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VITAMIN D ATTENUATED DOXORUBICIN-INDUCED CARDIAC DYSFUNCTION IN ADULT RATS: ROLE OF HEAT SHOCK PROTEIN-20

By

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ABSTRACT

Background: Doxorubicin-induced cardiotoxicity is a worldwide problem. Vitamin D receptors are present in cardiac tissue suggesting its impact on cardiovascular system. Heat shock proteins (HSP) are cardioprotective molecules that can be affected by vitamin D. O**bjective**: This research aimed to investigate effect of vitamin D supplementation on the Doxorubicin-induced cardiac dysfunction highlighting the relation with heat shock protein-20. **Materials and Methods:** Sixty adult male Albino rats, divided into 4 equal groups: control, Doxorubicin-treated group (2.5 mg/kg IP, 6 doses over 3 weeks, cumulative dose: 15 mg/kg), vitamin D-supplemented group (oral gavage, 500 IU/kg daily, 5 days per week, for 3 weeks) and combined Doxorubicin-treated vitamin D-supplemented group. **Results**: Vitamin D supplemented to doxorubicin treated rats significantly increased peak tension per left ventricle (PT/LV), myocardial flow rate per left ventricle (MFR/LV), cardiac total antioxidant capacity (TAC) and heat shock protein-20. Meanwhile, plasma Brain natriuretic peptide (BNP), cardiac malonaldehyde and troponin I were significantly decreased. Heat shock protein-20 showed significant positive correlation with vitamin D, PT/LV, TAC and a significant negative

correlation with BNP, cardiac troponin I and malonaldehyde. This was accompanied by presence of viable cardiac muscle fibers with scanty fibrosis. **Conclusion:** Vitamin D partially mitigated chronic doxorubicin-induced cardiac dysfunction by increasing heat shock protein-20 levels and improving the cardiac antioxidant state.

Key words: Cardiac dysfunction, Doxorubicin, isolated heart studies, vitamin D, brain natriuretic peptide, Heat shock protein-20.

INTRODUCTION

The chemotherapeutic agent doxorubicin has been widely used. Meanwhile, its use is limited due to its life-threatening cardiotoxic effects **(Leong et al., 2017)**. Chronic Doxorubicin use for few months led to irreversible cardiomyopathy that is characterized by progressive left ventricular dysfunction and congestive heart failure **(Wang et al., 2024)**. Doxorubicin-induced cardiotoxicity is strongly linked to cellular oxidative stress through increased generation of reactive oxygen and nitrogen species, lipid peroxidation, mitochondrial dysfunction and Ca^{2+} overload with concomitant apoptosis and necrosis (**Akolkar et al., 2017).**

The association between vitamin D and cardiovascular diseases had been reported by **Gonzalez-Parra et al (2012).** Vitamin D receptors are expressed in the cardiac myocyte and fibroblast which suggest that they might affect the development of cardiac hypertrophy, fibrosis, regulate blood pressure or suppress the development of atherosclerosis **(Gardner et al., 2013)**. **Awad (2015)** reported that Vitamin D decreased the levels of cardiac injury markers and reversed the alteration in ECG pattern in acute cardiotoxicity induced by doxorubicin. However, the role played by vitamin D on the doxorubicin induced chronic cardiotoxicity is not yet clarified.

Heat shock proteins are molecular chaperones that may serve as cell defenders against intracellular and extracellular stresses (**Ullah et al., 2021**). **Marcinkowska and Gocek (2010)** reported that vitamin D receptors interact with heat shock protein 90 (Hsp90). Therefore, the aim of this study is to investigate effect of vitamin D on the Doxorubicin-induced cardiac dysfunction highlighting the relation with heat shock protein-20.

MATERIALS AND METHODS

Chemicals: *Doxorubicin* (Oncodox – 50 mg vial) was purchased from regular pharmacy, manufactured in India by CIPLA Limited, dissolved in 25 ml saline and injected IP immediately after its preparation.

Vitamin D (cholecalciferol, vidrop) was purchased from regular pharmacy, manufactured in Egypt by Medical Union Pharmaceuticals and given by oral gavage.

Experimental animals: Sixty adult male local strain albino rats weighing 80-120 grams were purchased from an experimental animal farm (Feisal, Giza) and maintained in the Medical Research Center, Ain Shams University. Rats were housed under standard conditions of boarding at room temperature (24ºC±2) with natural light/ dark cycle. Rats were fed the regular meals of standard rat chow diet with free access to water. Rats were kept for 10 days for acclimation before starting the experiment. All rats were kept in similar conditions, in cages (five rats per cage: 35x35x30 cm).

All rats received care in accordance with the National Health Guidelines and the guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Experimental protocol and procedures were approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University. Sample size was estimated using the G * Power 3.1.9.7 for windows (F test) considering alpha error 0.05 and 0.9 % power to recognize 0.7 effect size. The result indicated a minimum of 14 rats per group.

Experimental groups: Rats were initially weighed ($_i$BW) and randomly assigned into 4 equal groups:

Group 1: Control group (Control) (n=15): Rats received six doses IP saline injections over 3 weeks and 0.1 ml water by gavage 5 days/ week for 3 weeks.

Group 2: Doxorubicin-treated group (Dox) (n=15): Rats received six doses of intraperitoneal injection of doxorubicin in a dose of 2.5 mg/kg body weight (cumulative dose: 15 mg/kg) over 3 weeks (**3**) and 0.1 ml water by gavage 5 days/ week for 3 weeks.

Group 3: Vitamin D-supplemented group (Vit D) (n=15): Rats received vitamin D via oral gavage (500 IU/kg daily 5 days a week) for 3 weeks and six doses IP saline injection over 3 weeks.

Group 4: Vitamin D-supplemented, doxorubicin-treated group (VitD+Dox) (n=15): Rats received concomitant doses of vitamin D and doxorubicin, using the same doses as mentioned above.

Experimental procedures: On the day of sacrifice, 24 hours after the last injection, overnight fasted rats were weighed and then injected with heparin sodium 5000 IU/Kg B.W., IP. Thirty minutes later, the rats were anaesthetized by sodium pentobarbital (40 mg/kg, IP) and subjected to electrocardiographic (EGG) recordings (Cardimax FX-2111, Fukuda Denishi Co., Ltd. Japan). Then, abdominal aorta was cannulated to collect blood samples in plastic centrifuge tubes for further determination of brain natriuretic peptide (BNP), cardiac troponin I (cTnI), vitamin D concentration (vit D) and total calcium level (Ca). Heart was excised and perfused in Langendorff preparation to record intrinsic in vitro activity of the heart under basal conditions. The procedure was performed according to the ordinary technique of Langendorff. Tension developed by the heart was measured by an isometric force transducer ***(UGO BASILE S.R.L., Model 7004-F, Serial N. 101014, Data EVO 14543, Italy***) that was connected via a USB interface to the data capsule–Evo four channel digital recorder ***(UGO BASILE S.R.L. Biological Research Apparatus 21036, Model 17304, Serial N. O448A15, Italy)*** and to a computer provided with ***iWorx LabScribe2 ^{TM} Data Recording and Analysis Software.*** Then, heart chambers were dissected, weighed in 5-Digit-Metler balance (AE 163) and the left ventricle (LV) of each rat was stored at -80ºC for later determination of malondialdehyde (MDA), total antioxidant capacity (TAC) and heat shock protein-20 (HSP 20). A piece of left ventricle of one rat from each group was kept in formaldehyde for histological examination.

Tissue homogenization: The left ventricular tissue was homogenized by phosphate buffer solution (pH 7.4). Then, the supernatants were carefully collected after centrifugation for 20 min at 3000 rpm and stored at -80ºC for later determination of MDA, TAC and HSP 20.

During the outcome assessment, the researchers performing biochemical measurements and histological examination were unaware of the treatment each animal received.

Biochemical measurements: Elisa kits supplied by MyBioSource, USA to measure Plasma brain natriuretic peptide (BNP) (**Ku et al., 2011**), plasma cardiac troponin I (cTnI) (**Eerola et al., 2013**), Plasma vitamin D (**Zerwech,2008**), Cardiac tissue heat shock protein-20 (HSP-20) (**Christians et al., 2012**) and kits supplied by Biodiagnostic, Egypt measure cardiac tissue malondialdehyde (MDA) , Cardiac tissue total antioxidant capacity (TAC) and plasma calcium levels**.**

Cardiac tissue histopathological studies: heart tissue was fixed in 10% formalin, dehydrated through a series of graded alcohols, cleared in xylenes and embedded in paraffin. The serial sections were cut at 7µm and stained hematoxylin&eosin and masson's trichome stain for determination of fibrosis.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). The significance of differences was determined by sample t-test for paired data and by one-way ANOVA LSD & post hoc test for multiple comparisons. Correlation coefficients were calculated by linear regression analysis, R for coefficient of correlation, R^2 for coefficient of determination using the least square method using SPSS 20.0 software. P value of < 0.05 was considered statistically significant.

RESULTS

Changes in body and cardiac weights:

The final body weight (fBW) and its percentage change significantly decreased in the Dox group and in the Dox+vit D group compared to the control group, **figure (1)**.

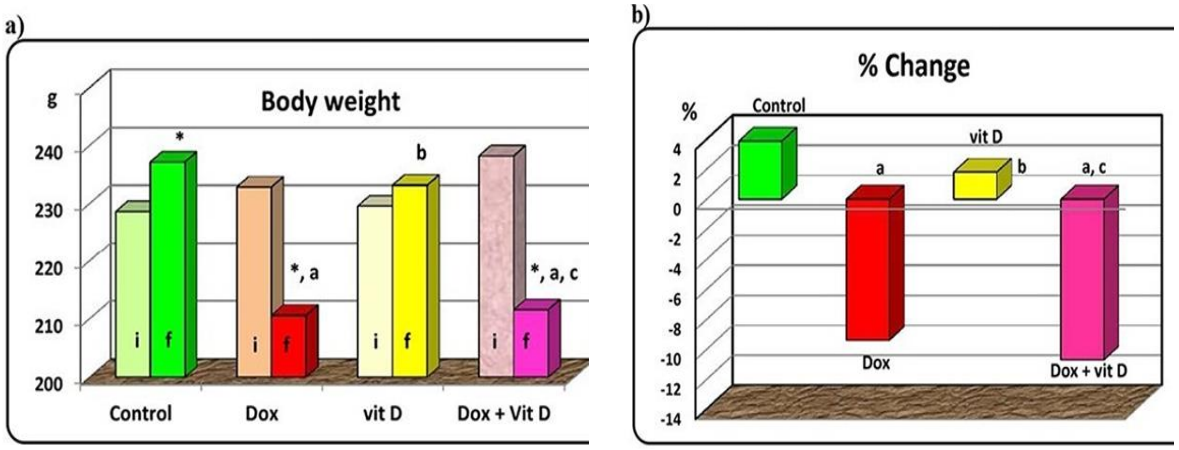

Figure1: a) Initial (i) and final (f) body weight(g) and b) % change in body weight (%) in the different studied rat groups. ***:** significance of difference between initial body weight (iBW) and final body weight(fBW) calculated by sample t test for paired data by SPSS. **a:** from the control group, **b:** from the Dox treated group, **c:** from the vitamin D supplemented group. The significance of difference was calculated by LSD of 1- way ANOVA at P< 0.05.

The atrial body weight ratio (AT/BW), right ventricular body weight ratio (RV/BW) and whole heart body weight ratio (WH/BW) showed a significant increase in the Dox group compared to the control group. Meanwhile, in the Dox+Vit D group relative cardiac weights were non-significantly changed compared to other groups, **figure**(**2**).

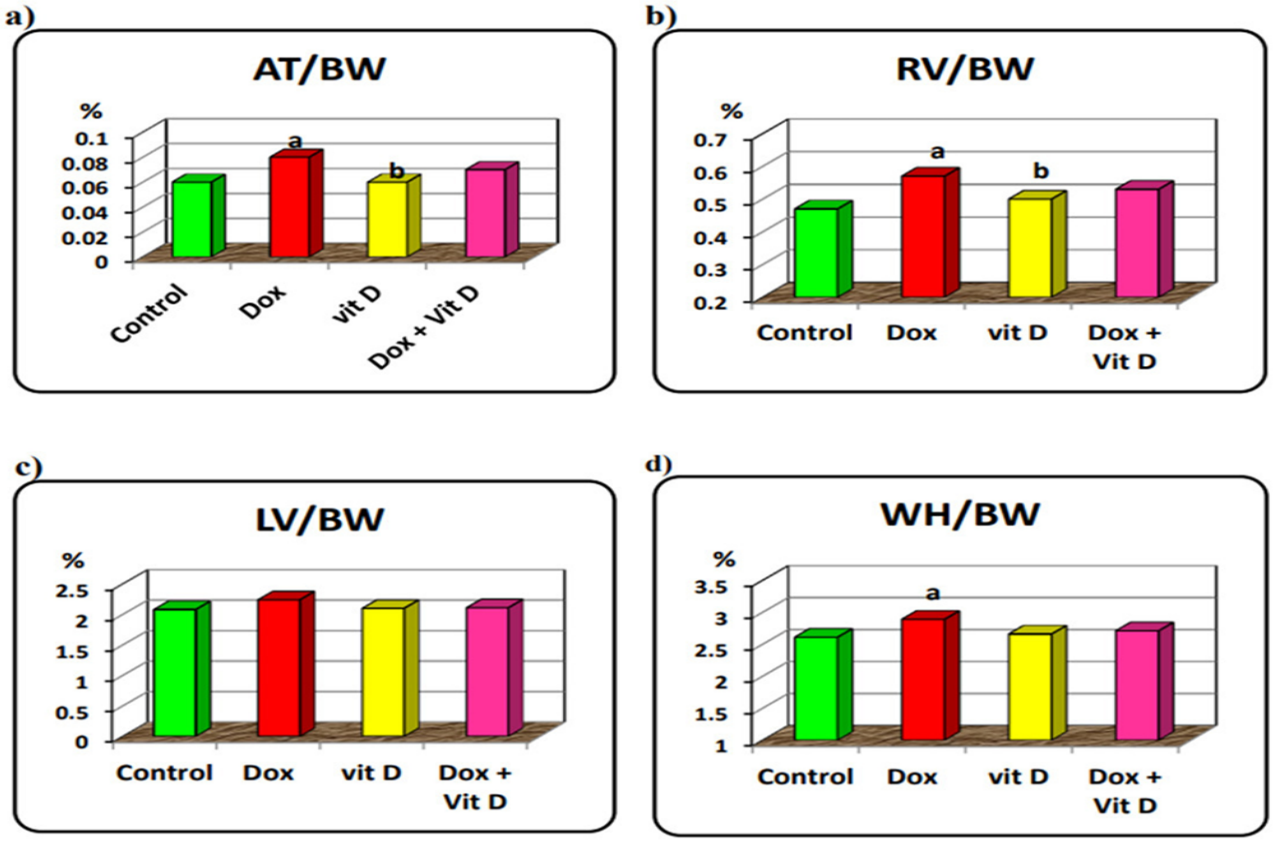

Figure 2: a) Atrial body weight ratio (AT/BW), b) Right ventricular body weight ratio (RV/BW), c) Left ventricular body weight ratio (LV/BW) and d) Whole heart body weight ratio (LV/BW) in the different studied rat groups. **a:** from the control group, **b:** from the Dox treated group. The significance of difference was calculated by LSD of 1- way ANOVA at $P< 0.05$:

ECG changes: Compared to the control group, the Dox group showed a significant prolongation in PR interval, QRS duration, observed Q-T (Q-T_O) and corrected Q-T (Q-T_c) intervals with a significant reduction in R voltage. In the Dox+Vit D group, both PR interval and QRS duration were significantly decreased compared to the Dox group and being non-significant from the control group. But, observed Q-T (Q-T_O) and corrected Q-T (Q-T_c) intervals were still significantly prolonged. (**Figure3 & figure 4**)

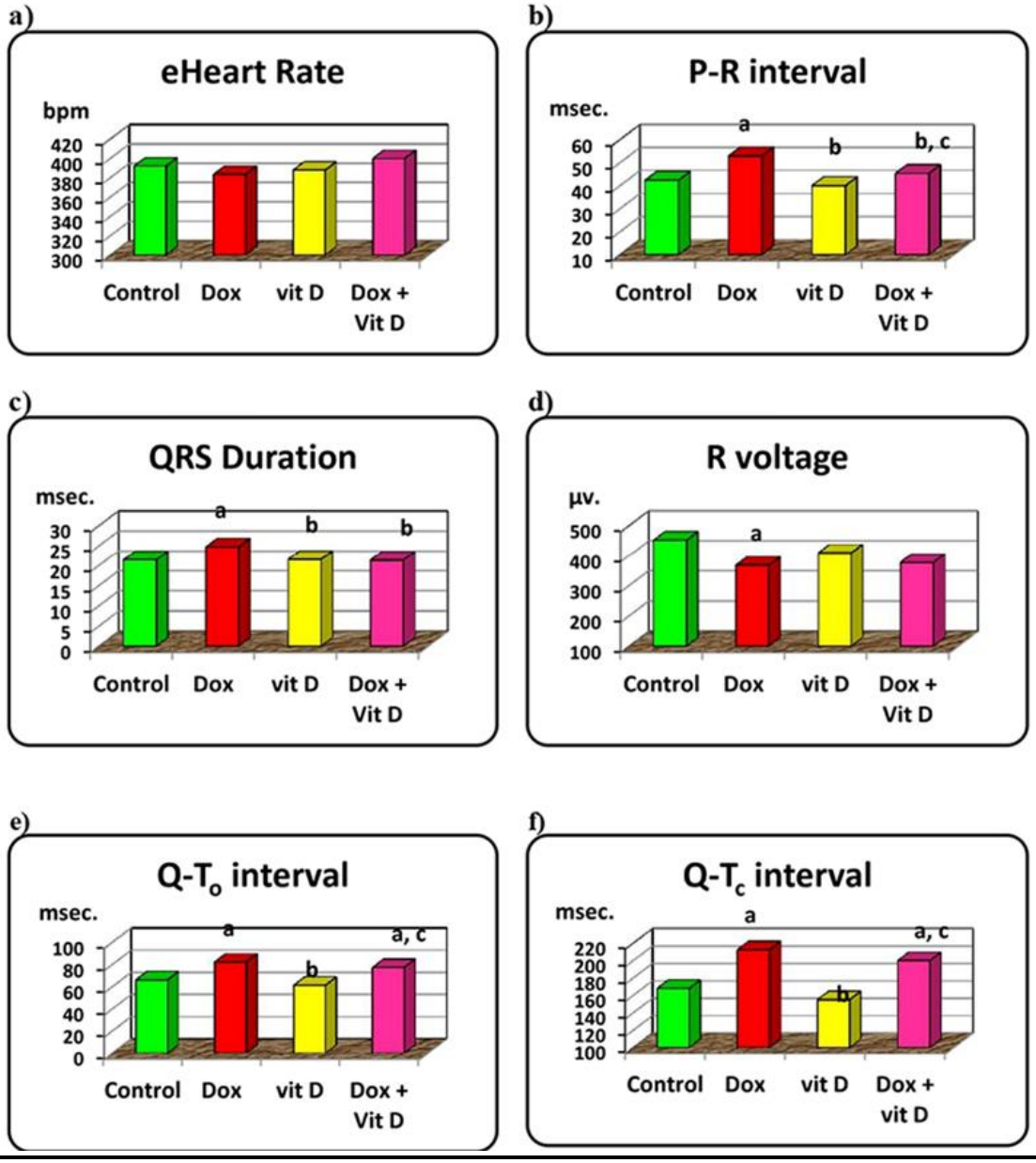

Figure 3: a) ECG recorded heart rate(bpm), b) P-R interval (msec), c) QRS duration (msec), d) R voltage(µV), e): Observed Q-T (Q-To) interval (msec) and f) Corrected Q-T (Q-Tc) interval (msec) in the different studied rat groups. . **a:** from the control group, **b:** from the Dox treated group, **c:** from the vitamin D supplemented group. The significance of difference was calculated by LSD of 1- way ANOVA at P< 0.05:

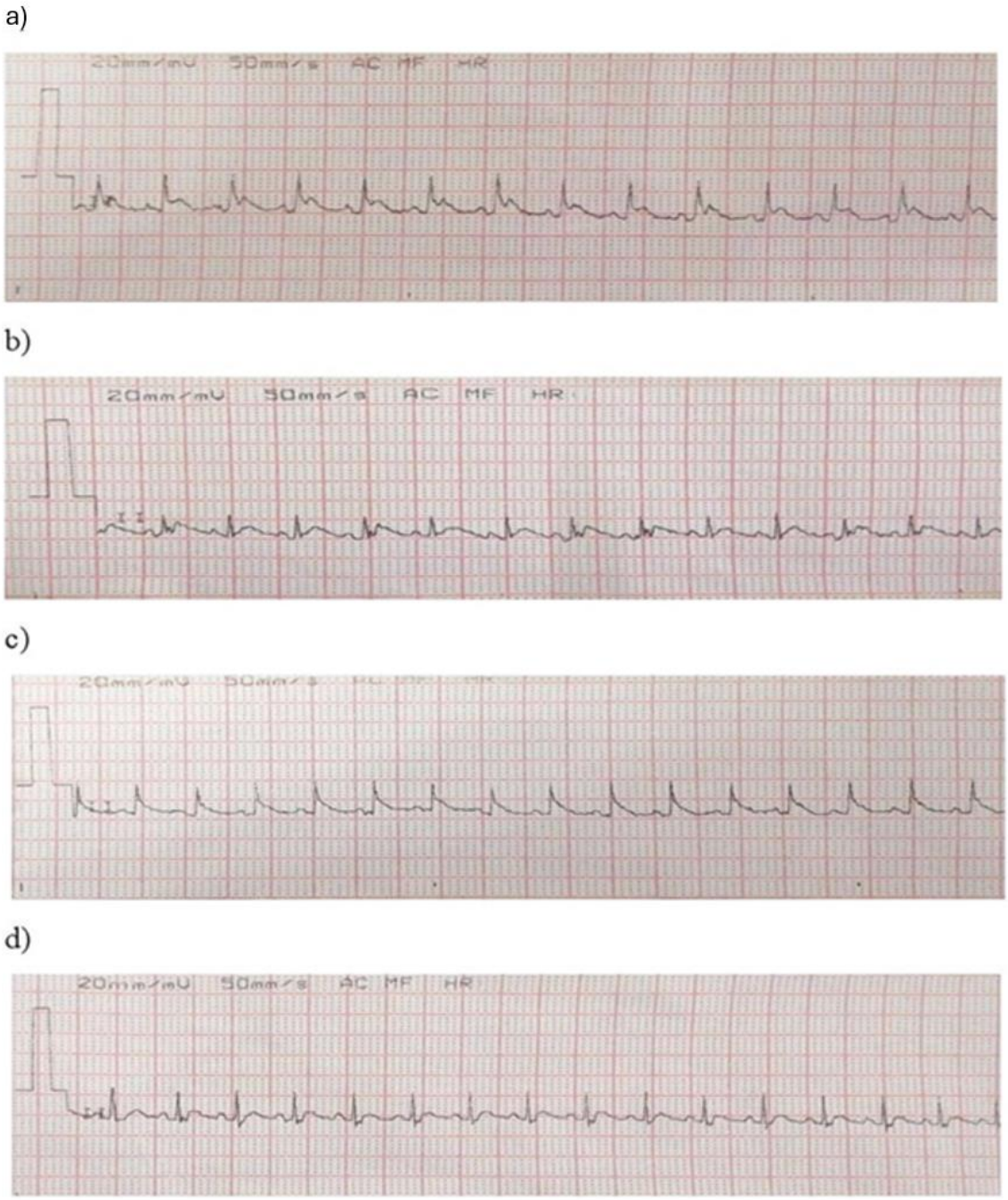

Figure 4: Tracing (1): ECG recording in a) control group, b) Dox group c) Vit D group and d) Dox+Vit D group.

Changes in isolated heart study: Compared to the control group, in the Dox group, in vitro heart rate (HR), peak developed tension (PT), peak developed tension per left ventricular weight (PT/LV) and Myocardial flow rate (MFR) were significantly decreased, also time to peak tension (TPT), half relaxation time (HRT) and the contraction time (CT) were significantly prolonged.

The Dox+Vit D group showed significant increase in PT, PT/LV, MFR and MFR/LV, non-significant increase in HR and non-significant shortening in HRT compared to the Dox group, being non-significant from the control. Meanwhile, both TPT and CT were still significantly prolonged compared to the control group **(figure 5 & figure 6)**.

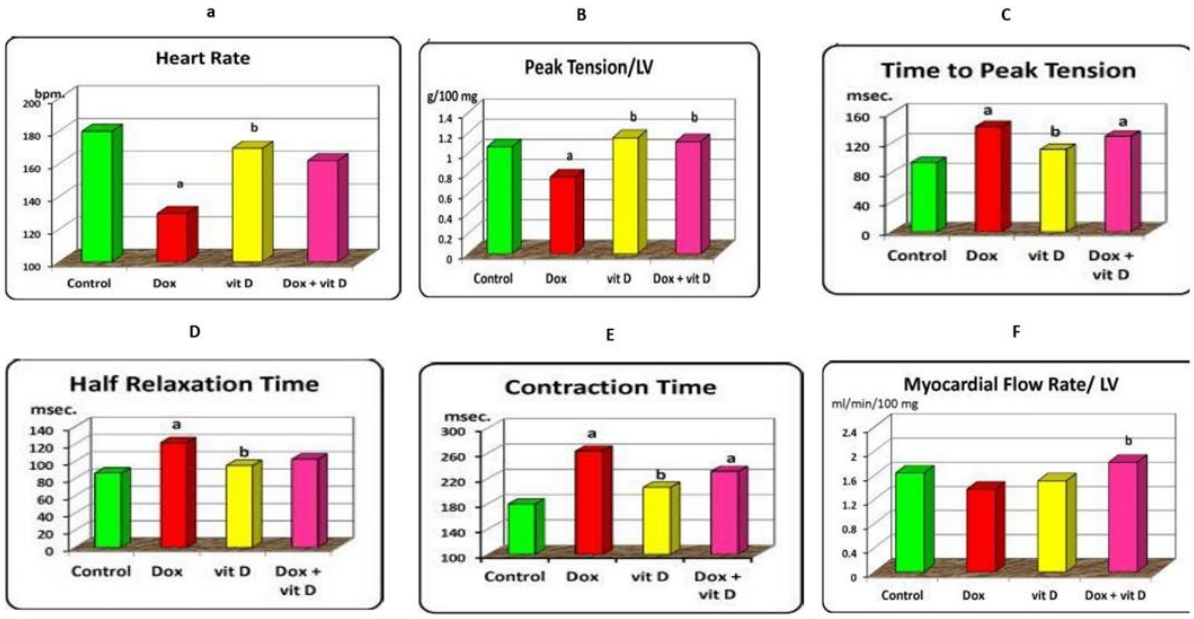

Figure 5: a) in vitro recorded heart rate(bpm) and b) peak tension per left ventricular weight (g/100mg), c) time to peak tension(msec) d) half relaxation time(msec) e) contraction time(msec) f) myocardial flow rate per left ventricle (ml/min/100mg) in the different studied rat groups. **a:** from the control group, **b:** from the Dox treated group. The significance of difference was calculated by LSD of 1- way ANOVA at $P< 0.05$:

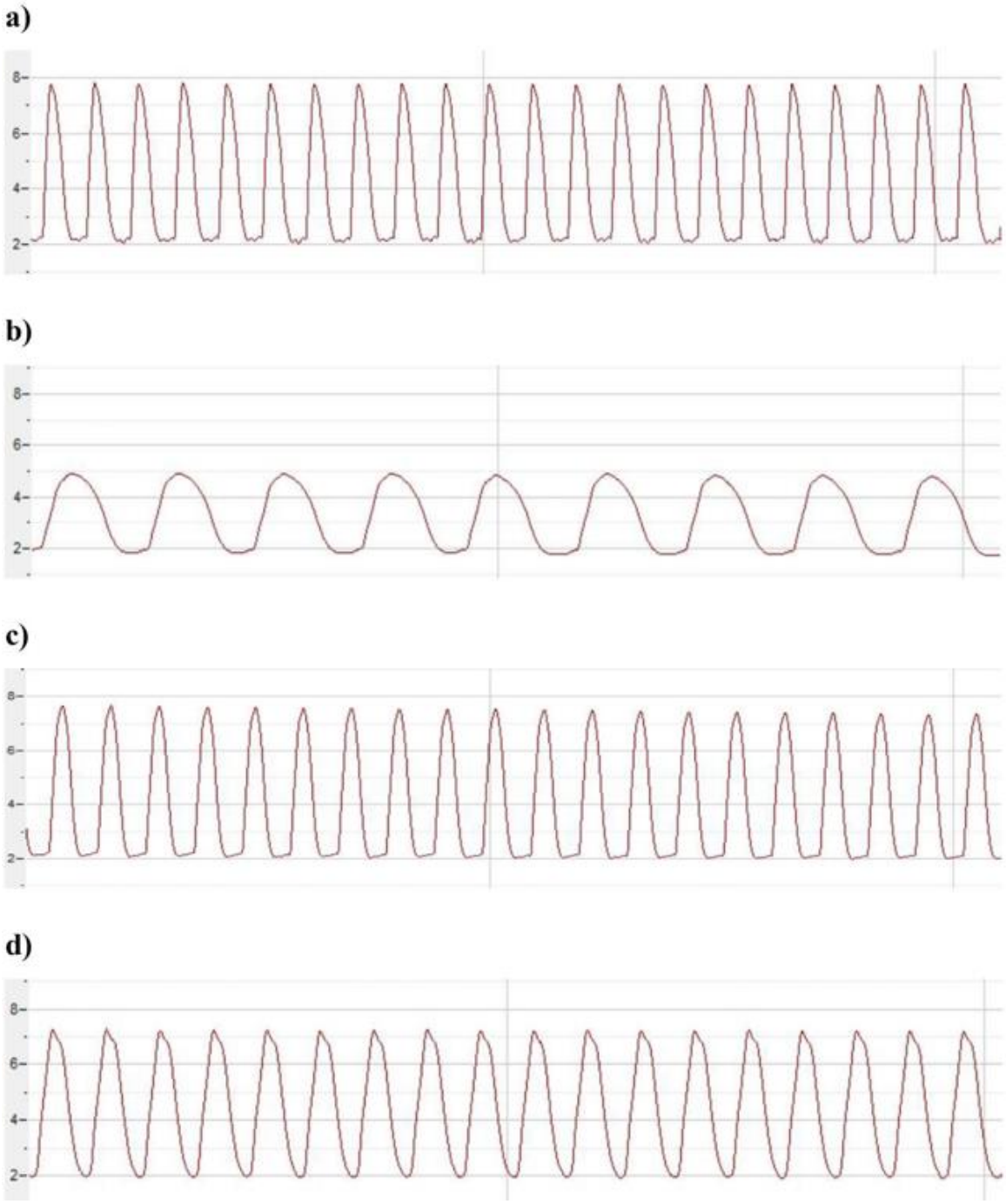

Figure 6: Tracing (2): showing the peak developed tension in a) control group, b) Dox group, c) Vit D group and d) Dox+Vit D group.

Changes in biochemical parameters: Compared to the control group, Dox group showed a significant increase in plasma brain natriuretic peptide (BNP), plasma cardiac troponin I (cTnI), cardiac tissue malondialdehyde (MDA) with a significant decrease in plasma vitamin D, total calcium, total antioxidant capacity (TAC) and the heat shock protein-20 (HSP20).

In Dox+ vit D group, compared to Doxo group, BNP, cTnI and MDA were significantly decreased while vit D, Ca level, TAC, HSP20 were significantly increased. Vit D, Ca level, MDA and TAC were all non-significant from the control group. In vitamin D treated group, Vit D and HSP20 were significantly higher than other studied groups. (**Figure 7**)

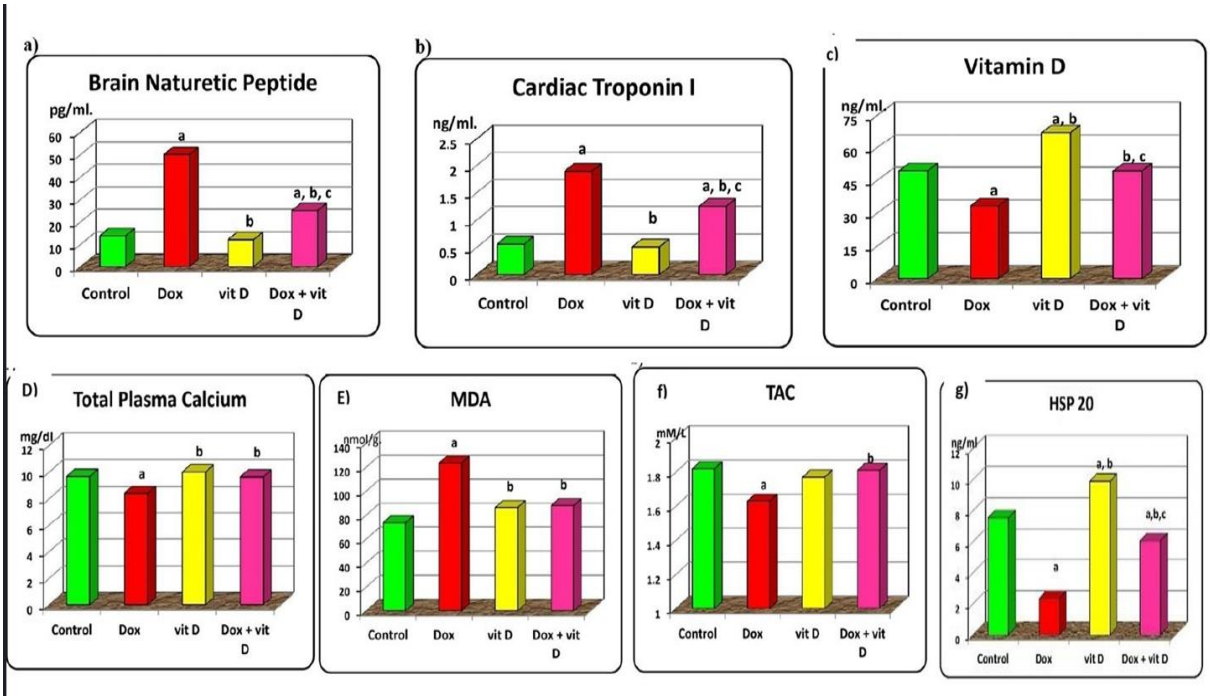

Figure 7: a) plasma levels of brain natriuretic peptide (pg/ml), cardiac troponin I (ng/ml), vitamin D (ng/ml), total calcium (mg/dl), cardiac tissue malondialdehyde (MDA, nmol/g), total antioxidant capacity of the heart (TAC, mM/L) and heat shock protein 20 (HSP20, ng/ml) in the different studied rat groups. **a:** from the control group, **b:** from the Dox treated group, **c:** from the vitamin D supplemented group. The significance of difference was calculated by LSD of 1-way ANOVA at $P< 0.05$:

CORRELATION STUDIES

As shown in **table (1),** BNP showed significant positive correlation with PR interval, QRS duration, QTc interval, TPT and HRT in addition to a significant negative correlation with PT/LV.

In Table (2): Vitamin D showed significant positive correlation with PT/LV, total antioxidant capacity (TAC), heat shock protein 20 (HSP-20), while it showed significant negative correlation with PR interval, QRS duration, QT-c interval, TPT and cardiac troponin I (cTnI)

In Table (3): Heat shock protein-20 showed significant positive correlation with peak tension per left ventricle (PT/LV), Total antioxidant capacity (TAC), PR interval while it showed significant negative correlation with QRS duration, QT-c interval, time to peak tension (TPT), half relaxation time (HRT), brain natriuretic peptide (BNP), cardiac troponin I (cTnI) and malonaldehyde (MDA)

Table (1): Correlation coefficients between Brain natriuretic peptide (BNP) and PR interval, QRS duration, QT-c interval, TPT, HRT and PT/LV

N= 60	**PR interval**	**QRS duration**	**QT-c interval**	**TPT**	**HRT**	**PR/LV**
P	<0.001	<0.01	<0.001	<0.05	<0.05	<0.001
R	+0.773	+0.433	+0.542	+0.325	+0.33	-0.554
R^2	0.597	0.187	0.294	0.106	0.109	0.307

N=number, P=significance of difference with P <0.05, R=coefficient of correlation, R^2= coefficient of determination.

Table (2): Correlation coefficients between vitamin D and PT/LV, total antioxidant capacity (TAC), heat shock protein 20 (HSP-20), PR, QRS duration, QT-C, TPT and cTnI

N=60	PT/LV	TAC	HSP20	PR interval	QRS duration	QT-c	TPT	cTnI
p	<0.01	< 0.05	<0.001	<0.05	<0.05	<0.01	<0.01	< 0.01
R	+0.5	+0.314	+0.592	-0.37	-0.386	-0.445	-0.407	-0.45
R^2	0.25	0.099	0.351	0.137	0.149	0.198	0.165	0.202

N=number, P=significance of difference with P <0.05, R=coefficient of correlation, R^2= coefficient of determination.

Table (3): Correlation coefficients between heat shock protein-20 and peak tension per left ventricle (PT/LV), Total antioxidant capacity (TAC), PR interval, QRS duration, QT-c interval, time to peak tension (TPT), half relaxation time (HRT), brain natriuretic peptide (BNP), cardiac troponin I (cTnI) and malonaldehyde (MDA)

N=60	PT/LV	TAC	PR	QRS	QT-c	TPT	HRT	BNP	cTnI	MDA
p	< 0.001	<0.05	<0.001	<0.01	< 0.001	<0.01	<0.05	<0.001	<0.001	<0.05
R	+0.588	+0.340	-0.654	-0.434	-0.647	-0.488	-0.328	-0.704	-0.722	-0.324
R^2	0.346	0.116	0.428	0.1889	0.419	0.238	0.108	0.496	0.521	0.105

N=number, P=significance of difference with P <0.05, R=coefficient of correlation, R^2= coefficient of determination.

Histopathological changes in the cardiac tissue:

In the control group: In Hematoxylin and eosin-stained sections, the cardiac wall showed intact pericardium, viable cardiac muscle fibers. Masson trichrome stain showed that the cardiac wall has normal collagen distribution around blood vessels and in between muscle fibers (endomysium). **Figure (8)**

In the Dox group: In Hematoxylin and eosin-stained sections, the cardiac wall showed shredded pericardium, markedly dilated blood vessel, markedly distorted muscle fibers and interstitial aggregates of inflammatory cells. Masson trichrome stain showed that the cardiac wall has increased collagen fibers around blood vessels and thick irregular collagen fibers in between the muscle fibers. **Figure (9)**

In the Vit D group: the cardiac wall showed intact pericardium, viable cardiac muscle fibers. Masson trichrome stain showed that the cardiac wall has normal collagen distribution around blood vessels and in between muscle fibers (endomysium) (**Figure 10**).

In the Dox+Vit D group: the cardiac wall showed some cardiac muscle fibers with indistinct cell borders, bright eosinophilic cytoplasm and mild interstitial edema. Masson trichrome stain showed that the cardiac wall has normal collagen distribution around blood vessels and some irregular collagen fibers in between the muscle fibers (**Figure 11**).

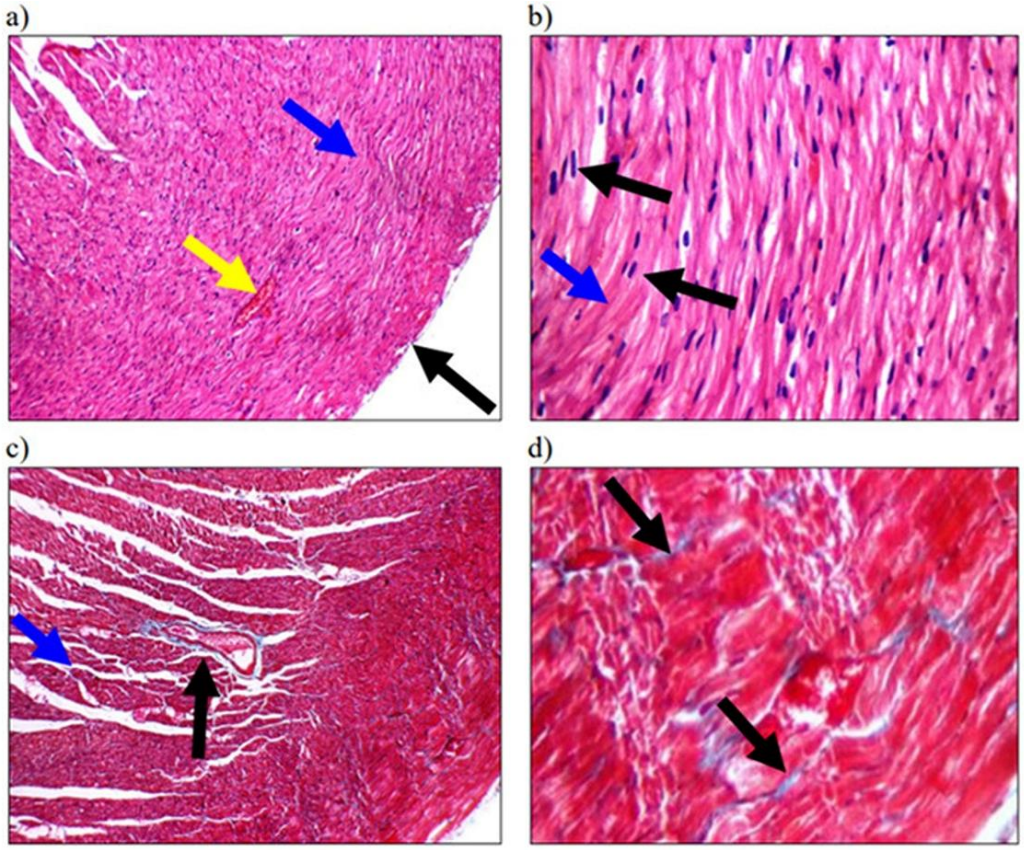

Figure 8: In the Control group: Cardiac tissue in a) H&E X 200 & b) 400, cardiac wall showing intact pericardium (black arrow), and viable cardiac muscle fibers with preserved striations (blue arrow) with scattered congested blood vessels (yellow arrow). Cardiac wall in c) Masson trichrome stain X 200& d) 400 showing normal collagen distribution around blood vessels (black arrow) and in between muscle fibers (blue arrow).

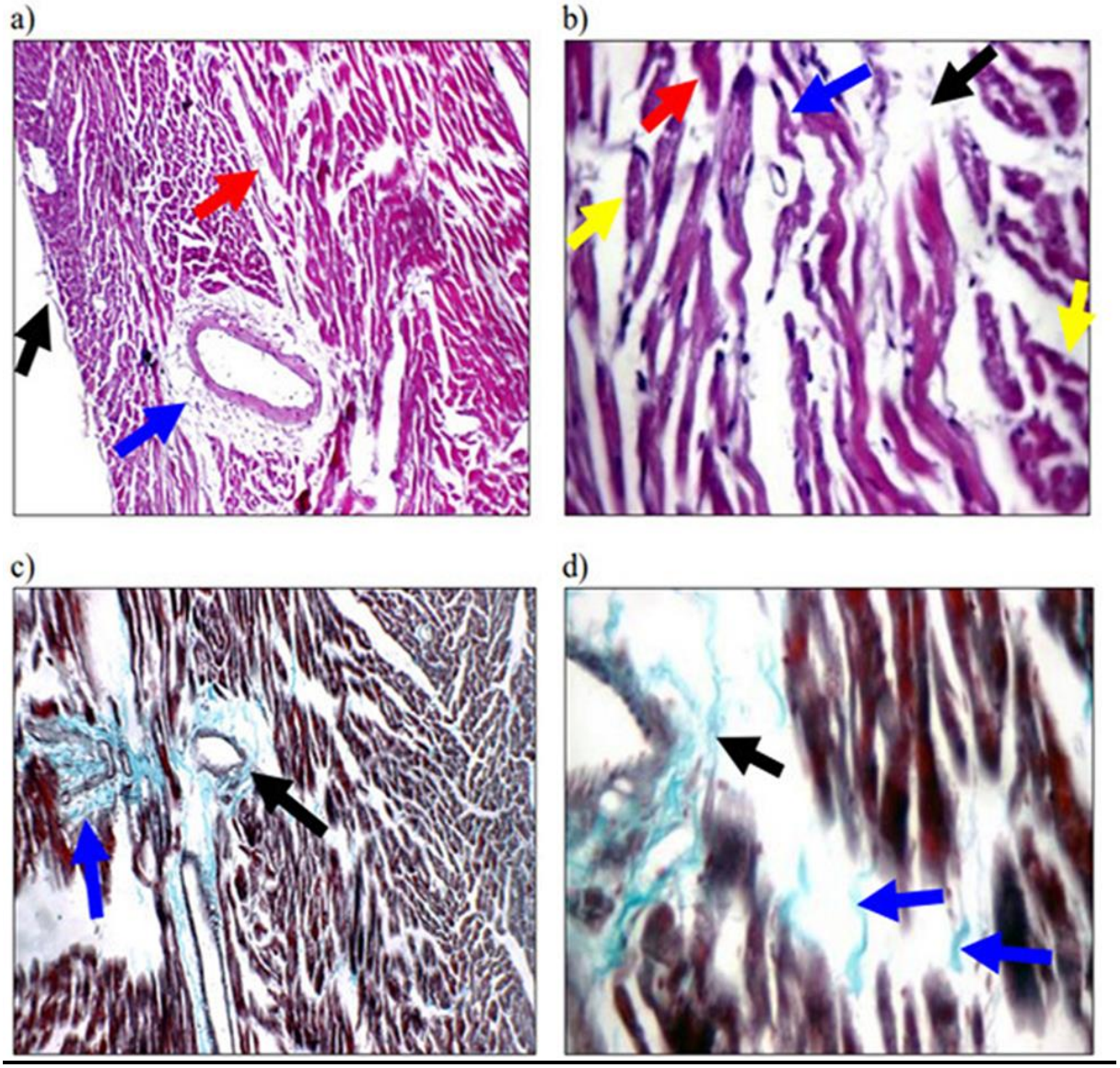

Figure 9: In Doxorubicin-treated group:

a) H&E X 200 Cardiac wall showed shredded pericardium (black arrow), markedly dilated blood vessel (BV) with marked peri-vascular (blue arrow) and interstitial edema (red arrow). In b) H&E X 400 There was marked interstitial edema (black arrow), markedly distorted muscle fibers (blue arrow), and some fibers showed indistinct cell borders with bright eosinophilic cytoplasm and small pyknotic nuclei (red arrow) and cytoplasmic vacuoles (yellow arrow). c) Masson trichrome stain X 200 and d) 400. There were normal collagen distribution around blood

vessels (black arrow) and thick irregular collagen fibers extending in the interstitium (blue arrow).

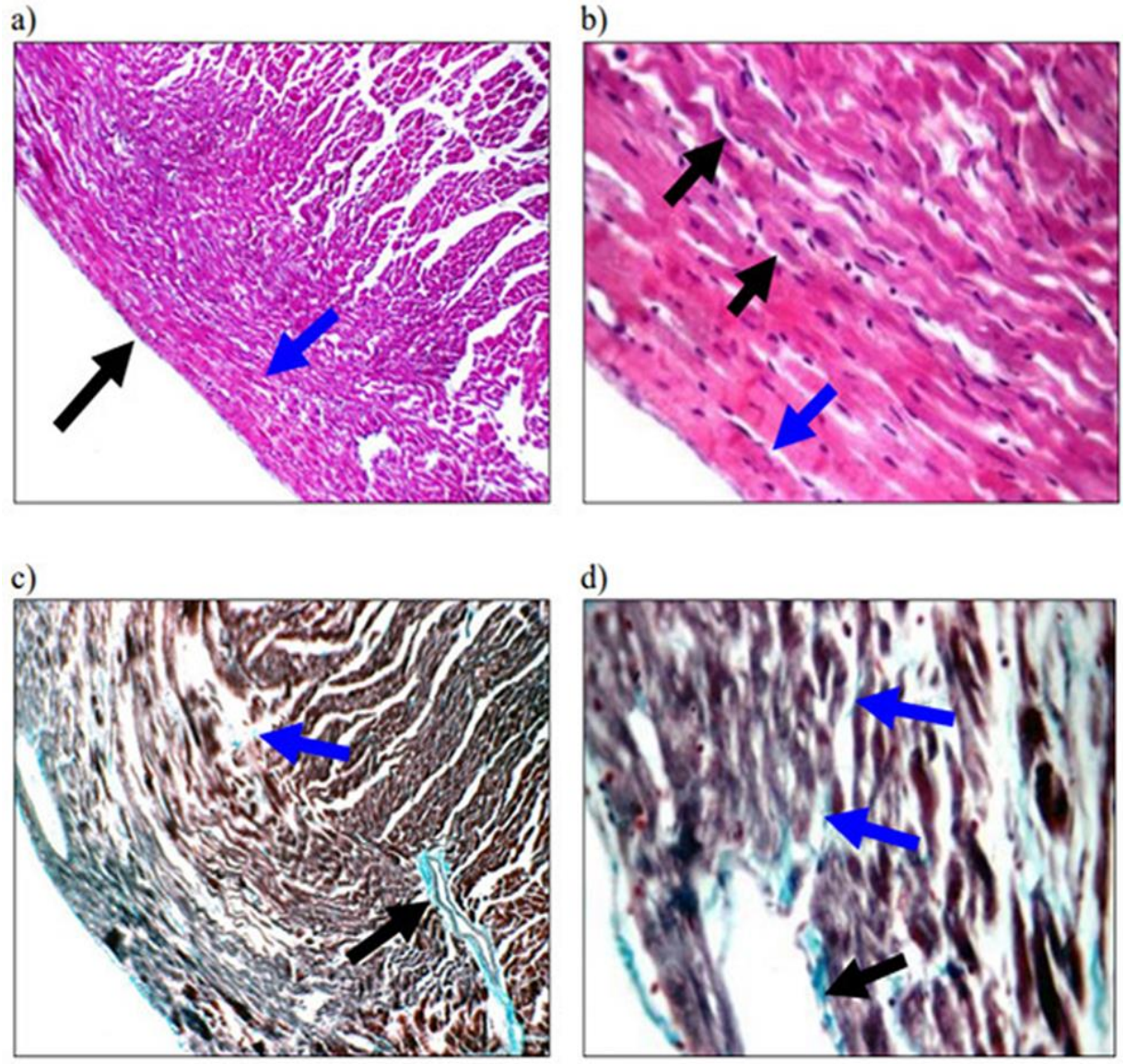

Figure 10: In Vitamin D supplemented group:

a) H&E X 200, b) 400 Cardiac wall showed intact pericardium (black arrow), and viable cardiac muscle fibers (blue arrow). C) Masson trichrome stain X 200 d) 400 showing normal collagen distribution around blood vessels (black arrow) and in between muscle fibers (blue arrow).

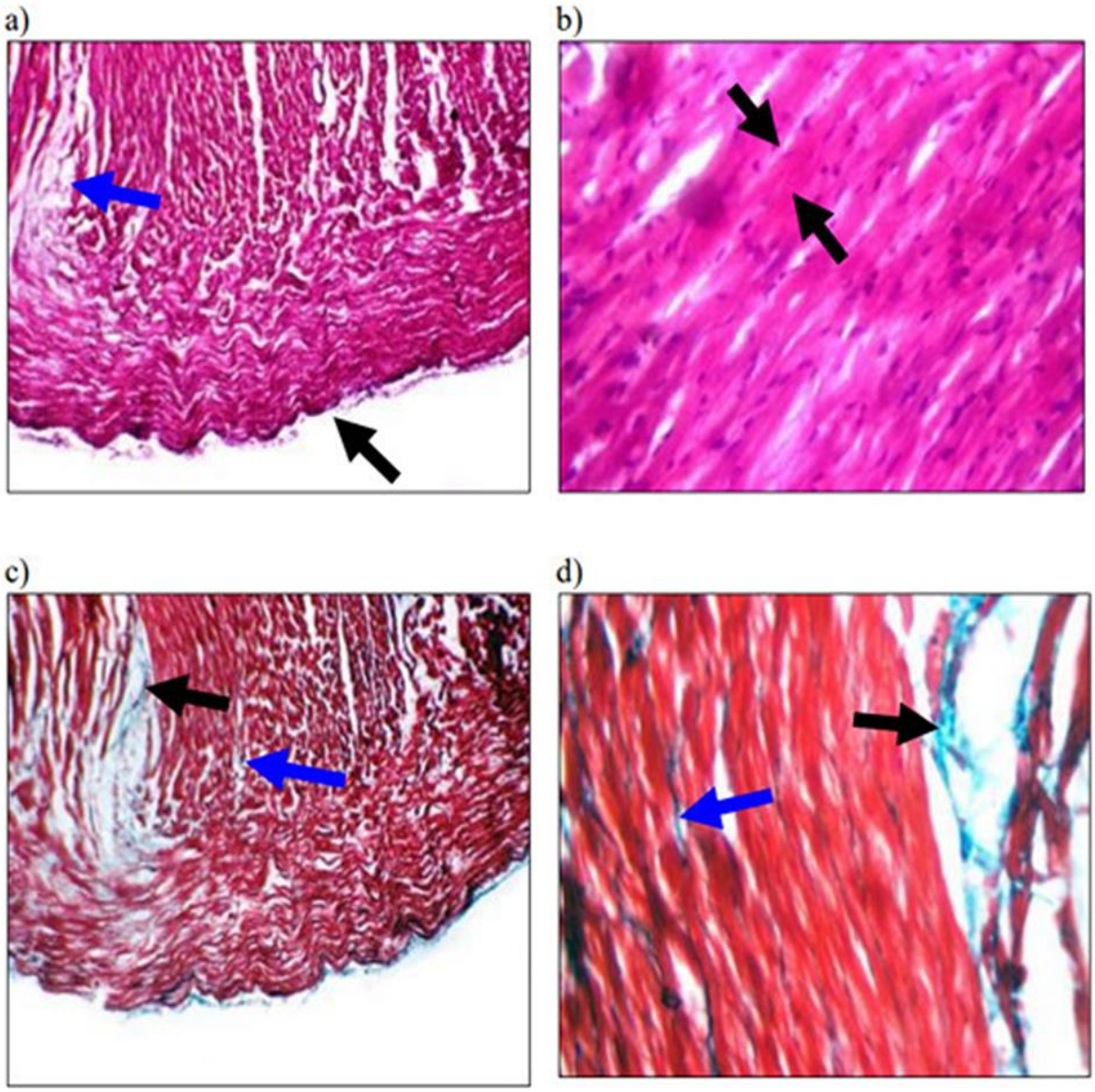

Figure 11:

In Doxo+ vitamin D group:

a) Cardiac wall showed shredded pericardium (black arrow), with mild interstitial edema (red arrow) (H&E X200).

b) Some cardiac muscle fibers with indistinct cell borders, bright eosinophilic cytoplasm and small pyknotic nuclei (black arrows) (H&E X 400).

c) Masson trichrome stain X 200 d) 400 showing cardiac wall showing normal collagen distribution around blood vessels (black arrow) and in between muscle fibers (blue arrow).

DISCUSSION

The present work illustrated that the effect of vitamin D on Doxorubicin-induced cardiotoxicity highlighting the role of heat shock protein-20. Doxorubicin reduced body weight due to its anorexic effects, damaging effect on GIT epithelium or lipogenesis impairment **(Jaćević et al., 2018)**. This reduction explained the significant increase in relative cardiac weights AT/BW, RV/BW and WH/BW in spite of the insignificant changes in absolute cardiac weights.

Vitamin D ability to increase the basal metabolic rate explain the significant reduction in final body weight in combined group and the less weight gain with vitamin D supplementation only **(Sajjadi et al., 2018).** In the combined group, the decrease in absolute whole heart weight was related to vitamin D anti-hypertrophic effect and it was paralleled with body weight reduction resulting in loss of significant increase in WH/BW. Vitamin D antihypertrophic effect was reported by **Bae et al (2011)** and goes with the associated significant decrease in brain natriuretic peptide (BNP) which is a potential marker for cardiac hypertrophy (**Sergeeva and Christoffels , 2013**) and with the decrease in collagen fibers deposition in histopathological sections. Heat shock protein-20 that was significantly increased in this group and positively correlated with vitamin D, showed a significant negative correlation with BNP pointing to its role in this effect.

In doxo group, R wave voltage depression reflects myocardial cell apoptosis. P-R interval and QRS duration prolongation denotes slow atrioventricular conduction and prolonged ventricular depolarization. This can be attributed to doxorubicin-induced myocardium remodeling and fibrosis and the subsequent sodium channel remodeling **(Gintant et al., 2011).** This postulation was evidenced by significant positive correlation between ECG parameters and both BNP and cTnI and by the presence of thick irregular collagen fibers between the distorted muscle fibers.

Myocardium remodeling can be linked to the significant decrease in plasma vitamin D due to dox induced GIT malabsorption or due to deficient activation owing to the Dox induced oxidative stress organ damage **(He et al., 2020).** Hypocalcemia secondary to vitamin D deficiency can lead to prolonged Q-T_c**.**

Vitamin D corrected the R wave voltage depression owing to its cardioprotective anti-apoptotic effect and significantly shortened P-R interval and QRS duration due to the attenuated cardiac remodeling. This attenuation could be related to the improved oxidative stress state. The associated Dox oxidative stress may induce cardiac hypertrophic remodeling by activating renin angiotensin system (RAS) and promoting calcium overload (**Mongirdienė et al., 2022**). Both vitamin D and heat shock protein-20 showed a significant negative correlation with P-R interval and QRS duration and were significantly positively correlated with total antioxidant capacity.

Insignificant changes in the in vivo recorded heart rate reflects intact normal nervous control, meanwhile Dox depressed the in vitro heart rate due to the reduction in SAN excitability or after doxorubicin arrhythmogenic remodeling and SAN damage (**Hartupee and Mann, 2017).** Vitamin D improved bradycardia due to its potential inhibitory effect on Na^+/K^+ -ATPase and modulation of L-type Ca^{2+} channels or by suppressing RAS and remodeling **(Majeed et al., 2016)**.

Doxorubicin depressed cardiac inotropy and deteriorated systolic and diastolic functions which was evidenced by the significant depression in PT/LV with TPT and HRT prolongation. BNP and cTnI were negatively correlated with PT/LV and positively correlated with TPT & HRT. Doxorubicin altered the oxidative state that was evidenced by a significant increase in MDA and decrease in TAC. Cardiac troponin I showed significant positive correlation with MDA, and a negative one with TAC. There was a significant negative correlation between MDA and PT/LV. This denotes the involvement of doxorubicin-induced oxidative stress and mitochondrial dysfunction in cardiac dysfunction. Doxorubicin-induced oxidative stress increases mitochondrial calcium overload and opens the mitochondrial permeability transition pore (mPTP) releasing the apoptotic factor cytochrome C. Systolic dysfunction was a result of Doxo-induced T-tubular and L-type calcium channels damage or from myofibrillar proteins oxidative modifications**.** Diastolic dysfunction can be related to diastolic calcium overload by either suppressing sarcoplasmic reticulum Ca^{2+}-ATPase (SERCAII), up-regulating phospholamban (PLB) expression or ROS generation **(He et al., 2020)**. Moreover,

Doxorubicin induced cardiotoxicity could be related to the significant reduction in the heat shock protein-20 (HSP-20), being a cardioprotective molecular chaperon that was significantly positively correlated with PT & PT/LV and negatively correlated with PR interval, QRS duration, Q-T interval, TPT, HRT, CT, BNP and cTnI.

Vitamin D improved cardiac contractility that was evidenced by the significant increase in PT/LV being non-significant from the control and the significant decrease in BNP and cTnI, added to significant negative correlation of vitamin D with cardiac troponin I and TPT. Cardiac systolic function and lusitropy were partially improved with HRT insignificant from both doxo and control groups. This can be explained by vitamin D antioxidant effects as it significantly decreased plasma MDA and increased TAC or its anti-apoptotic effects supported by less cardiac damage in histopathological study. Also, vitamin D increased plasma calcium by activating its intestinal absorption which may increase systolic intracellular calcium **(Bikle, 2017).**

Further, vitamin D exerted cardio-protection through the significant increase in cardiac HSP-20, and the positive correlation between them designates vitamin D as HSP inducer. HSP-20 showed significant positive correlations with PT & PT/LV and significant negative correlations with PR interval, QRS duration, Q-T interval, TPT, HRT, CT, BNP and cTnI. HSP-20 antioxidant, anti-inflammatory and anti-apoptotic actions goes with the significant positive correlation between cardiac HSP-20 & TAC, and the significant negative one with MDA.

Doxorubicin-induced oxidative stress leads to endothelial dysfunction which is aggravated by vitamin D deficiency explaining the significant reduction in MFR **(He et al., 2020).** MFR/LV was insignificantly reduced due to parallel decrease in left ventricular mass detected in the Dox group.

Vitamin D improved coronary blood flow with a significant increase in MFR/LV. This could be related to vitamin D antioxidant, anti-atherosclerotic effects and to its ability to upregulate endothelial NO synthase **(29)**. Further, the associated increase in HSP-20 may increase MFR/LV owing to its ability to combat doxorubicin oxidative stress and the related endothelial dysfunction) and its role in activating e NOS, thus increasing NO production (**Fan and Kranias, 2011).**

Conclusion:

Vitamin D attenuated chronic cardiotoxicity of Dox via improving oxidative state and induction of cardiac HSP-20. Few literatures were found describing the relationship between HSP-20, vitamin D and Dox-induced cardiomyopathy. Further studies are needed to examine the Hsp-20 efficacy in the treatment of heart disease and to add natural agents that can induce Hsp-20.

Declarations:

Ethics approval:

Experimental protocol and procedures were approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University. All rats received care in accordance with the National Health Guidelines and the guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Consent for publication:

All authors read and approved the final manuscript to be published.

Data Availability Statement: The data supporting the conclusion of this study are available in this manuscript.

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Authors contribution:

Diab F designed the study. Khaled Y, Nassef F and Said M performed the experimental procedure. All authors analyzed and interpreted the data, contributed to the writing of the manuscript and read , approved the final manuscript.

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إعطاء فيتامين (د) يخفف من الخلل الوظيفي في عضلة القلب الناجم عن الدوكسوروبيسين فى الجرذان البالغة: دور بروتين الصدمة الحرارية ـ ٢٠

فاتن محمود على دياب, نهى عبد العزيز ناصف, ياسمين محمد خالد أمين , منـال سعيـد عبد الحميـد

قسم الفسيولوجيا الطبية-طب عين شمس

خلفية البحث: يعد تسمم القلب الناجم عن الدوكسوروبيسين هو مشكلة عالمية. توجد مستقبلات فيتامين د في أنسجة القلب مما يشير إلى تأثيره على نظام القلب والأوعية الدموية. أيضا" بروتينات الصدمة الحرارية (HSP) وهي جزيئات وقائية للقلب يمكن أن تتأثر بفيتامين د.

الهدف من البحث: دراسة تأثير مكملات فيتامين د على الخلل الوظيفي القلبي الناجم عن الدوكسوروبيسين وتسليط الضوء على العلاقة مع بروتين الصدمة الحرارية 20.

مواد وطرق البحث: أجريت هذه الدراسة على ستين من اناث الجرذان مقسمة الى أربعة مجموعات: مجموعة ضابطة , مجموعة معالجة بالدوكسوروبيسين ٢ و نصف ميلي غرام لكل كيلوغرام من وزن الجسم، 6 جرعات على مدى 3 أسابيع (الجرعة التراكمية: 15 ميلي غرام لكل كيلوغرام)، المجموعة المكملة بفيتامين د: تغذية فموية، 500 وحدة دولية/كغ يومياً، 5 أيام في الأسبوع، لمدة 3 أسابيع، مجموعة مكملة بفيتامين د المعالجة بالدوكسوروبيسين. وقد تم تقييم الاوزان في بداية ونهاية البحث لحساب كتلة الجسم. في نهاية التجربة تم تسجيل الرسم الكهربائي لعضلة القلب وتسجيل وظائف القلب بجهاز لانجندورف و قياس اوزان القلب و الفحص النسيجي لعضلة القلب.

نتائج البحث: استطاع فيتامين د الذي تم اعطاؤه للجرذان المعالجة بالدوكسوروبيسين ان يؤدي إلى زيادة كبيرة في ذروة التوتر لكل البطين الأيسر (PT/LV)، ومعدل تدفق عضلة القلب لكل البطين الأيسر (MFR/LV)، والقدرة الكلية لمضادات الأكسدة القلبية (TAC) وبروتين الصدمة الحرارية -20. وفي الوقت نفسه، انخفض الببتيد الناتريوتريك في بلازما الدماغ (BNP)، والمالونالدهيد القلبي والتروبونين بشكل ملحوظ. أظهر بروتين الصدمة الحرارية 20 ارتباطًا إيجابيًا كبيرًا مع فيتامين D وPT/LV وTAC وارتباطًا سلبيًا كبيرًا مع BNP والتروبونين القلبي I والمالونالدهيد. وقد صاحب ظهور عضلات القلب في فحص الانسجة بطريقة منتظمة وانخفاض في ظهور التلييف.

الاستنتاج: فيتامين (د) يخفف جزئيا من خلل القلب المزمن الناجم عن الدوكسوروبيسين عن طريق زيادة مستويات بروتين الصدمة الحرارية 20 وتحسين حالة مضادات الأكسدة القلبية.

الكلمات الدالة: خلل وظيفي في القلب -عقار دوكسوروبيسين -دراسات القلب المعزولة- فيتامين د- الببتيد الدماغي الناتريوتريكBNP -بروتين الصدمة الحرارية -20.