DOI 10.4149/BLL_2024_42

Bratisl Med J 2024; 125 (5):
281–288

EXPERIMENTAL STUDY

Electrocardiographic, biochemical, and scintigraphic evidence for the cardioprotective effect of paricalcitol and vitamin D^3 on doxorubicin-induced acute cardiotoxicity in rats

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ABSTRACT

AIM: We aimed to investigate the possible cardioprotective effects of paricalcitol (PR), its vitamin D receptor agonist, and vitamin D^3 (VIT-D3) on an experimental model of doxorubicin (DX) cardiotoxicity by ^{99m}Tc-PYP scintigraphy, electrocardiographic (ECG) and biochemical methods.

METHOD: Forty-two male Wistar/Albino rats (250–300 g; aged 10–12 weeks) were randomly separated into six groups, namely into control (CN), doxorubicin (DX), paricalcitol (PR), vitamin D^3 (VIT-D^3), paricalcitol + doxorubicin (PR+DX), and vitamin D^3 + doxorubicin (VIT-D^3+DX) groups. Cardiotoxicity was induced by three doses of DX (18 mg/kg, i.p.) at 24-hour intervals on days 18, 19 and 20. PR (0.5 ug/ kg, i.p) and VIT-D^3 (5,000 IU/kg, i.p) were injected for 20 days before and after the application of DX (18 mg/kg, i.p.). On day 21 of the experiment, biochemical parameters [tumor necrosis factor TNF-alpha (TNF-α); interleukin-6 (IL-6), nitric oxide (NO), and cardiac troponin T (cTnT)], as well as ECG and scintigraphic (^{99m}Tc-PYP) features were assessed.

RESULTS: Compared to CN, DX significantly raised TNF-α, IL-6, and NO in heart tissue, cTnT in serum, ^{99m}Tc-PYP uptake in the myocardium, and ECG parameters, specifically QRS complex duration, QT interval duration, and ST-segment amplitude, while also reducing heart rate ($p<0.001$). Pretreatment with PR and VIT-D^3 mitigated these abnormalities produced by DX in the heart ($p<0.001$).

CONCLUSION: Results show that vitamin D receptor agonist paricalcitol and vitamin D protect against DX-induced cardiotoxicity through anti-inflammatory and antioxidant effects *(Fig. 4, Ref. 59)*. Text in PDF *www.elis.sk*

KEY WORDS: paricalcitol, doxorubicin, vitamin D, ECG, 99mTc-PYP scintigraphy, cardiotoxicity, inflammation.

Introduction

Doxorubicin (DX) is one of the most effective chemotherapeutic agents in cancer treatment, but its clinical use is hampered by cardiotoxicity (Swain et al, 2003; Chicco et al, 2006). Clinically, DX-induced cardiotoxicity is characterized by aberrant arrhythmias, acute left ventricular dysfunction, and congestive heart failure (Volkova et al, 2011; Zhang et al, 2022). Numerous experimental studies have been conducted to investigate the mechanisms of DX-induced cardiotoxicity and reduce its cardiotoxic effects with possible treatment modalities (Songbo et al, 2019; Singh et al, 2023). The best-recognized mechanisms for acute and chronic cardiac side effects caused by DX are lipid peroxidation, apoptosis, oxidative stress, mitochondrial dysfunction, inhibition of nucleic acids, and inflammation (McGowan et al, 2017; Singh et al, 2023).

Recently, the use of vitamins to alleviate DX-related toxicity has received much attention. Vitamin D (VIT-D^3) is critical in regulating the cardiovascular system. Deficiency of VIT-D^3 is closely related to cardiovascular diseases such as coronary artery disease, hypertension, myocardial infarction (MI), cardiomyopathy, and heart failure (Aleksova et al, 2015; Anderson et al, 2010). Studies indicated that VIT-D^3 at normal levels improves hypertension and reduces the risk of coronary artery disease (Witte et al, 2016). VIT-D^3 modulates cell functions through the vitamin D receptor (VDR). It has been shown that VDR receptors are widely distributed in the cardiovascular system (cardiomyocytes, vascular smooth muscle cells, and endothelial cells). Therefore, the stimulation of VDR can directly affect the cardiovascular system (Li et al, 2002). Paricalcitol is a new VIT-D^3 receptor agonist. It was revealed that upregulation of VDR expression by paricalcitol can ameliorate left ventricular hypertrophy and alleviate myocardial

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compression (Qu et al, 2021). Tamayo et al (2020) showed that paricalcitol reduces ventricular arrhythmias and improves electrophysiological changes in patients with heart failure.

The literature review suggests that VIT-D^3 supplementation and direct stimulation of the VIT-D^3 receptor may affect the prevention of DX-induced cardiotoxicity (DXIC). For this reason, we investigated the possible protective effects of VIT-D^3 and paricalcitol in DXIC by scintigraphic, electrocardiographic, and biochemical methods in this study.

Material and method

Experimental Protocol

Animals

Wistar Albino rats (n = 42, 250–300 g) included in the study were allowed to acclimate to the laboratory conditions for one week. To maintain the diurnal rhythm, all procedures conducted on rats were performed during a period between 09:00 and 11:00 AM daily. Rats were housed in polypropylene cages. Room temperature was 22–25 °C, and a 12/12-hour light/dark cycle was provided. The animal had free access to food (standard pellet diet) and tap water. The Local Ethical Committee approved the study for animal experiments (2019-HADYEK-13, 04.04.2019).

Experimental design

In this study, DX (18 mg/kg, i.p) was used to induce cardiotoxicity, and VIT D (5,000 IU/kg) and paricalcitol (0.5 µg/kg, i.p) were preferred for the treatment. The study was conducted on six groups of Wistar rats, each consisting of seven animals as follows:

Group I (CN): healthy untreated rats,

Group II (PR): rats receiving only paricalcitol (PR) for 20 days (0.5 ug/ kg/i.p.),

Group III (VIT-$D^{3)}$: rats receiving only vitamin D^3 (VIT-D^3) for 20 days (0.5 ug/ kg/i.p.),

Group IV (DX): rats injected intraperitoneally with doxorubicin (18 mg/kg) dissolved in 2 mL of saline three times at an interval of 24 h (on days 18, 19 and 20),

Group V (PR+DX): rats pre-co-treated with 0.5 paricalcitol (PR) daily for 20 days and injected with DX (18 mg/kg) three times at 24-hour intervals (on days18, 19 and 20),

Group VI (VIT-D^3+ DX): rats pre-co-treated with 5,000 IU/kg vitamin D^3 daily for 20 days and injected with DX (18 mg/kg) three times at 24-hour intervals (on days 18, 19 and 20).

At the end of the experimental period, 24 hours after the last DX injection (i.e, on day 21), all the Wistar rats were anesthetized with a dose of ketamine/xylazine (75/10 mg/kg of body weight) and scintigraphic imaging, and ECG were performed. Blood samples were collected in tubes. Then the rats were sacrificed by cervical decapitation and heart tissues were excised.

Electrocardiogram

Electrocardiograms were recorded after the scintigraphy procedure at a standard limb leads position under ketamine/xylazine anesthesia (Ketalar vial, 75 mg/kg, Pfizer/Rompun vial, 10 mg/kg, Bayer) for 1 minute. The leads were attached to the rats' left arm, right arm, and left leg. An ECG processor for heart rate, P wave duration, QT interval duration, QRS complex duration, and ST segment interval were used to analyze each ECG in the limb lead II position.

Biochemical analysis

Blood samples were collected from the heart after the ECG recordings. Then the blood samples were collected into tubes and centrifuged (at 3,000 rpm for 10 min). The plasma was stored at −20 °C for later analysis. The rats were killed under anesthesia, and the heart tissue was removed. The heart tissue was frozen in liquid nitrogen and stored at –70 °C until assaying. The heart tissue samples were washed with cold saline (0.9%) and weighed while wet. Then, the heart tissues were cut into pieces with a scalpel, placed into a tube and homogenized with stainless steel balls in cold PBS (pH 7.4). To obtain the supernatant, the tissues were incubated and centrifuged at 2,500 rpm for 20 min. The results were normalized to wet tissue weights and expressed as ng/L for TNF-alpha and IL6 and as µmol/L for NO. The level of nitric oxide (NO), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) level were studied with ELISA kits (Bioassay Technology Laboratory) according to the manufacturer's instructions. (Rat Nitric Oxide ELISA Kit, E0703Ra; Rat Interleukin 6 ELISA Kit; E0135Ra; Rat Tumor Necrosis Factor Alpha ELISA Kit, E0764Ra).

Scintigraphic imaging

One millicurie (mCi) ^{99m}Tc-PYP radiopharmaceutical (TechneScan PYP, Mallinckrodt) was diluted for scintigraphic imaging with 5 ml of isotonic. Then, 0.1 ml of ^{99m}Tc-PYP radiopharmaceutical was injected into the rats intraperitoneally. One hour after the injection, the rats were imaged on a gamma camera (SIEMENS Syimbia, USA) with a static study. The rats were placed on their backs while the images were taken. Then, the affected heart muscle region was measured (7 separate measurements) for regions of interest (ROI) evaluation, and the average was taken.

Statistical analysis

SPSS 16.0 software was used for statistical evaluation in the present study. The Kolmogorov–Smirnov test was used to determine whether the data fit the normal distribution. The one-way ANOVA post hoc Tukey test was used to analyze customarily distributed data to determine the differences between groups. For data analysis that did not fit the normal distribution, the difference between the groups was determined by Kruskal–Wallis analysis of variance, and then paired comparisons were made with the Mann–Whitney U test. While $p<0.05$ was considered significant for the post hoc test, Bonferroni correction was applied to determine the p-value in non-parametric analyses.

Results

ECG assay

Figure 2 a–f shows the ECG patterns of CN, PR, PR+DX, and VIT-D^3+DX rats. The difference in heart rates between groups CN, PR, VIT-D^3, PR+DX, and VIT-D^3+DX was not statistically significant. However, the difference in heart rate between groups CN,

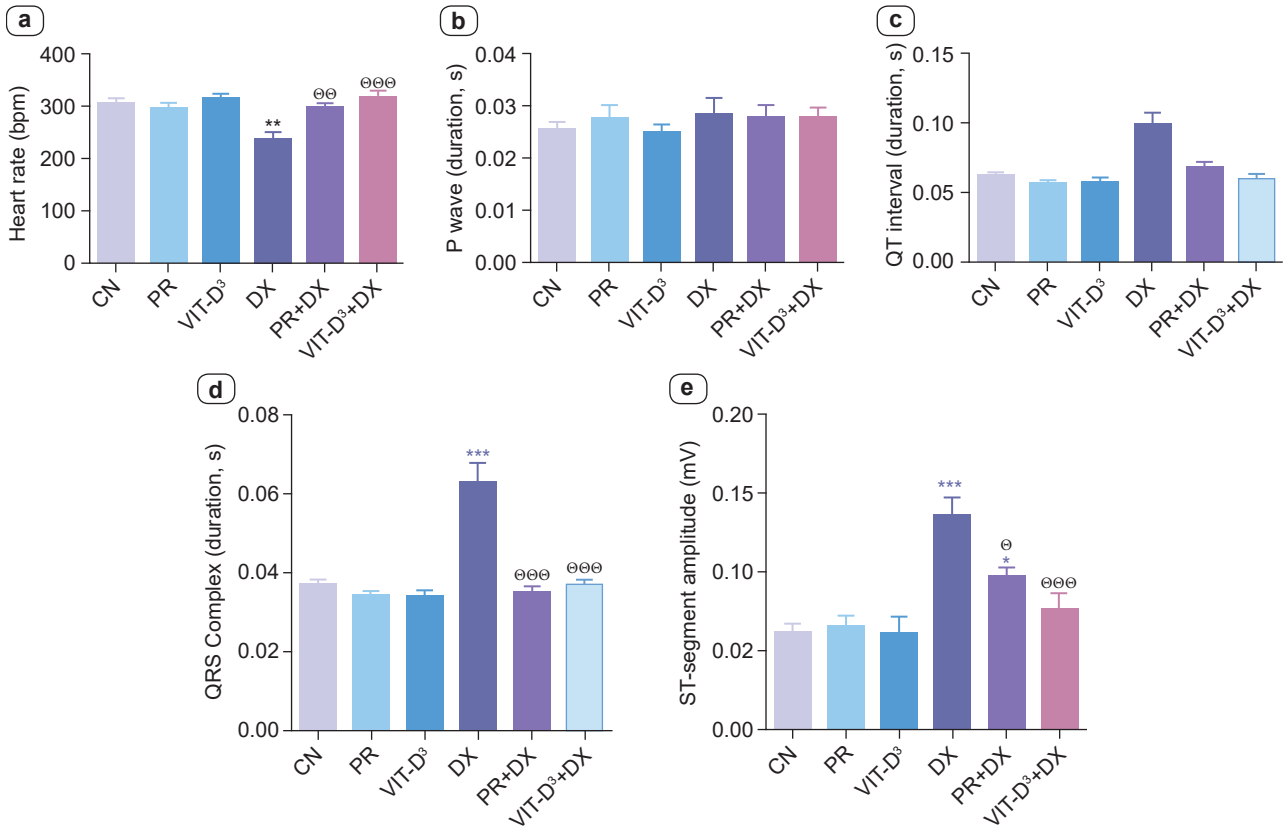

Fig. 1. Effects of DX-induced cardiotoxicity on duration and interval of ECG waves; CN, PR, VIT-D³, DX, PR + DX, and VIT-D³ + DX. (a) heart rate (bpm); (b) P wave (duration, s); (c) QT interval (duration, s), (d) QRS complex (duration, s), ST-segment amplitude (mV). Results are expressed as mean ± SEM. * p<0.05, **p<0.01, ***p<0.001 versus control group; Θ p<0.05, ΘΘ p<0.01, ΘΘΘ p<0.001 versus DX group.

PR+DX, VIT-D³+DX, and group DX was significant. Bradycardia was observed in DX-administered rats. However, pretreatment with 0.5 µg/kg PR and 5,000 IU/kg VIT-D³ suppressed the DX-induced bradycardia (Fig. 1a).

The P wave duration difference between all experimental groups was not found to be significant (Fig. 1b).

The difference in QRS complex duration and QT interval duration between the five groups (CN, PR, VIT-D³, PR+DX, and VIT-D³+DX) was not significant. Nevertheless, the DX application significantly increased QRS complex and QT interval as compared to the CN group. However, the administration of 0.5 µg/kg PR and 5,000 IU/kg VIT-D³ significantly helped reduce the DX-induced increase in QRS complex and QT interval (Fig. 1c, d).

CN, PR, VIT-D³, and VIT-D³+DX groups yielded normal ST-segments. DX-induced cardiotoxicity rats could be observed in form of elevated ST segments (Fig. 1e). ST-segment elevation was also recorded in the PR+DX group compared to the CN group. However, the pretreatment of DOX-induced cardiotoxicity rats with 0.5 µg /kg PR and 5,000 IU/kg VIT-D³ minimized the ST-segment elevation (Fig. 1e).

Biochemical assay

The serum cardiac troponin T (cTnT), IL-6, TNF-α, and NO in the experimental groups is as shown in the figure. The

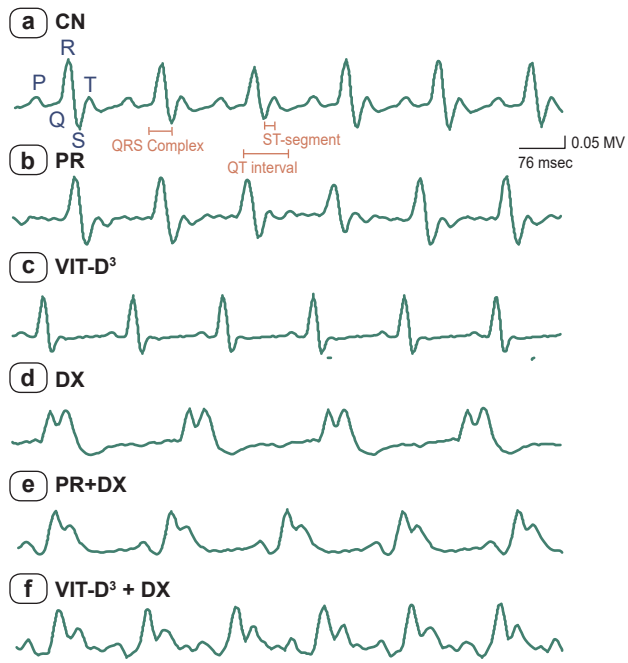

Fig. 2. Limb lead II ECGs. (a) ECG pattern of CN, (b) PR, (c) VIT-D3, (d) DX, (e) PR + DX, and (f) VIT-D³ + DX-group rat's heart showed in the figure.

difference in levels of cTnT between the four groups (CN, PR, VIT-D^3, and VIT-D^3 + DX) was insignificant. The administration of DX increased the cTnT level as compared to the CN group. However, pretreatment of cardiotoxic rats with 0.5 µg/kg PR and 5,000 IU/kg VIT-D^3 helped reduce the cTnT levels increased by DX (Fig. 3a).

The difference in levels of IL-6 and NO between the five groups (CN, PR, VIT-D^3, PR+DX, and VIT-D^3+DX) was not found significant. The administration of DX increased IL-6 and NO levels compared to the CN group. However, pretreatment of cardiotoxic rats with 0.5 µg/kg PR and 5,000 IU/kg VIT-D^3 helped reduce the IL-6 and NO levels increased by DX (Fig. 3b, d).

The difference in TNF-α levels between the three groups (CON, PR, VIT-D^3) was not found significant. DX, PR+DX, and VIT-D^3+DX groups had significantly increased TNF-α levels compared to the CN group. Nevertheless, PR+DX and VIT-D^3+DX groups had significantly reduced TNF-α levels compared to the DX group (Fig. 3a).

Scintigraphic assay

Representative ^{99m}Tc-PYP scintigraphic images of experimental groups are shown in Figure 4a. On scintigraphy, DX rats showed heart uptake, representing a circulating blood pool. The ^{99m}Tc-PYP uptake was not observed in the hearts of rats in the CN, PC, and VIT-D^3 groups. DX, PR+DX, or VIT-D^3+DX groups had a significantly increased ^{99m}Tc-PYP uptake level in comparison with the CN group ($p<0.001$, $p<0.05$, $p<0.05$, respectively). However, the pretreatment with 0.5 µg/kg PR or 5,000 IU/kg VIT-D^3 suppressed the ^{99m}Tc-PYP uptake elevation in comparison with the DX group ($p<0.001$, $p<0.001$, respectively) (Fig. 4b).

Discussion

In this study, cardiotoxicity induced by DX was evidenced by elevation of ^{99m}Tc-PYP, cTnT, NO, and inflammatory cytokines IL-6 and TNF-α, as well as by conduction abnormalities in ECG in DX-administered rats. Furthermore, the present study has illustrated that PR and VIT-D^3 have a cardioprotective effect on DX-induced cardiotoxicity in the rat by improving plasma and heart tissue biomarkers, ECG parameters, and scintigraphic changes.

It has been well established in the literature that the cardiotoxic effects of DX are a necessary clinical condition. However, there is no effective pretreatment to reverse all cardiac changes caused by DX. Many mechanisms play a role in the formation of cardiotoxicity caused by DX, with oxidative stress being one of the most important factors. Also, DX treatment causes inflammation by raising proinflammatory cytokines and, consequently, damages the myocardial cell membrane (Chiosi et al, 2007). Moreover, in many experimental and clinical studies, it has been shown that TNF-α and IL-6, which are potent proinflammatory cytokines, play an essential role in DX-induced cardiotoxicity (Chiosi et al, 2007; Kanda et al, 2004). Kanda et al. noted that the increase in IL-6 in DX-induced cardiomyopathy may begin within 24 hours after the first dose of DX (Kanda et al, 2004). Another study by Lou et al (2004) showed that DX caused elevation of TNF-α serum levels in rats. Compared to the published studies, in the presented study, it was likewise observed that the administration of DX to rats increased TNF-α and IL-6 in the heart tissue.

Previous studies have shown that VIT-D^3 has a promising cardioprotective effect by suppressing proinflammatory cytokines TNF-α and IL-6 and increasing anti-inflammatory IL-10 (Canning et al, 2001). In preclinical and experimental studies, it has been reported that VIT-D^3 has a promising cardioprotective effect by reducing proinflammatory cytokines (Saleh et al, 2020; El-Bassiouny et al, 2022). In a clinical study, supplementation with VIT-D^3 was shown to reduce serum IL-6 levels in

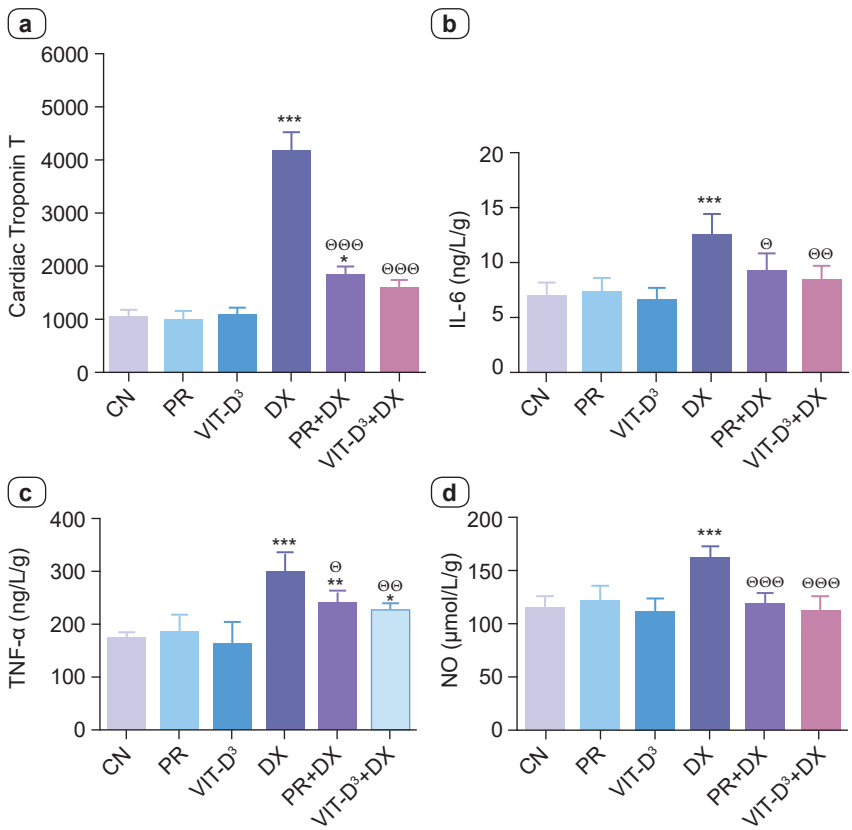

Fig. 3. Effects of DX-induced cardiotoxicity on cTnT in blood samples and IL-6 (ng/L/g), TNF-α (ng/L/g), NO (mmol/L/g) in the heart tissue in CN, PR, VIT-D^3, DX, PR + DX, and VIT-D^3+ DX groups. Results are expressed as mean ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus control group; $^{\Theta}$ $p<0.05$, $^{\Theta\Theta}$ $p<0.01$, $^{\Theta\Theta\Theta}$ $p<0.001$ versus DX group.

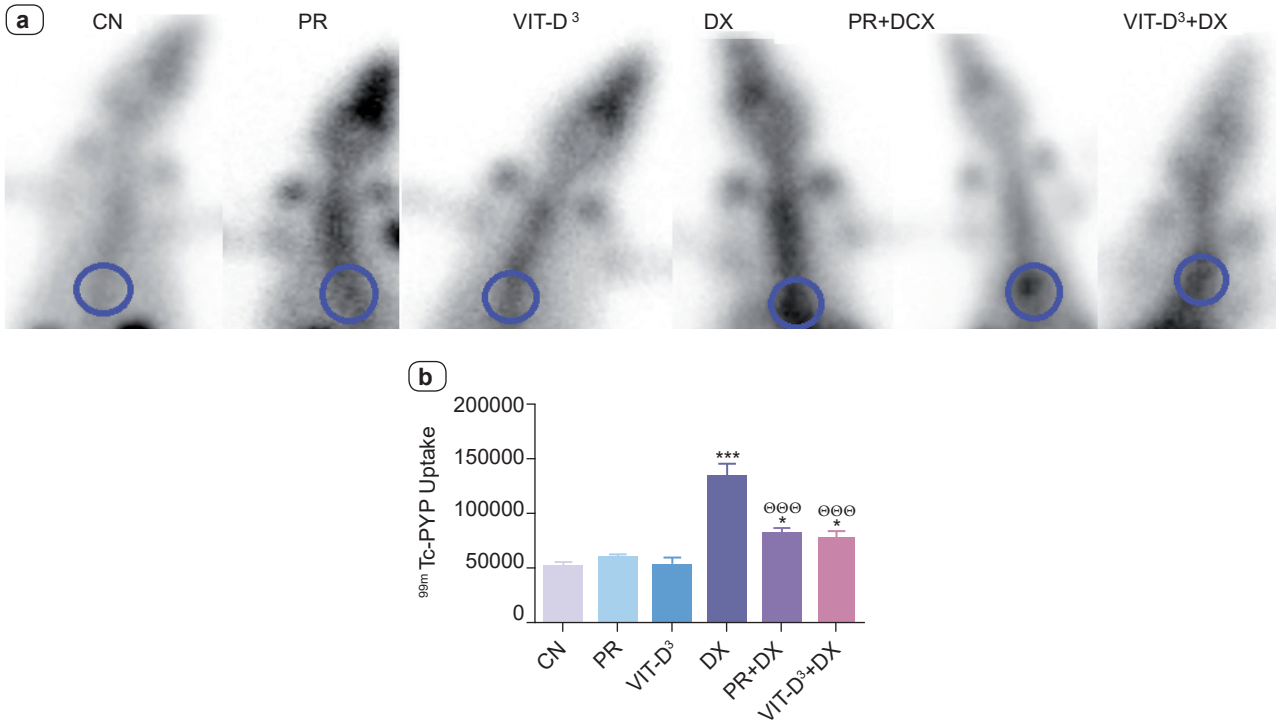

Fig. 4. ^{99m}Tc-PYP radiopharmaceutical uptake images (a) and findings (b) in CN, PR, VIT-D^3, DOX, PR + DX, and VIT-D^3 + DX groups. * p<0.05, **p<0.01, ***p<0.001 versus control group; ^{ΘΘΘ} p<0.001 versus DX group.

breast cancer patients receiving DX (El-Bassiouny et al, 2022). Experimental studies have shown that VIT-D^3 supplementation significantly reduces inflammatory IL-6 and TNF-α compared to rats injected with only DX, which supports our finding (Saleh et al, 2020; Awad et al, 2021; Ghaleb et al, 2021). In addition, various studies indicate that PR reduces oxidative stress and cellular inflammation (Izquierdo et al, 2012; Husain et al, 2015). A study reported that PR treatment reduced cyclohexyl-genes-2, TNF-α, inducible nitric oxide synthase (iNOS) and raised antioxidant capacity in apolipoprotein E-deficient mice (Suarez-Martinez et al, 2014). A clinical study indicated that PR raised anti-inflammatory and antioxidant activities in hemodialyzed patients by reducing TNF-α, IL-6, IL-18, and malondialdehyde, c-reactive protein, NO, and protein carbonyl groups (Izquierdo et al, 2012). In our current study, PR and VIT-D^3 treatment significantly reduced TNF-α and IL-6 compared to the DX group. These effects provide additional evidence that VIT-D^3 and PR protect the myocardium by reducing proinflammatory cytokines.

The cTnT is a very sensitive and specific bio-indicator of cardiac damage (Higgins and Higgins, 2003; Bakshi et al, 2002). Increased oxidative stress and inflammation damage cell membrane structure, causing rupture (Kiyan et al, 2006). As a result of this, cTnT is released into the bloodstream. Drug-induced toxicities significantly raise cTnT levels (Higgins and Higgins, 2003). Many studies have been reported in the literature on increased cTnT levels due to DX cardiotoxicity (Peng et al, 2005). Also, studies posited that increased cTnT levels in plasma indicate the severity of DX-induced myocardial injury (Peng et al, 2005; Wakade et al, 2008). In the present study, the plasma levels of cTnT were raised in DX-induced cardiotoxicity. This result was consistent with previous studies.

The inverse correlation between cTnT and VIT-D^3 blood levels was reported in many clinical studies, where low VIT-D^3 levels were associated with raised CTnT levels (Hur et al, 2009; Michos et al, 2016). Likewise, a recent study showed that supplementation of VIT-D^3 significantly reduced cTnT in breast cancer treatment with adjuvant DX chemotherapy (El-Bassiouny et al, 2022). In addition, it was reported that in experimental models of cardiotoxicity with various drugs, VIT-D^3 and PR supplementation reduced serum cTnT (Saleh et al, 2020; Basol et al, 2019). PR and vitamin D may show anti-inflammatory activity by reducing proinflammatory cytokines in the myocardium, and this activity may explain why PR and VIT-D^3 treatment reduces DX-induced serum cTnT elevation.

NO is an essential free radical responsible for the cardiotoxic effect of DX. NO is involved in DX-related cardiotoxicity by increasing its synthesis in plasma and heart tissue (Sayed-Ahmed et al, 2001; Guerra et al, 2005). Moreover, Barnabe et al (2003) reported that pretreatment with NOS inhibitors, L-NAME and NG-monomethyl-L-arginine, could prevent DX-induced myocyte injury. Furthermore, a study reported that serum NO could indicate DXCI in rats (Guerra et al, 2005). In the present study, we also showed that the NO level increased in the heart tissue of DX-administered rats.

In one study, in a mouse triple-negative breast cancer model (TNB), the mice were administered with DX for treating the tumor and concurrently with VIT-D^3 supplements. The results of the study have shown that VIT-D^3 supplementation reduced DXCI by reducing mitochondrial damage and reactive oxygen species and did not reduce the anticancer efficacy of DX against TNB. (Lee et al, 2021). Experimental studies have shown that PR treatment reduces iNOS and NO activities in uremic rats (Suarez-Martinez et al, 2014; Finch et al, 2011). A previous study observed that PR and VIT-D^3 supplementation decreased NO levels in rats with induced nephrotoxicity with DX (Demir et al, 2020). As shown in experimental and clinical studies, these potent anti-inflammatory and antioxidant effects of PR may protect the heart by preventing myocardial damage caused by DX.

Changes in ECG after DX treatment in rats have been shown in many studies. Previous studies reported that DX-induced ECG waves abnormalities, specifically the changes in the P wave, heart rate, R amplitude, and QRS-complex, are frequently transient and cannot be marked as specific predictors of DXCI (Suzuki et al, 1999; Koti et al, 2013; Bhatt et al, 2017). However, prolonged QT interval and ST-segment elevation are considered an important biomarker for DXCI (Roden, 2004; Hazari et al, 2009). A wide QRS complex is an indicator of myocardial ischemia, cardiac insufficiency, and intraventricular conduction abnormalities (Emeka and Al-Ahmed, 2017; Hazari et al, 2009). A prolonged duration of the QT interval and raised ST segment amplitude indicate myocardial ischemia and are associated with disruption of the cell membrane structure in the heart resulting from DX-induced overproduction of ROS in the mitochondria (Parker et al, 2001; Bărcan et al, 2016).

In the present study, VIT-D^3 and PR significantly reverse all ECG abnormalities. Lee et al (2011) showed that 75% of myocardial infarction patients with ST-segment elevation had VIT-D^3 deficiency. Similarly, Separham et al (2017 showed an inverse correlation between elevated VIT-D^3 levels and ST-segment elevation in patients with myocardial infarction. Additionally, experimental studies indicated that VIT-D^3 treatment reduced the isoprenaline or DX-induced ST-segment elevation (El-Gohary et al, 2017; Aygun and Gul, 2019). Owing to the antioxidant and anti-inflammatory properties of VIT-D^3 and PR, they may have exerted a membrane-stabilizing effect on the heart.

As the ratio of systole and diastole duration decreases, the reduction in heart rate usually prolongs the QT (Ahnve, 1985; Funck-Brentano and Jaillon, 1993). NO is a potent vasodilator that lowers blood pressure (Izquierdo et al, 2012). In the presented study, NO was observed to increase in cardiac tissue. The increase in NO may have caused vasodilation, decreased heart rate, and prolonged QT interval in DX-treated rats. At the same time, the increase in NO in heart tissue may have caused damage to the cell membrane by increasing oxidative stress. Many experimental and clinical studies showed that treatment with PR and VIT-D^3 reduced NO levels (Finch et al, 2011; Izquierdo et al, 2012; Suarez-Martinez et al, 2014). In this study, treatment with VIT-D^3 and PR helped normalize the QT interval and heart rate by decreasing the NO level in the heart.

In acute myocardial infarction, technetium (Tc)-99m-pyrophosphate (PYP) accumulates in myocardial cells that are irreversibly damaged by calcium crystals or subcrystals. Pyrophosphate adsorbs these calcium crystals in the necrotic myocardium (Bonte et al, 1974). The ^{99m}Tc-PYP radiopharmaceutical scintigraphy method can provide direct visualization of myocardial infarction in animals and humans (Bonte et al, 1974). As a result, the ^{99m}Tc-PYP scintigraphy method has been considered helpful in the clinical setting for diagnosing the acute phase of myocardial infarction and in measuring infarct size (Stokely, 1976; Kawano et al, 2001). Moreover, the degree of radiopharmaceutical accumulation of ^{99m}Tc-PYP serves as an essential index of tissue damage severity (Affleck et al, 2001; Chang et al, 2001; Walker et al, 2007). Lee et al reported cardiac damage observed with the ^{99m}Tc-PYP method in rats with cardiotoxicity induced with DX, which was also compatible with histopathological data. Furthermore, many recent studies have demonstrated cardiotoxicity induced by DX and amitriptyline (an antidepressant drug) using the PYP scintigraphic method (Aygun, 2020; Basol et al, 2019). The present study indicates that the ^{99m}Tc-PYP uptake has been raised in the myocardium damaged by DX-induced necrosis. Pretreatment with PR and VIT-D^3 reduces ^{99m}Tc-PYP uptake in the heart. When evaluating both previous studies and the presented study, the use of the ^{99m}Tc-PYP scintigraphic method can be essential in evaluating drug-induced cardiotoxicity.

Conclusion

The pretreatment with VIT-D^3 and PR helped reverse doxorubicin-induced changes in the heart. They were shown to help normalize the QT interval, QRS complex time, heart rate, and ST segment, as well as help lower the TNF-α, IL-6, NO, and PYP uptake levels in the heart.

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Received June 15, 2023.
Accepted December 22, 2023.