ORIGINAL ARTICLE

POTENTIAL ROLE OF VITAMIN D3 IN AMELIORATING DOXORUBICIN INDUCED CARDIOTOXICITY IN MALE RATS

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ABSTRACT

The aim: The goal of this study was to asses if vitamin D3 protect rats against doxorubicin-induced cardiotoxicity.

Materials and methods: Overall twenty-one male rats were divided randomly into three groups (7 rats in each group). Control group in which rats received 0.9% normal saline for two weeks. Doxorubicin group (induced group): rats received 2.5mg/kg three times a week for two weeks and Vitamin D3 group (treated group): vitamin D3 was given in a dose 60000 IU/kg IP as single dose on the first day of the procedure.

Results: Doxorubicin caused cardiotoxicity as indicated by a significant elevation ($P < 0.01$) in TNF-α, IL-6, MDA, cTnI and caspase-3 level, while TAC and Bcl-2 levels significantly ($P < 0.01$) reduced in cardiac tissues of rats in the doxorubicin group as compared with control group, also doxorubicin caused histological lesions. Vitamin D3 administration show cardioprotective effect reported by significant decrease ($P > 0.01$) incTnI, Bcl2 and TAO as compared with DOX group, also show significant improvement ($P > 0.01$) in cardiomyopathy histological lesions score.

Conclusions: At their applied doses in the present study, vitamin D3 exerted a significant heart protective effect against cardiotoxicity induced by doxorubicin in rats probably by intrusive with Oxidative-stress, inflammatory response in addition to apoptotic pathway

KEY WORDS: Vitamin D3, Doxorubicin, cardiotoxicity, inflammatory markers, oxidative stress, apoptosis

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INTRODUCTION

Doxorubicin is highly effective in the management of different malignancies, but its use is limited due to their potential cardiotoxicity [1]. Cardiotoxicity is a term used to describe "toxicity that affects the heart", as it is a state when there is destruction to the heart muscle which led to cardio-toxicity. The heart may not be intelligent to force the blood throughout the body, also this description refers to a direct influence of the chemotherapy on the whole cardiovascular system, however this also due to an indirect effect of a thrombogenic status or alteration in hemodynamic flow [2]. Since Anthracyclines discovery they have become the backbone for the management of many tumors [3] due to its high antitumor efficacy so becomes an important antineoplastic agent but it's used limited because of cardiotoxicity [2]. Doxorubicin an important anthracyclines drugs, studies well-known that the amassed dose received from DOX was the chief risk factor for cardio-toxicity, DOX is associated with cardiotoxicity in 3–26 % of treated patients [4]. Many theories have been proposed to explain these mechanisms of DOX cardiotoxicity. Until now, the best widely recognized hypothesis was the cardio-toxicity caused by anthracyclines is because the reactive oxygen species (ROS) produced by the Quinone moiety corporate to all anthracyclines group [5]. These reactive oxygen species may lead to damage of mitochondrial functional, energy imbalances and apoptosis of cardiomyocyte [6]. Also inflammation consider as a mechanism of DOX induced cardio-toxicity as it produced chronic and much disparaging inflammation process that generally involves; the activation the signaling pathway of TNF, massive ROS generation in addition to insistent expression of multiple pro-inflammatory cytokines like TNF, IL-1β and IL-6 which elevated significantly following DOX administration [7]. Apoptosis is another mechanism of cardiotoxicity, as the main apoptotic way of the anticancer medications acts by the damage of the integrity of mitochondrial membrane. Sudden increase impermeability of mitochondrial membrane, which is named Mitochondrial Permeability Transition, is a vital coordination episode in the apoptotic progression. Mitochondrial Permeability Transition causes cytochrome c release from mitochondria; which then stimulates the effector caspase to encourage the DNA ladder formation [8-9]. Vitamin D3 is an endogenous element, which mainly produced in skin during the revelation to sunlight. Certain dietary supplements also entertainment as precursors for vitamin D3 synthesis [10]. Pleiotropic effects of Vit D3 made it as a prophylactic agents against cardio-toxicity mediated by DOX . One of these pleiotropic effects is antioxidant effects as Vit D3 considered as key managers of the mitochondrial respiratory function, oxidative stress and systemic inflammation. Cellular and molecular actions of the active form of vitamin D decrease oxidative stress

Table I. Effect of Cardiac Troponin I (cTnI) in three experiment groups after 2 weeks

Group	cTnI
Control	176.53±1.64 d
DOX	469.52 ± 4.47a
VIT-D3	264.03 ± 11.02 b

Table II. Effect of Vit. D3 on MDA and TAO in three experiment groups after 2 weeks

Group	MDA	TAO
Control	255.5 ± 14.30 d	5.99 ± 0.75 a
DOX	502.3 ± 21.45 a	1.74 ± 0.16 c
Vit.D3	371.4 ± 17.06 b	4.00 ± 0.25 a

Table III. Effect of Vit. D3 on TNF-a and IL-6 in three experiment groups after 2 weeks

Group	TNF-a	IL-6
Control	56.03 ± 2.47 c	72.27 ± 8.85 c
DOX	82.63 ± 8.81 a	131.70 ± 9.60 a
Vit.D3	63.04 ±2.17 b	84.37 ± 7.37 c

Table IV. Effect of Vit D3 on Caspase-3 and Bcl2 in three experiment groups after 2 weeks

Group	CASPASE-3	Bcl2
Control	18.28 ± 1.18 c	89.29±1.43 a
DOX	49.21 ± 1.19 a	31.21 ± 1.98 c
Vit.D3	30.00 ± 1.01 b	51.97 ± 1.73 b

Table V. Effect of Vit D3 on cardiomyopathy severity scores (CMY) in three experiment groups after 2 weeks

Group	cardiomyopathy severity scores (CMY)
Control	0.00 ±0.00 d
DOX	3.83 ± 0.16 a
Vit.D3	0.85 ± 0.14 c

and damage of cell and tissue [11]. Tohari et al. propose that 1, 25-(OH) 2D3 defends the human retinal pigment epithelial cell from the damage caused by oxidative stress. Also vit.D3 has anti-inflammatory effect, as numerous studies both in vitro and vivo established. Vit. D prevents the kidney tissue inflammation via reducing the production of pro-inflammatory cytokines like IF N-g γ, TNF-a, IL-6, IL-8 and IL-12 [12]. Also many studies prove that vit.D3 has anti-apoptotic effect and it can be used as cardio-protective against anthracyclines toxicity [13].

THE AIM

The goal of this study was to asses if vitamin D3 protects rats against doxorubicin-induce cardiotoxicity.

MATERIALS AND METHODS

Twenty-one rats (adult males) were equally divided into three groups (each group included 7 rats); control group received a daily of 0.9% normal saline for two weeks; DOX group (induced group) received 2.5mg/kg three time a week for two weeks by i.p. route (a cumulative dose 15 mg/kg) [14]; Vitamin D3 (Cholecalciferol) plus (DOX) group (treated group), D3 given in a dose 60000 IU/kg IP as single dose on the first day of procedure [15], and DOX given in the same way in group 2 as in DOX group. At end of the two weeks of experimental period; all rats were sacrificed. Blood sample was collected unswervingly from left ventricle of the heart. The serum was prepared for assessing indices of cardiac toxicity; cardiac troponin I (cTnI), inflammatory parameters [tumor necrosis factor-alpha, Interleukin-1 beta] as well as apoptotic markers caspase-3 and Bcl2 by ELISA. Hearts were removed, and the basal part of heart used to prepare tissue homogenate to determine the parameters of oxidative stress which [malondialdehyde (MDA) and total antioxidant capacity (TAC)], and the apical part was used for histopathological examinations.

RESULTS AND DISCUSSION

Doxorubicin administration cause cardiotoxicity manifested by significant elevation (P>0.01) in cTnI, caspase-3, TNF-α, IL-1β and MDA as compared as doxorubicin group, also there are significant decrement (P >0.01) in Bcl2 and TAO as compared as Control group, in addition to that Dox caused histological lesions also. Vitamin D3 administration decrease cardiotoxicity reported by significant decreasing (P >0.01) incTnI, caspase-3, TNF-α, IL-1β and MDA, significant elevation in (P >0.01) Bcl2 and TAO as compared as DOX group, also significant improvement (P >0.01) in cardiomyopathy histological lesions score.

The scores of cardiomyopathy (CMY) severity were classified from 0 to 4 by the histopathological examination: 0 denotes no Cardiomyopathy, 1 denotes mild Cardiomyopathy, 2 denote moderate Cardiomyopathy and 3 or more denotes sever Cardiomyopathy (P<0.01)

EFFECT OF VITAMIN D3 ON CARDIOTOXIC INDICES TROPONIN I (CTNI)

In the current study ,troponin I level was increased significantly in DOX treated rats, comparing to Control group because of DOX mediated cardiomyocytes damage, While in vit.D3 treated group, cTnI level decreased significantly as compared with DOX group so impart the cardio-protective effect of Vit.D3 in DOX treated rats [15], also there is agreement with study by [16-18] reported that Vit. D3 significantly conserved the cardiac conductivity and biochemical cardiac markers and also conserved normal cardiomyocytes architecture which indicating the hopeful cardio-protective actions of Vit D3 against DOX-induced myocardial damage.

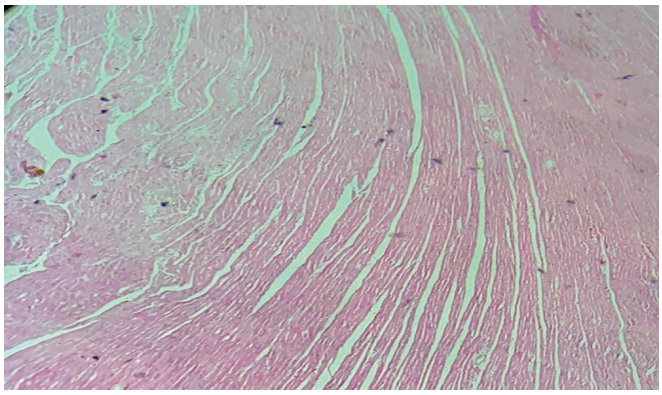

Fig 1. Group score zero damage normal histology H&E × 400

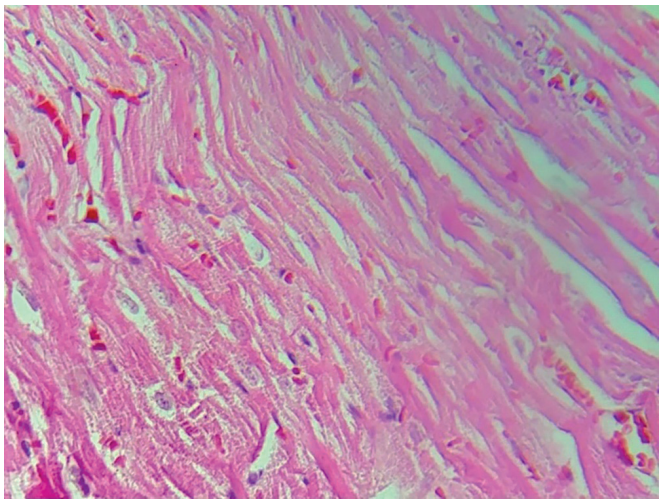

Fig 2. Vit.D3 score 1 damage mild interstitial edema and cellular swelling H&E × 400

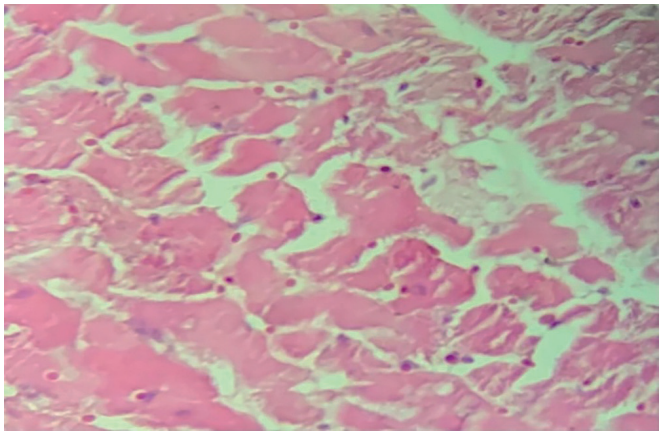

Fig 3. DOX group score 4 damage necrotic myocardial cells, cellular swelling, increased cytoplasmic eosinophilic and karryolysis H&E × 400

EFFECT OF VITAMIN D3 ON APOPTOSIS MARKERS (CASPASE-3 AND BCL2)

In Vit D3 treated group, caspase-3 level decreased significantly when comparing with DOX group. this improve the cardio protective effect of Vit D3 against DOX cardiotoxicity that substituted on the mitochondrial-dependent apoptotic, as Vit.D3 has established potent antiapoptotic action which determined by down-regulating of the markers of apoptosis(caspase-3) and up-regulation of Bcl2. There is agreement with a recent study by [18-19]. While in Bcl2 level, there is significant increment in Vit.D3 treated group, this result agree with a recent study by [18], which reported that the potent antiapoptotic properties of Vit.D3 as a main contrivance for its cardio-protective capability as demonstrated by up-regulating the antiapoptotic marker Bcl-2, while down-regulating of the apoptotic markers Bax and caspase 3 enzyme, thus maintaining mitochondrial membrane integrity.

EFFECT OF VITAMIN D3 ON MARKERS OF INFLAMMATION (TNF-A AND IL-6)

In current study, TNF-α level in Vit.D3 decrease significantly when compared to DOX group, because Vit D3 could reduce inflammation and oxidative stress , as Vit.D3 plays an important part in the inflection of inflammation and immune function via inhibit the generation of proinflammatory cytokines like TNF-a, this result agree with recent study by 13.

EFFECT OF VITAMIN D3 ON OXIDATION MARKERS (MDA AND TAO)

In the current study Vit.D3 treated group, there is significant inhibition in MDA level, the antioxidant actions of Vit D may be endorsed to the removal of excess mitochondrial ROS [19]. This result is agreement with a study by [20]. In Vit.D3 treated group, there is significant increment in TAO when compared to DOX group, due to significantly decreased lipid peroxidation ,suppress overproduction of ROS and preserve the antioxidant status of the heart tissue as indicated in reduction of MDA level and elevation of TAC level, this result agree with recent study by [20-21].

EFFECT OF VITAMIN D3 ON HISTOPATHOLOGICAL CHANGES

While the histopathological examination of DOX revealed sever cardiotoxicity according to CMY scoring due to DOX mediated apoptosis ,inflammation and oxidative stress, the histopathological examination of Vit.D3 treated group revealing an improving in the CMY score, as the degree of toxicity is mild toxicity as show cellular swelling without necrosis, disorganization nor vacuolization, there is an agreement with a recent study by [22] reported an improvement on renal histopathological changes due to Vit.D3 effect.

CONCLUSIONS

At their applied doses in the present study, vitamin D3 exerted a significant heart protective effect against cardiotoxicity induced by doxorubicin in rats probably by intrusive with Oxidative-stress, inflammatory response in addition to apoptotic pathway.

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Conflict of interest:

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