Research in Pharmaceutical Sciences, February 2025; 20(1): 55-64
Received: 20-10-2023
Peer Reviewed: 06-11-2023
Revised: 07-04-2024
Accepted: 01-06-2024
Published: 20-02-2025

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Cardioprotective potential of protocatechuic acid against doxorubicin-induced cardiotoxicity in rats

Leila Safaeian[1,*], Zahra Haghighatian[2], and Mohammadreza Zamani[1]

[1]*Department of Pharmacology and Toxicology and Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*
[2]*Department of Pathology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, I.R. Iran.*

Abstract

Background and purpose: Chemotherapy with doxorubicin (DOX) is associated with toxicity in many organs including cardiac tissue. A large body of evidence has suggested that phenolic acids, such as protocatechuic acid (PCA), have beneficial effects on cardiovascular problems. This investigation was conducted to evaluate the ameliorative properties of PCA against DOX-induced cardiotoxicity in Wistar rats.
Experimental approach: Animals were treated with PCA (50, 100, and 200 mg/kg, orally) for 10 days. On the 7th day, a single injection of DOX (20 mg/kg/day, i.p.) was administered to induce cardiotoxicity. Electrocardiography, biochemical analysis of cardiac markers, and histological inspections were performed.
Findings/Results: Pretreatment with PCA, especially at the doses of 100 and 200 mg/kg for 7 days before the administration of DOX, significantly improved cardiac rhythm and pathological changes, reduced serum levels of creatine phosphokinase-MB, lactate dehydrogenase, aspartate aminotransferase, lipid peroxides and also prevented heart weight rise.
Conclusions and implications: The *in-vivo* findings of the current study revealed that PCA exhibits protective effects against DOX-induced cardiotoxicity. These results suggest that PCA, a natural phenolic acid, may serve as a promising candidate for cardioprotective interventions in clinical trials involving chemotherapy with DOX.

Keywords: Cardiotoxicity; Doxorubicin; Protocatechuic acid; Rat.

INTRODUCTION

Cardiotoxicity is one of the most important complications in patients treated with anticancer chemotherapeutic drugs (1). The most severe form of cardiomyopathy has been observed with doxorubicin (DOX). This antineoplastic agent belongs to the anthracyclines and effectively treats many cancers (2). Heart damage may occur instantly after a single dose or with a delay after repeated doses of DOX with different symptoms including heart palpitations, transient arrhythmias, hypotension, chest pain, dyspnea, and fluid retention in the lower limbs (3). Various mechanisms have been proposed for the development of cardiomyocyte toxicity induced by DOX, such as impairment of calcium homeostasis and mitochondrial function, formation of iron complexes, up-regulation of toll-like receptors (TLRs), and stimulation of nuclear factor kappa-B (NF-κB) which initiate oxidative, inflammatory and apoptotic cascades (4-6). Due to the prevalent utilization of DOX in chemotherapy protocols, extensive investigations have sought to discover novel chemotherapeutic agents aimed at preventing or alleviating the cardiotoxic effects associated with its administration.

Access this article online	
	Website: http://rps.mui.ac.ir
	DOI: 10.4103/RPS.RPS_220_23

*Corresponding author: L. Safaeian
Tel: +98-3137927087, Fax: +98-3136680011
Email: leila_safaeian@pharm.mui.ac.ir

Protocatechuic acid (PCA; 3, 4, dihydroxybenzoic acid) belongs to the hydroxybenzoic acid and is an eminent type of phenolic acid. PCA is found in many plant sources and is a main metabolite of anthocyanins and proanthocyanidins (7). There is growing evidence for various pharmacological actions of PCA including antioxidative, antimicrobial, analgesic, neuroprotective, hepato-protective, anti-tumor, and anti-inflammatory properties (8,9).

Some studies have reported the beneficial impact of PCA in cardiovascular disorders such as anti-atherosclerotic, antihypertensive, and anti-fibrotic actions (10-12). PCA has been able to reverse the biochemical alterations associated with diabetic cardiomyopathy and protect against cardiac toxicity caused by an environmental toxin (2,3,7,8-tetracholorodibenzo-p-dioxin; TCDD) (13,14). In a recent cellular assay, PCA alleviated oxidative stress and protected cardiomyocytes against toxicities caused by arsenic trioxide and DOX (15). Therefore, the current study aimed to investigate the potential effects of PCA on DOX-induced cardiotoxicity in an animal model.

MATERIALS AND METHODS

Animals and experimental design

Male adult Wistar rats, weighing 220 ± 20 g, were acquired from the animal house of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences. The rats were maintained at room temperature of about 20-25 ℃ and a 12/12-h light/dark cycle with free access to water and standard food. Animals were adapted under an experimental environment for 1 week before the beginning of the experiment. The research procedures were per international guidelines for laboratory animal use and care, and approval was obtained from the Institutional Research Ethics Committee of Isfahan University of Medical Sciences (Ethic Code: IR.MUI.AEC.1401.020).

Experimental protocol

Thirty rats were randomly distributed into 5 groups with 6 rats in each group as follows: group 1 as the normal control received oral administration of vehicle (normal saline) for 10 days and intraperitoneal (i.p.) injection of normal saline on day 7; group 2 as the cardiotoxicity control group received a single dose of DOX (20 mg/kg, i.p.) on day 7; groups 3-5 received oral administration of 50, 100, and 200 mg/kg/day of PCA (Cayman Chemical Co., USA) for 10 days and i.p. injection of DOX on day 7.

Doses of PCA were selected based on previous studies (16,17). A study timeline of drug administration is depicted in Fig. 1.

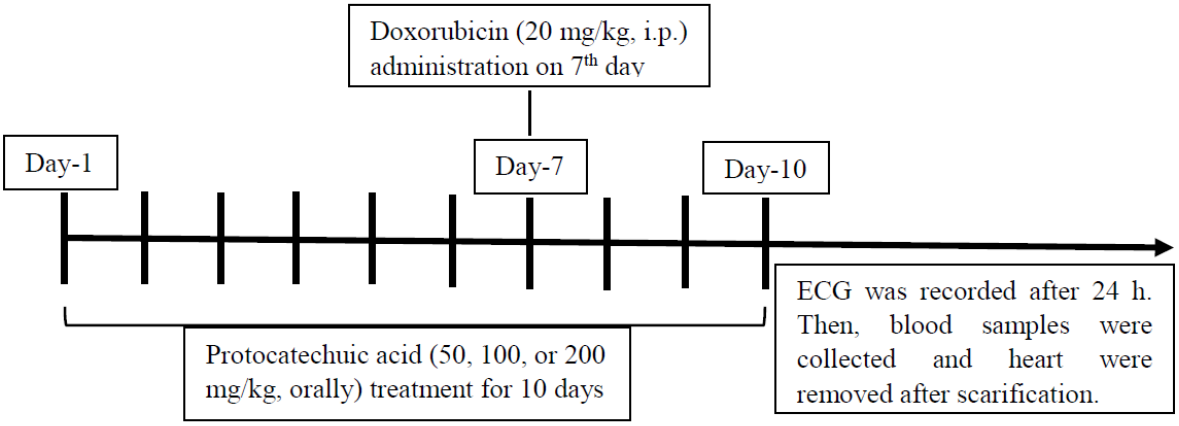

Fig. 1. A study schematic timeline showing drug (protocatechuic acid and doxorubicin) administration schedule.

Induction of DOX-induced cardiotoxicity

The model of cardiotoxicity was developed by a single high dose of i.p. injection of 20 mg/kg DOX which was used as an Ebedoxo injection vial (EBEWE Pharma GmbH Nfg KG Co., Austria) on the 7th day in rats (18,19). The weight of the animals was measured at the start of the experiment and then every other day. After 24 h of the last treatment, rats were anesthetized with ketamine (50 mg/kg)/xylazine (5 mg/kg), and a lead II electrocardiograph (ECG) was recorded by computerized data acquisition eWave system and analyzed with eProbe software (Science Beam; Parto Danesh Co., Iran). ECG was assessed for pattern, heart rate, ST segment, QRS complex, QT interval, and RR interval alterations. Then, blood samples were collected by retro-orbital technique under anesthesia, and serum was analyzed for biochemical markers of heart injury and oxidative stress. After sacrificing the rats under CO_2 exposure, hearts were removed, weighed, and immersed in formalin solution. Tissue samples were further processed and evaluated for histopathological changes.

Biochemical assay

The blood samples were centrifuged at 4000 rpm for 10 min to separate the serum to assess the biochemical parameters of cardiac injury. Serum concentrations of lactate dehydrogenase (LDH), creatine phosphokinase-muscle-brain (CK-MB), and aspartate aminotransferase (AST) were assessed according to the manufacturer's instructions by colorimetric method (at 340 nm) using standard kits manufactured by Pars Azmoon Co. (Iran) *via* an auto-analyzer (Nihon Kohden, Japan). Results are expressed in IU/L (20).

Lipid peroxidation assay

The malondialdehyde (MDA) content in serum specimens was determined to calculate lipid peroxidation. For this mean, the thiobarbituric acid reactive substances test was performed by colorimetric assay (at 532 nm) using a commercial kit (Hakiman Shargh Research Co., Iran). A standard curve was depicted and the lipid peroxides concentration was stated as µM of MDA equivalents (21).

Histopathological examination

The heart samples were fixed in a 10% buffered formalin solution. After additional processing, they were embedded in paraffin blocks, and sections of 5 µm thickness were stained with hematoxylin and eosin (H&E). The heart tissue slides were blindly checked for routine histopathological alterations including inter-muscular edema, infiltration of inflammatory cells, cardiomyocyte degeneration, myofibrillar rupture, hemorrhage, and congestion, at ×400 magnification under a light microscope (16).

Statistical analysis

Data were presented as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test using SPSS software (version 25.0). Significant differences were established at *P*-values less than 0.05.

RESULTS

Effect of PCA on ECG parameters and heart weight

As seen in Fig. 2, a single administration of DOX (20 mg/kg) caused significant changes in ECG pattern including elevation of the ST segment, prolongation of QT interval, a decrease in RR interval, and an increase in heart rate versus the normal pattern attained in the control group (Table 1).

Pretreatment with PCA at 100 and 200 mg/kg significantly reversed ST elevation and QT prolongation. All doses of PCA increased RR interval and reduced the heart rate in animals with DOX-induced cardiotoxicity, especially the dose of 200 mg/kg (Fig. 2 and Table 1).

Exposure to DOX also resulted in a significant increase in the relative weight of the heart (heart/body weight ratio). Administration of various doses of PCA could significantly prevent heart weight increase in this cardiotoxicity model (Table 1).

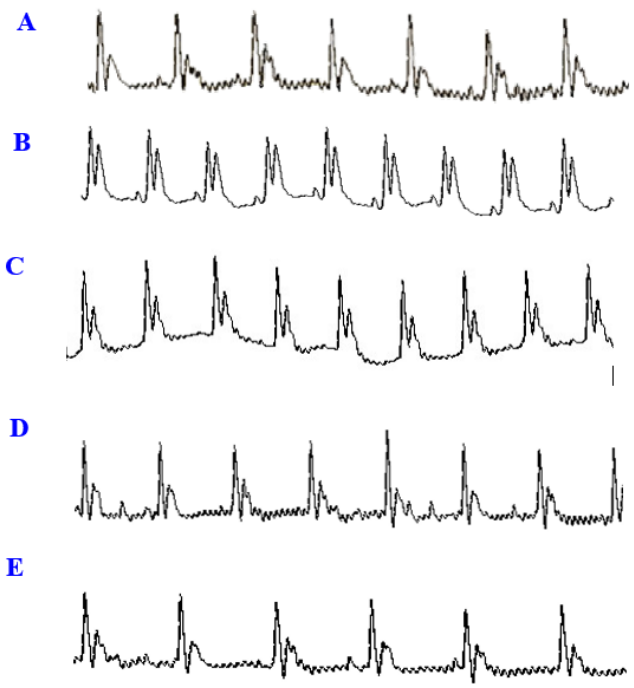

Fig. 2. Representative electrocardiograph traces of lead Π for (A) normal control group; (B) doxorubicin-induced cardiotoxicity control; (C-E) protocatechuic acid treated groups with doses of 50, 100, and 200 mg/kg, respectively.

Table 1. Effect of PCA on rats' electrocardiogram parameters and relative heart weight in DOX-induced cardiotoxicity. Values are expressed as mean ± SEM, n = 6. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ indicate significant differences in comparison with the normal control; $^{*}P < 0.05$, $^{*}P < 0.01$, and $^{***}P < 0.001$ versus DOX group.

Groups	Heart rate (BPM)	RR-interval (ms)	ST elevation (µV)	QRS (ms)	QT interval (ms)	Relative heart weight (%)
Normal	274.7 ± 9.6	218.3 ± 8.6	98.0 ± 6.7	43.3 ± 1.7	96.0 ± 4.6	0.38 ± 0.012
DOX	321.7 ± 8.7$^{\#\#}$	186.8 ± 5.1$^{\#}$	138.0 ± 8.2$^{\#\#}$	42.9 ± 1.5	117.8 ± 2.3$^{\#\#}$	0.48 ± 0.023$^{\#\#}$
DOX + PCA (50 mg/kg)	285.8 ± 4.3*	211.5 ± 3.2*	109. 6 ± 9.5	43.0 ± 1.3	108.3 ± 2.9	0.40 ± 0.016*
DOX + PCA (100 mg/kg)	280.5 ± 6.1*	213. 9 ± 4.5*	95.0 ± 5.7**	42.5 ± 1.8	97.4 ± 2.2**	0.41 ± 0.018*
DOX + PCA (200 mg/kg)	255.0 ± 9.4***	236.3 ± 7.7***	93.2 ± 4. 1**	41.2 ± 1.9	97.5 ± 5.6**	0.41 ± 0.011*

PCA, Protocatechuic acid; DOX, doxorubicin; BPM, beats per minute; ms, millisecond; µV, microvolts.

Effect of PCA on biochemical parameters

Figure 3 designates the effect of PCA on serum biochemical markers of cardiac injury in DOX-induced cardiotoxicity. Following exposure to DOX, the serum level of LDH activity considerably increased compared to that of the normal control group. Pretreatment with PCA at 100 and 200 mg/kg significantly decreased the activity of this enzyme when compared to the DOX control group (Fig. 3A).

An elevation in the serum activity of CK-MB was observed after the administration of DOX, which was meaningfully reduced after receiving PCA at 100 and 200 mg/kg (Fig. 3B).

DOX also led to a significant upsurge in AST activity. Administration of PCA (100 and 200 mg/kg) notably decreased AST levels compared with DOX control rats (Fig. 3C).

Effect of PCA on lipid peroxidation

As seen in Fig. 4, animals' exposure to DOX resulted in a major increase in the serum content of MDA compared to the normal control rats. Treatment with PCA at 100 and 200 mg/kg significantly attenuated the MDA concentration in rats' serum.

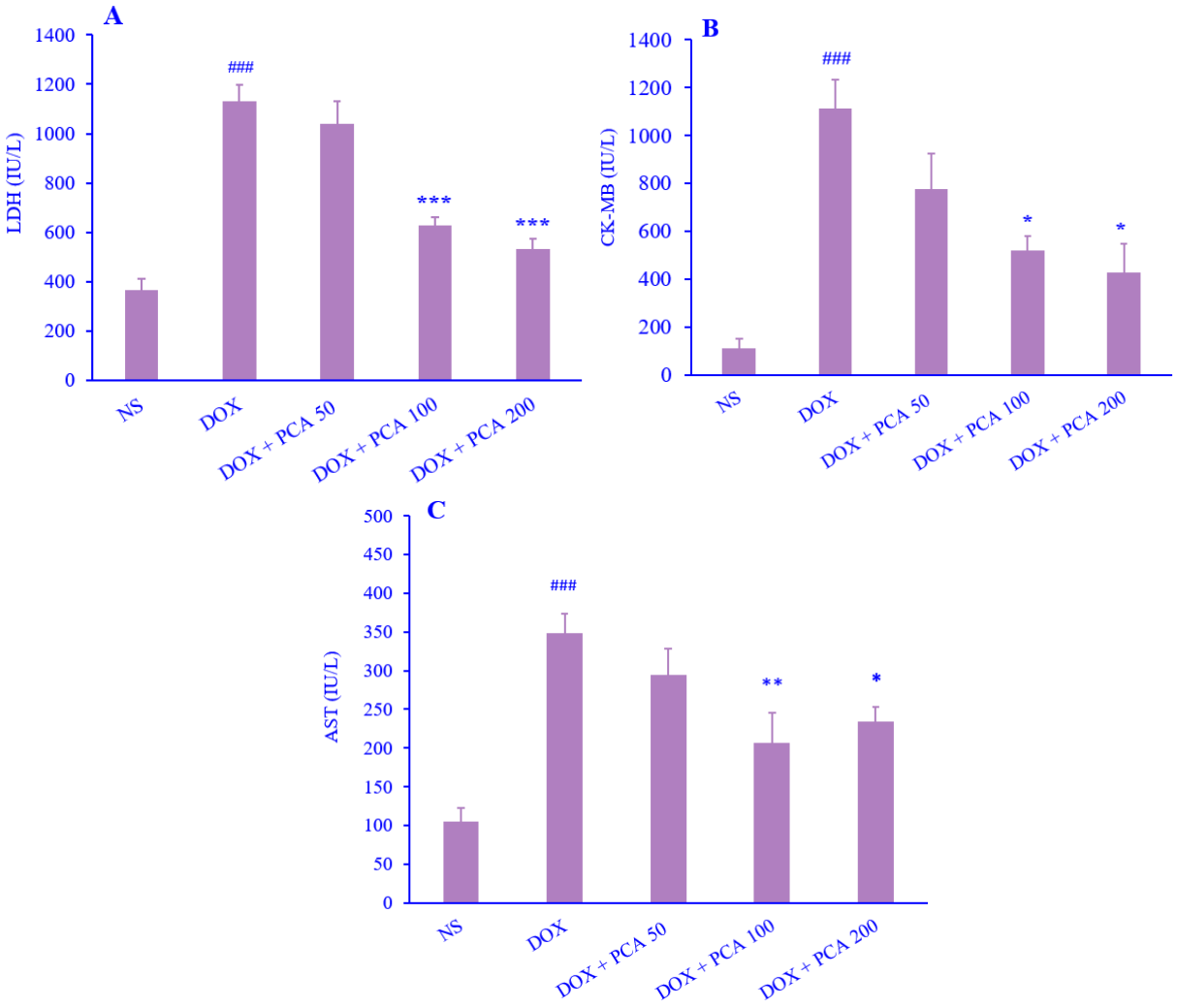

Fig. 3. Effect of PCA at 50-200 mg/kg on serum biochemical parameters of cardiac injury in rats with DOX-induced cardiotoxicity. (A) LDH, (B) CK-MB, and (C) AST. Values are expressed as mean ± SEM, n = 6. $^{###}P < 0.001$ indicates significant differences in comparison with the normal control receiving NS. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus DOX group. PCA, Protocatechuic acid; DOX, doxorubicin; LDH, lactate dehydrogenase; CK-MB, creatine phosphokinase-muscle-brain; AST, aspartate aminotransferase; NS, normal saline.

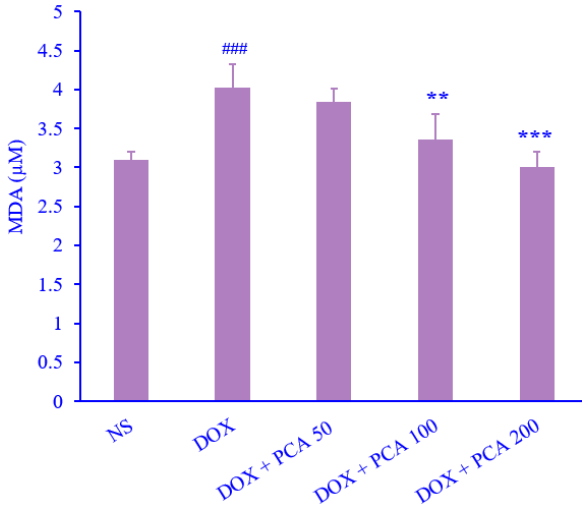

Fig. 4. Effect of PCA at 50-200 mg/kg on serum MDA level in rats with DOX-induced cardiotoxicity. $^{###}P < 0.001$ indicates a significant difference in comparison with the normal control receiving NS; $^{**}P < 0.01$ and $^{***}P < 0.001$ versus DOX group. PCA, Protocatechuic acid; DOX, doxorubicin; MDA, malondialdehyde; NS, normal saline.

Effect of PCA on body weight changes

As shown in Fig. 5, the body weight changes of all rats had a slight upward trend in the first 7 days of treatment. However, there was a strict fall in the rats' body weight after administration of DOX. Treatment with various doses of PCA was not able to prevent body weight loss induced by DOX.

Effect of PCA on heart histopathology

Assessment of the H&E-stained sections of heart tissue displayed a normal architecture of cardiomyocytes in the heart of normal control rats (Fig. 6A). Meanwhile, pathological alterations including degeneration and rupture of myocytes, multiple areas of hemorrhage and congestion of blood vessels were seen in the heart tissues of rats with DOX-induced cardiotoxicity (Fig. 6B). Pretreatment with PCA at 50 mg/kg had no effect (Fig. 6C). However, administration of PCA at 100 mg/kg (Fig. 6D) and 200 mg/kg (Fig. 6E) showed minimal myocyte rupture and fewer areas of hemorrhage.

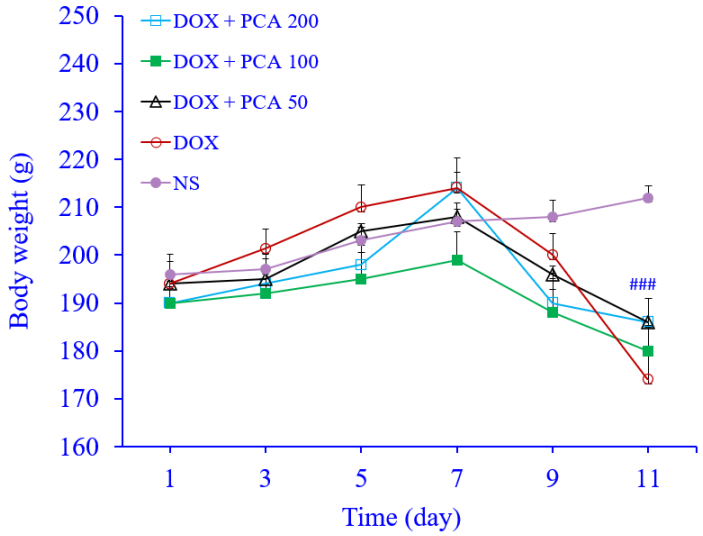

Fig. 5. Effect of PCA at 50-200 mg/kg on body weight changes in rats with DOX-induced cardiotoxicity. $^{\#\#}P < 0.01$ indicates a significant difference compared to the normal control receiving NS. PCA, Protocatechuic acid; DOX, doxorubicin; NS, normal saline.

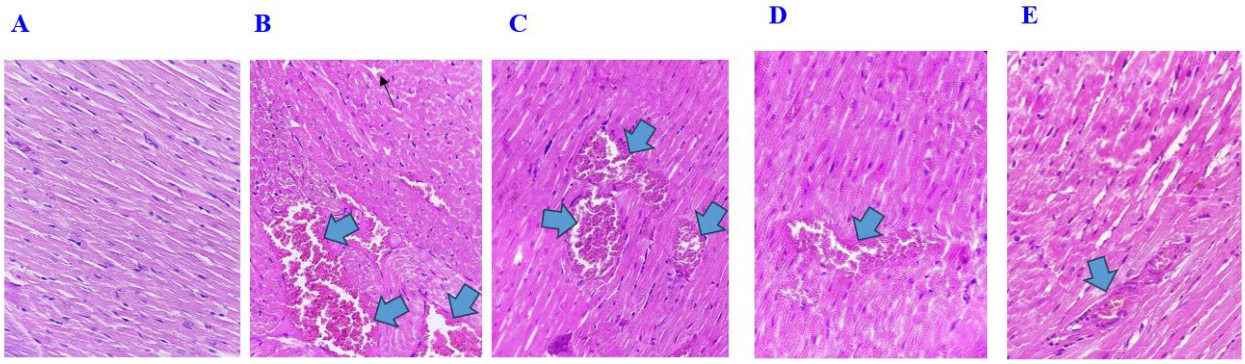

Fig. 6. Representative H&E sections of heart tissue for (A) the normal control; (B) doxorubicin-induced cardiotoxicity group; (C-E) protocatechuic acid-treated rats at doses of 50, 100, and 200 mg/kg, respectively. Magnification: ×400. Blue arrows indicate areas of hemorrhage and congestion and the black-thin arrow (in part B) shows the degeneration of myocytes.

DISCUSSION

In this study, pretreatment with PCA at 100 and 200 mg/kg ameliorated the DOX-induced cardiotoxicity in rats followed by the improvements in ECG pattern and heart histopathological changes, and declining MDA and biochemical markers of cardiac injury. The protective role of PCA as a natural phenolic acid has been proven in many organ damages such as hippocampal neuronal death caused by seizures, hepato-renal toxicities induced by various oxidant and anti-neoplastic agents, and endothelial dysfunction in diabetic conditions (22-25). A previous *in-vitro* study showed that PCA could protect cardiomyocytes against arsenic trioxide and DOX toxicities *by* regulating the oxidative stress pathway through declining TLR4 expression and hydroperoxide levels and increasing total antioxidant power (15).

The current *in-vivo* investigation examined the effect of PCA pretreatment on parameters of cardiac damage in rats after acute exposure to DOX. The DOX-induced cardiotoxicity was established by heart histopathological alterations, elevated ST segment, prolonged QT interval, lessened RR interval, and increased heart rate, as well as a significant increase in the activities of serum CK-MB, LDH, AST, and the content of lipid peroxides, and also cardiac weight gaining.

Myocardial cell toxicity caused by DOX is strictly associated with producing large amounts of oxidants and free radicals, inflammatory cytokines, and iron-DOX complexes leading to various forms of cell death (4,5). DOX induces intrinsic pathway, caspase-independent, and extrinsic apoptosis pathways in cardiomyocytes (26). This activates TLRs and the formation of inflammasomes and subsequent initiation of pyroptosis in cardiac tissue (27).

Cardiomyocyte injury caused by DOX resulted in the leakage of cardiac enzymes such as LDH, CK-MB, and AST into the circulation. Based on the data obtained from ECG, DOX increased heart rate in our study, however, contradictory effects on heart rate and QTc interval have been reported in different animal studies that may be affected by DOX dosages and time of the ECG recording (28,29). It is important to note that arrhythmia, such as tachycardia or bradycardia, has been mentioned as the side effect of doxorubicin in clinical studies (30). Moreover, although QT intervals are affected by human heart rate, studies have shown a weak correlation between QT and RR intervals in mice and rats (31,32).

Our findings also indicated that DOX administration induced weight loss in the animals. The effects of DOX on crucial factors of lipid and glucose metabolism, including peroxisome proliferator-activated receptor gamma (PPARγ) and 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK), may lead to body weight loss and atrophy of adipose tissue (33).

In the current study, pretreatment with PCA at 100 and 200 mg/kg exhibited protective effects against DOX-induced cardiotoxicity in rats as demonstrated by the improvements in cardiac rhythm and pathological changes, dropping serum levels of CK-MB, LDH, AST, and MDA, and the prevention of the increase in the weight of hearts.

Beneficial cardiovascular effects of PCA have been observed in several cellular and animal investigations (13-15). In a model of diabetic cardiomyopathy studied by Bhattacharjee *et al*. treatment with PCA (50 and 100 mg/kg, orally) showed beneficial cardiac effects through reversing the serum levels of LDH, C-reactive protein, CK, troponin I and II in rats. Their findings revealed the impact of PCA on the modulation of polyol enzyme expression, augmentation of intracellular ATP (adenosine triphosphate) concentration, and reduction of collagen deposition in the heart tissues besides anti-hyperglycemic and anti-hyperlipidemic activities. Moreover, they reported potent antioxidative effects for PCA since it lessened protein carbonylation, lipid peroxidation, and reactive oxygen species (ROS) production, enhanced glutathione, catalase, and superoxide dismutase levels, increased co-enzyme Q9 and Q10 and prevented DNA fragmentation and oxidation in the heart tissues (13).

In a study conducted by Ciftci *et al*. PCA (100 mg/kg) protected rats' cardiac tissue against oxidative injury caused by a toxic

environmental agent (TCDD) by reducing lipid peroxide, elevating antioxidant defense, and subsequently preventing hemorrhage and necrosis (14).

A recent investigation also displayed that pretreatment with PCA (10 and 20 mg/kg) could protect kidney tissue from toxicity induced by DOX by regulating the cyclooxygenase-2 and inducible nitric oxide activities, reducing the lipid peroxide levels, and enhancing the antioxidant enzymes (34).

The protective effects of PCA may be mediated through enhancing the antioxidant capacity, upregulation of nuclear factor erythroid 2-related factor 2, reducing ROS formation, decreasing NF-κB level, inhibition of inflammatory mediator expression such as tumor necrosis factor-α and interleukin-1β, diminution of mitochondrial dysfunction, suppression of caspase-3, lessening expression of Bax (Bcl-2-associated X protein) and increasing expression of Bcl-2 (B-cell lymphoma 2) and subsequently inhibition of apoptosis (35).

In addition to chemo-preventive properties, PCA has exhibited anti-neoplastic and anti-metastatic effects in some tumor cells by promoting apoptosis and hindering cell migration and invasion, suggesting its potential for use in combination chemotherapy to augment the therapeutic efficacy and decrease organ toxicities (36,37).

The major limitation of the present study included the lack of investigation of the possible mechanisms involved in the protective effects of PCA against DOX-induced cardiotoxicity. Although we evaluated various electrocardiography, biochemical, and histological parameters of cardiac toxicity, echocardiographic assessments may better prove the cardiomyopathy in this model.

CONCLUSION

The results of the present study confirmed that PCA alleviates DOX-induced cardiotoxicity in rats by improving ECG patterns and histopathological alterations of hearts and dropping serum levels of lipid peroxides and cardiac enzymes. Accordingly, PCA can be suggested as a potential candidate for cardio-protection in clinical trials during chemotherapy with DOX.

Acknowledgments

This paper is based on a Pharm.D thesis authored by M. Zamani and was financially supported by the Vice-Chancellery for Research and Technology at Isfahan University of Medical Sciences, Isfahan, I.R. Iran, under Grant No. 3401366.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

L. Safaeian contributed to the design and development of the study, supervision, project administration, and editing of the manuscript; Z. Haghighatian was responsible for histopathological analysis; M. Zamani performed the experiments, collected the data, and drafted the manuscript. All authors read and approved the finalized article.

REFERENCES

1. Schimmel KJ, Richel DJ, Van den Brink RB, Guchelaar HJ. Cardiotoxicity of cytotoxic drugs. Cancer Treat Rev. 2004;30(2):181-191. DOI: 10.1016/j.ctrv.2003.07.003.
2. Mostafa RE, Morsi AH, Asaad GF. Anti-inflammatory effects of saxagliptin and vildagliptin against doxorubicin-induced nephrotoxicity in rats: attenuation of NLRP3 inflammasome up-regulation and tubulo-interstitial injury. Res Pharm Sci. 2021;16(5):547-458. DOI: 10.4103/1735-5362.323920.
3. Sheibani M, Azizi Y, Shayan M, Nezamoleslami S, Eslami F, Farjoo MH, *et al*. Doxorubicin-induced cardiotoxicity: an overview on pre-clinical therapeutic approaches. Cardiovasc Toxicol. 2022;22(4):292-310. DOI: 10.1007/s12012-022-09721-1.
4. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Sci Rep. 2017;7(1):44735,1-11. DOI: 10.1038/srep44735.
5. Xinyong C, Zhiyi Z, Lang H, Peng Y, Xiaocheng W, Ping Z, *et al*. The role of toll-like receptors in myocardial toxicity induced by doxorubicin. Immunol Lett. 2020;217:56-64. DOI: 10.1016/j.imlet.2019.11.001.
6. Avagimyan A, Sheibani M, Pogosova N, Mkrtchyan L, Yeranosyan H, Aznauryan A, *et al*. Possibilities

of dapagliflozin-induced cardioprotection on doxorubicin+cyclophosphamide mode of chemotherapy-induced cardiomyopathy. Int J Cardiol. 2023;391:131331.
DOI: 10.1016/j.ijcard.2023.131331.

7. Zhang S, Gai Z, Gui T, Chen J, Chen Q, Li Y. Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: old wine in a new bottle. Evid Based Complement Alternat Med. 2021;2021:6139308,1-19.
DOI: 10.1155/2021/6139308.
8. Masella R, Santangelo C, D'Archivio M, LiVolti G, Giovannini C, Galvano F. Protocatechuic acid and human disease prevention: biological activities and molecular mechanisms. Curr Med Chem. 2012;19(18):2901-2917.
DOI: 10.2174/092986712800672102.
9. Khan AK, Rashid R, Fatima N, Mahmood S, Mir S, Khan S, *et al*. Pharmacological activities of protocatechuic acid. Acta Pol Pharm. 2015;72(4):643-650.
PMID: 26647619.
10. Deng JS, Lee SD, Kuo WW, Fan MJ, Lin YM, Hu WS, *et al*. Anti-apoptotic and pro-survival effect of protocatechuic acid on hypertensive hearts. Chem Biol Interact. 2014;209:77-84.
DOI: 10.1016/j.cbi.2013.11.017.
11. Tang XL, Liu JX, Dong W, Li P, Li L, Lin CR, *et al*. The cardioprotective effect of protocatechuic acid on myocardial ischemia/reperfusion injury. J Pharmacol Sci. 2014;125(2):176-183.
DOI: 10.1254/jphs.13247fp.
12. Wang D, Wei X, Yan X, Jin T, Ling W. Protocatechuic acid, a metabolite of anthocyanins, inhibits monocyte adhesion and reduces atherosclerosis in apolipoprotein E-deficient mice. J Agric Food Chem. 2010;58(24):12722-12728.
DOI: 10.1021/jf103427j.
13. Bhattacharjee N, Dua TK, Khanra R, Joardar S, Nandy A, Saha A, *et al*. Protocatechuic acid, a phenolic from *Sansevieria roxburghiana* leaves, suppresses diabetic cardiomyopathy via stimulating glucose metabolism, ameliorating oxidative stress, and inhibiting inflammation. Front Pharmacol. 2017;8:251.
DOI: 10.3389/fphar.2017.00251.
14. Ciftci O, Disli OM, Timurkaan N. Protective effects of protocatechuic acid on TCDD-induced oxidative and histopathological damage in the heart tissue of rats. Toxicol Ind Health. 2013;29(9):806-811.
DOI: 10.1177/0748233712442735.
15. Shafiee F, Safaeian L, Gorbani F. Protective effects of protocatechuic acid against doxorubicin- and arsenic trioxide-induced toxicity in cardiomyocytes. Res Pharm Sci. 2023;18(2):149-158.
DOI: 10.4103/1735-5362.367794.
16. Ye P, Li WL, Bao LT, Ke W. Mulberrin confers protection against doxorubicin-induced cardiotoxicity *via* regulating AKT signaling pathways in mice. Oxid Med Cell Longev. 2022; 2022:2967142,1-18.
DOI: 10.1155/2022/2967142.
17. Satyam SM, Bairy LK, Shetty P, Sainath P, Bharati S, Ahmed AZ, *et al*. Metformin and dapagliflozin attenuate doxorubicin-induced acute cardiotoxicity in *Wistar rats*: an electrocardiographic, biochemical, and histopathological approach. Cardiovasc Toxicol. 2023;23(2):107-119.
DOI: 10.1007/s12012-023-09784-8.
18. Krzysztoforska K, Piechal A, Wojnar E, Blecharz-Klin K, Pyrzanowska J, Joniec-Maciejak I, *et al*. Protocatechuic acid prevents some of the memory-related behavioural and neurotransmitter changes in a pyrithiamine-induced thiamine deficiency model of Wernicke–Korsakoff syndrome in rats. Nutrients. 2023;15(3):625,1-25.
DOI: 10.3390/nu15030625.
19. Thakare VN, Patil RR, Suralkar AA, Dhakane VD, Patel BM. Protocatechuic acid attenuate depressive-like behavior in olfactory bulbectomized rat model: behavioral and neurobiochemical investigations. Metab Brain Dis. 2019;34(3):775-787.
DOI: 10.1007/s11011-019-00401-8.
20. Zhao L, Tao X, Qi Y, Xu L, Yin L, Peng J. Protective effect of dioscin against doxorubicin-induced cardiotoxicity via adjusting microRNA-140-5p-mediated myocardial oxidative stress. Redox Biol. 2018;16:189-198.
DOI: 10.1016/j.redox.2018.02.026.
21. Safaeian L, Mirian M, Bahrizadeh S. Evolocumab, a PCSK9 inhibitor, protects human endothelial cells against H_2O_2-induced oxidative stress. Arch Physiol Biochem. 2022;128(6):1681-1686.
DOI: 10.1080/13813455.2020.1788605.
22. Lee SH, Choi BY, Kho AR, Jeong JH, Hong DK, Lee SH, *et al*. Protective effects of protocatechuic acid on seizure-induced neuronal death. Int J Mol Sci. 2018;19(1):187,1-12.
DOI: 10.3390/ijms19010187.
23. Habib SA, Suddek GM, Rahim MA, Abdelrahman RS. The protective effect of protocatechuic acid on hepatotoxicity induced by cisplatin in mice. Life Sci. 2021;277:119485,1-11.
DOI: 10.1016/j.lfs.2021.119485.
24. Owumi SE, Ajijola IJ, Agbeti OM. Hepatorenal protective effects of protocatechuic acid in rats administered with anticancer drug methotrexate. Hum Exp Toxicol. 2019;38(11):1254-1265.
DOI: 10.1177/0960327119871095.
25. Chook CY, Cheung YM, Ma KY, Leung FP, Zhu H, Niu QJ, *et al*. Physiological concentration of protocatechuic acid directly protects vascular endothelial function against inflammation in diabetes through Akt/eNOS pathway. Front Nutr. 2023;10:1060226,1-11.
DOI: 10.3389/fnut.2023.1060226.
26. Sangweni NF, Gabuza K, Huisamen B, Mabasa L, van Vuuren D, Johnson R. Molecular insights into the pathophysiology of doxorubicin-induced cardiotoxicity: a graphical representation. Arch Toxicol. 2022;96(6):1541-1550.
DOI: 10.1007/s00204-022-03262-w.
27. Tavakoli Dargani Z, Singla DK. Embryonic stem cell-derived exosomes inhibit doxorubicin-induced

TLR4-NLRP3-mediated cell death-pyroptosis. Am J Physiol Heart Circ Physiol. 2019;317(2):H460-H471. DOI: 10.1152/ajpheart.00056.2019.
28. Durdagi G, Pehlivan DY, Oyar EO, Bahceci SA, Ozbek M. Effects of melatonin and adrenomedullin in reducing the cardiotoxic effects of doxorubicin in rats. Cardiovasc Toxicol. 2021;21(5):354-364. DOI:10.1007/s12012-020-09625-y.
29. Ammar ESM, Said SA, Suddek GM, El-Damarawy SL. Amelioration of doxorubicin-induced cardiotoxicity by deferiprone in rats. Can J Physiol Pharmacol. 2011;89(4):269-276. DOI: 10.1139/y11-020.
30. Xie Z, Wu W, Xing H, Cai Y, Wang X, Chen J. Bradycardia associated with pegylated liposomal doxorubicin administration: a case report. Eur J Hosp Pharm. 2017;24(2):128-130. DOI: 10.1136/ejhpharm-2015-000817.
31. Roussel J, Champeroux P, Roy J, Richard S, Fauconnier J, Le Guennec JY, *et al*. The complex QT/RR relationship in mice. Sci Rep. 2016;6(1):25388,1-9. DOI: 10.1038/srep25388.
32. Mulla W, Murninkas M, Levi O, Etzion Y. Incorrectly corrected? QT interval analysis in rats and mice. Front Physiol. 2022;13:1002203,1-8. DOI: 10.3389/fphys.2022.1002203.
33. Arunachalam S, Kim SY, Kim MS, Yi HK, Yun BS, Lee DY, *et al*. Adriamycin inhibits adipogenesis through the modulation of PPAR gamma and restoration of adriamycin-mediated inhibition of adipogenesis by PPAR gamma over-expression. Toxicol Mech Methods. 2012;22(7):540-546. DOI: 10.3109/15376516.2012.692110.
34 Molehin OR, Adeyanju AA, Adefegha SA, Oyeyemi AO, Idowu KA. Protective mechanisms of protocatechuic acid against doxorubicin-induced nephrotoxicity in rat model. J Basic Clin Physiol Pharmacol. 2019;30(4):1-10. DOI: 10.1515/jbcpp-2018-0191.
35. Kassab RB, Theyab A, Al-Ghamdy AO, Algahtani M, Mufti AH, Alsharif KF, *et al*. Protocatechuic acid abrogates oxidative insults, inflammation, and apoptosis in liver and kidney associated with monosodium glutamate intoxication in rats. Environ Sci Pollut Res Int. 2022;29(8):12208-12221. DOI: 10.1007/s11356-021-16578-4.
36. Tanaka T, Tanaka T, Tanaka M. Potential cancer chemopreventive activity of protocatechuic acid. J Exp Clin Med. 2011;3(1):27-33. DOI: 10.1016/j.jecm.2010.12.005.
37. Lin HH, Chen JH, Chou FP, Wang CJ. Protocatechuic acid inhibits cancer cell metastasis involving the down-regulation of Ras/Akt/NF-κB pathway and MMP-2 production by targeting RhoB activation. Br J Pharmacol. 2011;162(1):237-254. DOI: 10.1111/j.1476-5381.2010.01022.x.