydrated using alcohols, cleared in xylene and embedded in paraffin. Myocardial damage was evaluated for interstitial oedema and cardiomyocyte degeneration (eosinophilic cytoplasm, vacuolization, and pyknotic nuclei) in various heart areas with hematoxylin and eosin (H-E).

Statistical Analysis

From the calculations, it was determined that a minimum of 7 subjects per group was required to detect a statistically significant difference, with a predicted effect size of 0.05 Type I error (alpha), 0.8 test power (1-beta) and 1.80 GSH (nmol). Therefore, a total sample size of at least 28 is necessary. The Shapiro-Wilk test was employed to evaluate the conformity of the quantitative data to the normal distribution. However, the data did not demonstrate a normal distribution; consequently, they were presented with a median and interquartile range. The Kruskal-Wallis H test was utilized for intergroup comparison of data, and the Conover test was employed for post-hoc analysis. All analyses were performed using IBM SPSS Statistics 28.0 for Windows (New York; USA).

RESULTS

A statistically significant difference was observed between the groups with regard to GSH levels ($p < 0.05$). However, no such difference was observed for MDA, Cu-Zn SOD and CAT ($p > 0.05$). GSH tissue levels were higher in the CAPE group ($p < 0.05$), but not in the other groups ($p > 0.05$). Table 2 provides a comprehensive overview of the alterations in biochemical and molecular analyses of the heart. The study found no significant difference between the groups in terms of MMP-2 gene expression ($p > 0.05$). Furthermore, a significant statistical difference in Troponin I levels (pg/ml) was detected among the groups ($p < 0.05$). No significant difference was observed between the CAPE+DOX and DOX groups ($p > 0.05$), but Troponin I levels were higher in the CAPE+DOX and DOX groups compared to the Control and CAPE groups ($p < 0.05$).

Table 3 shows a statistically significant difference between the groups in terms of HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), QR, QRS and QT waves ($p < 0.05$). The CAPE+DOX group had a higher HR than the CAPE group ($p < 0.05$). However, there were no statistically significant differences in the other groups ($p > 0.05$). The SBP was higher in the DOX group than in the CAPE and control groups ($p < 0.05$). However, there was no significant difference between the DOX and CAPE+DOX groups ($p > 0.05$). The DBP increased in the CAPE+DOX group compared to the CAPE and Control groups ($p < 0.05$). There were no significant differences between the DOX and CAPE+DOX groups or between DOX and the control group ($p > 0.05$). The MBP was

TABLE 3. Electrocardiographic changes in the treatment groups

Parameters*	Groups**				p
	CAPE (n=10)	CAPE+DOX (n=10)	DOX (n=10)	Control (n=10)	
HR (beats/min)	234 ^a (45.5)	296.5 ^b (65.75)	251 ^{ab} (45.75)	258 ^{ab} (29.25)	0.048
SBP (mm-Hg)	86 ^a (4)	101.5 ^b (8.25)	102.5 ^b (7.75)	84.5 ^a (13.5)	<0.001
DBP (mm-Hg)	45.5 ^a (9.75)	72.5 ^b (13.75)	70.5 ^{bc} (10.25)	60.5 ^c (15.25)	<0.001
MBP (mm-Hg)	57.5 ^a (15.25)	85.5 ^b (10.25)	85.5 ^b (8.5)	73 ^c (14)	<0.001
PR interval (ms)	46 ^a (8.5)	45 ^a (5)	48 ^{ab} (5)	52 ^b (5)	0.018
QRS duration (ms)	53 ^a (15.5)	46 ^b (3.5)	44 ^b (5.5)	66 ^a (28)	<0.001
QT interval (ms)	92 ^a (5.5)	86 ^{bc} (6.5)	81 ^b (4)	90 ^{bc} (27)	0.007

*: Data are summarized as median (interquartile range); **: There is a statistically significant difference in group categories that do not contain the same letter in each line. CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MBP: Mean blood pressure

significantly elevated in the CAPE group compared to the other two groups ($p < 0.05$), yet no significant difference was observed between the CAPE+DOX and DOX groups ($p > 0.05$). The PR interval was higher in the control group than in the CAPE+DOX and CAPE groups ($p < 0.05$). No significant difference was observed between DOX and the other groups ($p > 0.05$). The QRS interval increased significantly in the control group compared to the CAPE+DOX and DOX groups ($p < 0.05$), while no significant difference was observed between the control-CAPE and CAPE+DOX-DOX groups ($p > 0.05$). In addition, the QRS interval was significantly higher in the CAPE group than in the CAPE+DOX and DOX groups ($p < 0.05$). The QT interval was significantly increased in the CAPE group compared to the CAPE+DOX and DOX groups ($p < 0.05$), whereas no significant differences were observed among the Control-CAPE, Control-CAPE+DOX, and CAPE+DOX-DOX groups ($p > 0.05$). There was no arrhythmia in the subjects in the groups (100%). ST depression was only seen in 1 (10%) subject in the DOX group. ST elevation was seen in 7 (70%) subjects in the DOX group, in 2 (20%) subjects in the CAPE group, and in 3 (30%) subjects in the CAPE+DOX group. T negativity was seen in 1 (10%) subject in each of the DOX, CAPE and CAPE+DOX groups. While block was observed in 4 (40%) subjects in the control group, it was not observed in the other groups. Table 4 shows the descriptive statistics regarding the qualitative variables in the ECG.

The myocardium in the control and CAPE groups exhibited a typical normal histological structure, as de-

picted in Figures 1a and 1b. Normal cardiomyocytes with eosinophilic cytoplasm and euchromatic nuclei were seen in the cardiac tissue of these groups. A thin connective tissue (the interstitial tissue) was seen between cardiomyocytes. However, the presence of intensely degenerative cardiomyocytes was marked in the myocardium of the DOX group. In the DOX group, more edematous interstitial connective tissue was also seen between cardiomyocytes (Fig. 1c). The histopathological alterations that occurred in the DOX group were clearly reduced in the CAPE+DOX group (Fig. 1d).

DISCUSSION

Oxidative damage plays a significant role in DOX toxicity. It has been reported that DOX increases lipid peroxidation in plasma by causing excessive production of naturally occurring ROS in the cellular environment [36]. Yarmohmmadi et al. [37] reported that DOX causes cardiomyopathy by inducing cardiac dysfunction, increasing the levels of oxidative stress products, proinflammatory cytokines and proapoptotic factors. Oxidative stress is described as an oxidative balance disorder in which the balance between the increase in ROS formation and the antioxidant system that detoxifies them is disrupted in favor of reactive species [38]. During oxidative stress, reactive species alter and destroy various intracellular components such as DNA, RNA, lipids, and proteins. This occurs when the balance between pro-oxidants and antioxidants shifts in favor of oxidants. Biological membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) can all be adversely impacted by the

TABLE 4. Descriptive statistics for qualitative data on ECG

Parameters	Category	Control		DOX		CAPE		CAPE+DOX	
		n	%	n	%	n	%	n	%
Arrhythmia	Absent	10	100.00	10	100.00	10	100.00	10	100.00
	Exist	0	0.00	0	0.00	0	0.00	0	0.00
ST depression	Absent	10	100.00	9	90.00	10	100.00	10	100.00
	Exist	0	0.00	1	10.00	0	0.00	0	0.00
ST elevation	Absent	10	100.00	3	30.00	8	80.00	7	70.00
	Exist	0	0.00	7	70.00	2	20.00	3	30.00
T negativity	Absent	10	100.00	9	90.00	9	90.00	9	90.00
	Exist	0	0.00	1	10.00	1	10.00	1	10.00
Block	Absent	6	60.00	10	100.00	10	100.00	10	100.00
	Exist	4	40.00	0	0.00	0	0.00	0	0.00

ECG: Electrocardiography; DOX: Doxorubicin; CAPE: Caffeic acid phenethyl ester

damaging process of oxidative stress [39, 40]. Because membrane phospholipids are key targets of ROS, lipid peroxidation is a vital indicator of oxidative damage [41]. MDA is commonly used to evaluate lipid peroxidation and oxidative stress levels [42]. The findings of present study indicate that there is no alteration in MDA levels among the groups suggesting that no lipid peroxidation is present in the cardiac tissues of DOX -treated group. Antioxidants inhibits oxidative damage to the target molecules by reacting with free radicals and neutralizing the free radicals [43]. The body has developed two categories of endogenous antioxidant defense mechanisms: enzymatic and non-enzymatic. The enzymatic defense system includes various endogenous enzymes, such as glutathione reductase, CAT, glutathione peroxidase, and SOD. Additionally, a non-enzymatic defense system, like GSH, is also present [44]. In this study, Glutathione levels were significantly higher in the CAPE group compared to the control group ($p < 0.05$). However, no statistically significant differences were found in the pairwise comparisons of the other groups. Additionally, there was no significant difference in SOD and CAT activities between groups, indicating that CAPE did not affect the antioxidant status of the cardiac tissue in the rats across all groups. Troponin I is suggested to be a suitable method for the detection of myocardial necrosis. It seems to be as sensitive to cardiac damage as CK-MB, and it has a high specificity for myocardium [45]. Troponin I levels were significantly elevated in the DOX group compared to the control group, indicating myocardial damage in the

DOX-treated group. On the other hand, CAPE treatment in DOX group (CAPE+DOX) did not affect Troponin I level compared to DOX group.

Matrix metalloproteinases plays a crucial part in the processes of tissue remodeling because of their high affinity for extracellular matrix (ECM) components. The ECM regulates the alignment of cardiac cells and the overall stability of the myocardium. In a healthy heart, the balance between the synthesis and degradation of the ECM is tightly controlled [46]. A variety of developing cardiovascular disease states have recently been found to have altered *MMP* expression patterns and myocardium undergoes changes in collagen structure and content, which may have an impact on left ventricular geometry [47]. Myocyte alignment is disturbed as a result of increased metalloproteinase activity, which also speeds up the remodeling of the left ventricle in congestive heart failure [46]. Polegato et al. [48] reported that *MMP-2* gene expression is increased in acute DOX toxicity, and cardiac dysfunction is associated with myocardial *MMP-2* activation. Similarly, Shaker and Souror [46] reported that DOX significantly increased the *MMP-2* level in the plasma of DOX treated rats. In our study, there were no alterations in *MMP-2* gene expressions among the groups.

SBP increased in DOX-treated rats compared to control animals, without any change in HR. The HR remained unchanged in the DOX group when compared to the control and CAPE+DOX groups. SBP was elevated

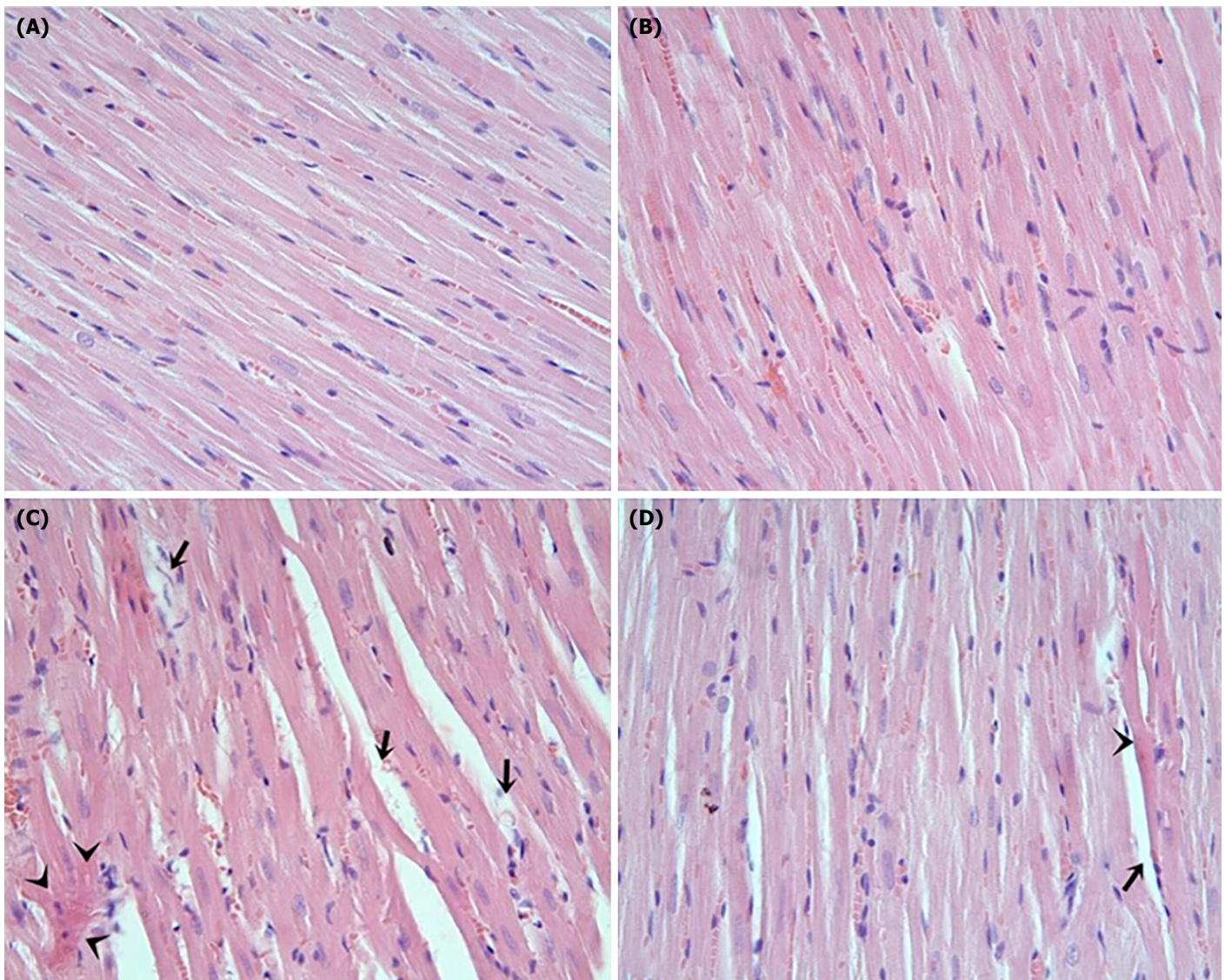


FIGURE 1. Photomicrographs of the longitudinal section of the myocardium **(A-D)**. Control **(A)** and CAPE **(B)** groups exhibit normal histological appearance. DOX group **(C)** draws attention to the presence of degenerate cardiomyocytes (arrowheads) and interstitial edema (arrows). CAPE+DOX group **(D)** demonstrates a prominent decrease in the intensity of degenerate cardiomyocytes (arrowheads) and interstitial edema (arrows) and marked improvement in the myocardial structure (H-E, x40).

CAPE: Caffeic acid phenethyl ester; DOX: Doxorubicin.

in both the DOX and CAPE+DOX groups compared to the control, while DBP showed no difference between the DOX group and the control. We also evaluated the ECG changes in the treatment groups. While duration of PR wave was not changed between DOX group and control, duration of QRS and QT waves was decreased in DOX group compared to control. These alterations may reflect intraventricular conduction defects as a result of myocardial ultrastructural changes. However, ECG parameters appear to be not affected by CAPE treatment meaningfully to report a clear conclusion

from our results. Villani et al. [49] reported that DOX causes prolongation of QRS complex in a dose dependent manner. Similarly, Shekari et al. [50] reported that DOX treatment resulted in significant prolongation of QRS complex and QT interval compared to the control group. However, no alterations in the ST segment were observed between the treated groups and the control group. It has been reported that prolongation of the QT interval, widening of the QRS and flattening of the T-wave were observed in rats given DOX as well as causing body weight loss and death during drug treatment [37].

In terms of histopathological alterations in the present study, the presence of dense degenerative cardiomyocytes in the myocardium of the DOX group was noted. Furthermore, increased interstitial connective tissue and edema were seen between cardiomyocytes in the DOX group. Our results showed that DOX causes damage to cardiomyocytes. It was observed that CAPE showed a significant decrease in histopathological changes and scoring in the CAPE+DOX group compared to DOX group. According to Shekari et al. [50] DOX caused loss of myofibrils, inflammation with mononuclear cell infiltration, perinuclear and cytoplasmic vacuolization and hypertrophy of myocardium. Yarmahmoudi et al. [37] reported similar changes including mononuclear inflammation and cytoplasmic vacuolization in the cardiomyocytes of DOX-treated rats. A study investigating the protective effect of CAPE on DOX-induced cardiotoxicity reported that CAPE pretreatment significantly reduced DOX-induced cardiac damage. Biochemical parameters and electron microscopy results revealed a significant protection where DOX caused swelling of mitochondria and cristae disappearance and matrix clear out ultrastructurally. However, CAPE treatment ameliorated these ultrastructural alterations [51]. In general, DOX 6 times every other day at 2.5 mg/kg led to the infiltration of leukocyte cells, inflammation, and unevenly spaced myocardium fiber [52].

Limitations

The absence of Western blot analysis, immunohistochemistry findings, echocardiographic analysis and levels of B-type natriuretic peptide may be one of the limiting factors of the study. Another limiting factor is that CAPE was administered for as short as 10 days. In our study, longer CAPE administration instead of 10 days could have positively changed many parameters.

CONCLUSIONS

CAPE treatment ameliorated histopathological changes induced by DOX while other parameters including oxidative stress, MMP-2 gene expression, Troponin I and ECG studied in our study were not altered remarkably. CAPE did not affect these parameters significantly. Improved histopathological alterations due to CAPE treatment may be associated with other mechanisms that were not studied in this study. Although histologically the benefits of cape are seen, it has been observed that it causes changes in the ECG and leads to changes in the interventricular conduction system. Therefore, further research is needed for cape in terms of arrhythmia and resynchronization disorder.

Ethics Committee Approval: The Inonu University Animal Experiments Local Ethics Committee granted approval for this study (date: 06.01.2022, number: 2022/1-2).

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