European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 02, 2021

Renal Protective Effect Of Vitamin D3 In Isoproterenol-Induced Myocardial Infarction In Rats

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ABSTRACT: Acute kidney injury is a common complication of myocardial infarction (MI) and scarce data were available on the effect of vitamin D3 on heart and kidney functions in MI. In this study, we investigated the potential protective effect of vitamin D3 on cardiorenal functions in isoproterenol-induced MI in rats and the possible mechanisms involved. It was shown that in MI rats, there was a significant increase in serum levels of (creatine kinase myoglobin binding, lactate dehydrogenase, creatinine, malondialdehyde, interlukin-6 and tumor necrosis factor alpha) and urine levels of (total protein and albumin), with a significant decrease in urine creatinine level, creatinine clearance and serum levels of [reduced glutathione and 1, 25 dihydroxy vitamin D] in comparison with the control rats. On treatment of rats with vitamin D3 prior to induction of MI, these changes were significantly improved in comparison with the MI rats. Histopathological and immunohistochemical examinations of heart and kidney in MI rats reflected the deterioration in their structures with presence of marked apoptosis which were ameliorated on treatment of rats with vitamin D3 prior to induction of MI. In conclusion, vitamin D3 has a protective effect on heart and kidney functions in the rat model of myocardial infarction and this beneficial effect could be related to its anti-inflammatory, antioxidant and anti-apoptoticactions.

Keywords: Myocardial infarction, Vitamin D3, Acute kidney injury, Isoproterenol,Apoptosis.

1. INTRODUCTION:

Acute myocardial infarction (AMI), commonly known as a heart attack, is one of the common acute diseases which is characterized by ischemic injury and necrosis of the cardiac muscle**[23].**

Isoproterenol)ISO), β-adrenergic agonist, induces myocardial infarction in rat models when it is used in overdose that exhibits many metabolic and morphological aberrations in the heart tissue of rats similar to those seen in humans with myocardial infarction **[56]** through causing overexcitation of cardiac β- adrenergic receptors and overload of calcium in myocardial cells that results in increasing heart rate, cardiac contractility and myocardial oxygenconsumption **[28]** causing myocardial ischemia that increases the release of free radicals which cause lipid peroxidation of cellular membrane that leads to destruction of cell membrane or even cell death **[14].**

Acute kidney injury (AKI) is a term that has replaced the concept of acute renal failure which was defined as an abrupt decrease in kidney function and included both injury (structural damage) and impairment (loss of function) **[61].** Mansfield et al. **[33]** stated that AKI is a common complication which increases days of hospitalization and mortality rates in AMI. Also, de Almeida et al. **[10]** reported that impairment of cardiac function caused renal damage. Vitamin D is a fat- soluble vitamin, obtained from sun exposure, food, and supplements **[5].** It promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone [46]. Also, vitamin D has many other roles in the body, including modulation of cell growth, neuromuscular and immune function**[26].**

Expression of vitamin D receptors is widely spread in tissues and cells such as heart, kidney, immune cells, brain and muscle **[1].** 1,25 dihydroxy vitamin D (1, $25(OH)_2D$) is the active form of vitamin D which was found to be significantly decreased in patients with AKI compared to hospitalized patients without AKI **[2]**, but the previous studies on the effect of vitamin D3 on kidney function in myocardial infarction (MI) were few and still its action in such case is unclear. Thus, this study was performed to clarify the changes that occurred in heart and kidney functions in a rat model of MI, and to assess the potential protective effects of vitamin D3 on heart and kidney functions in such rat model and the possible mechanisms involved.

2. MATERIALS AND METHODS

Twenty-seven healthy, adult male albino rats of local strains weighing 100- 150 g, were obtained from the Animals house, Faculty of Veterinary Medicine, Zagazig University. The animals were kept under hygienic conditions in steel wire cages (50x30x20 cm), 4-5 rats per cage, on ordinary diet obtained from Zagazig College of Agriculture. All animals had free access to water and were kept at a comfortable temperature (20 to 24 ºC) and were maintained on normal light-dark cycle. The rats were accommodated to animal house conditions for 2 weeks before the experiments. The experimental protocol was approved by Physiology Department Committee and the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC). Approval number is ZU-IACUC/3/F/67/2018.

Experimental Design: Rats were subdivided equally and randomly into control, myocardial infarction (MI) and MI pre-treated with vitamin D3 groups. In the control group, each rat was injected intraperitoneally with 0.1 ml castor oil once daily for seven days, while injected subcutaneously once daily with 2 ml normal saline on the sixth and seventh days. Regarding MI group, each rat was injected intraperitoneally with 0.1 ml castor oil once daily for seven days, while injected subcutaneously once daily with isoproterenol hydrochloride (at a dose of 150 mg/kg body weight) **[44]**, dissolved in 2 ml of normal saline on the sixth and seventh days to induce MI. ISO was purchased from Sigma-Aldrich, St Louis, USA (catalog No. I6504). In the third group (MI pretreated with vitamin D3), each rat was treated intraperitoneally with 0.1 ml vitamin D3 (at a dose of 0.5 µg/kg body weight) **[22]**, diluted in castor oil once daily for seventh days, while injected subcutaneously once daily with isoproterenol hydrochloride (at a dose of 150 mg/kg body weight) dissolved in 2 ml of normal saline on the sixth and seventh days to induce MI. Vitamin D3 was purchased from Memphis Co. for Pharm. & Chem. Ind in the form of [Devarol-S 200000 IU (5000 µg)/2mlampoule].

Table-1: Experimental Design

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`	Control	MI	MI pretreated with vitamin D3
Number of rats	9	9	9
Duration	7 days	7 days	7 days
Ordinary diet	□	□	□
Free access to water	□	□	□
Castor oil i.p. 0.1 ml/rat/day along the 7 days	□	□	
ISO s.c. 150 mg/kg/day on the 6th and 7th days		□	□
Vitamin D3i.p.0.5 g/kg/day along the 7days			□
Normal saline s.c. 2.0 ml/rat/day on the 6th and 7th days	□		

MI, myocardial infarction; i.p., intraperitoneal; ISO, isoproterenol hydrochloride; S.C., subcutaneous.

Electrocardiogram (ECG) recording was done using invasive ECG monitor and recording results in power Lab 4/20 (data acquisition system), AD Instruments Pty Ltd, Australia, one hr after the 2nd dose of ISO injection. Each rat was placed on a suitable not electrically conductive flat surface, after being anesthetized with intraperitoneal sodium thiopental 60 mg/kg body weight **[4]**. Three bipolar leads were used to record ECG, positive, negative, and reference ECG electrodes were placed at the left foreleg, right foreleg, and left thigh, respectively **[59]**. Calibration of the voltage (millivolts) was performed, and the results were automatically calculated based on the calibration value.

In the 7th day of the study, 24 h urine collection was done. In metabolic cages, rats were housed individually with free access to water. At the bottom of the metabolic cages, funnels of suitable sizes, were arranged for collection of urine, with perforated plastic discs in the funnels to retain fecal matter **[11]**. The 24 h urine sample was collected for each rat in a beaker that was arranged at the bottom of the funnel, and centrifuged at 3000 revolutions per minute (rpm) for 10 minutes **[32]**. The supernatants were transferred into another set of clean and dry tubes and stored at -20°C until analysis.

At the end of the experiment, blood samples were collected under sodium thiopental anesthesia (60mg/kg body weight i.p.) from the retro-orbital plexus using microhematocrit tubes **[12]**, allowed to clot for 30 minutes at room temperature and then, centrifuged at 3000 revolutions per minute (rpm) for 15 minutes. Using automatic pipettes, the clean, clear sera were separated and stored in Eppendorf tubes at -20˚C until analysis**[38]**.

Chemical analysis was performed at Biochemistry Department, Faculty of Medicine, Zagazig University. For estimation of serum 1,25 dihydroxy vitamin D [1, $25(OH)_2D$], commercial kits were purchased from BioVendor.com (catalog No. RIS021R). Also, commercial kits were purchased from Sigma-Aldrich, St Louis, USA. for estimation of; serum and urine creatinine (catalog No. MAK080), and serum tumor necrosis factor-alpha (TNF-) (catalog No. MBS355371). Moreover, from Biodiagnostic-Egypt, kits for estimation of serum reduced glutathione (GSH) (catalog No. MBS724319), malondialdehyde (MDA) (catalog No. MBS738685), interleukin-6 (IL-6) (catalog No. MBS355410), lactate dehydrogenase (LDH) (catalog No. MBS269777) and creatine kinase-MB (CK-MB) (catalog No. MBS2515061) were purchased.

Kits for urinary levels of both total protein and albumin were purchased from Chondrex Inc., Redmond, USA (catalog No. 9040 and 3020, respectively).

Creatinine clearance (ml/min) = [Urine creatinine (mg/dl) x Urine volume (ml/day)]/ [Serum creatinine (mg/dl) x Time (min)] **[29]**

Histopathological examination and immunohistochemical study: were performed in collaboration with Pathology department, Faculty of Medicine, Zagazig University. After decapitation, kidney and heart specimens were obtained. Kidney specimens were fixed in 4% paraformaldehyde solution, embedded in paraffin and 4 μm sections were cut with a microtome and deparaffined with xylene **[8]**. The heart specimens were fixed in formalin, dehydrated in graded alcohol, embedded in paraffin wax and 4 μm sections were cut at with a microtome and deparaffined with xylene **[53]**. Deparaffinated heart and kidney sections were stained with Hematoxylin and Eosin dyes (H&E) and examined for morphological analysis to asses pathological changes **[3]**. Masson's Trichrome was used as a collagen bundle assay for distinguishing muscle from interstitial connective tissue **[35]**. After complete preparation, images were analyzed under light microscope at magnifications of 20 to 40×10 by an experienced investigator pathologist. Bax Immunostaining was performed on serial sections (4 μm thickness) of paraffin blocks of heart and kidney. To block nonspecific peroxidase reaction, deparaffinized tissue sections were treated with hydrogen peroxide for 10 min. Microwave antigen retrieval was done in citrate buffer 0.01 M (pH 6.0) for 20 min. The slides were incubated for 60 min at room temperature with rabbit monoclonal; Anti-Bax antibody (E63, 1:250, abcam, UK) after their washing with phosphate buffer saline. The DakoEnVision ™ kit (Dako, Copenhagen, Denmark) was used to visualize the binding site of primary antibodies. The peroxidase reaction was assessed by 15 min incubation of the sections with diaminobenzidine. With Mayer's hematoxylin, the sections were counterstained. Positive and negative controls were stained with the selected slides at the same setting. Negative controls were done using antibody diluents in phosphate buffer saline instead of primary antibodies and subsequently stained with the positive controls. Specimens were considered positive for Bax when more than 20% of cells were positively stained and they were scored as (mild, moderate and marked)**[34].**

Statistical Analysis: The data obtained were expressed as mean ± standard deviation (SD). For statistical significance, one-way analysis of variance (ANOVA) and Tukey HSD for post hoc multiple comparisons were used to compare means. The software, IBM SPSS Statistics (Version 26 Software for Windows), was used for that purpose. Also, GraphPad Prism (Version 8 Software for Windows) was used to analyze the Pearson's correlation between serum 1,25 $(OH)_2D$ and some studied parameters within MI group. Significance was considered with P value ≤ 0.05.

3. RESULTS

In this study, it was found that out of 30 adult male albino rats used in the present study, 3 rats died in MI group constituting a death rate of 10%. The remaining rats were 27 which were involved in the statisticalanalysis.

Changes in ST- segment depression (mV) and R wave amplitude (mV) one hour after the second dose of ISO injection in different studied groups: (Table-2 and Fig. 1)

There was a significant increase in the amplitude of ST-segment depression, with a significant decrease in R wave amplitude in MI group when compared with control group. In MI pretreated with vitamin D3 group, there was a significant decrease in the amplitude of ST-segment depression, with a significant increase in R wave amplitude in comparison with MIgroup.

Table-2: Changes in ST- segment depression (mV) and R wave amplitude (mV) one hour after the second dose of ISO injection in different studied groups (Number of rats in each group= 9)

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Groups Parameters	Control	MI	MI pretreated with vitamin D3
ST- segment depression (mV)	**0.03±0.002**	**0.16±0.03[a]**	**0.06±0.001[a&b]**
R- wave amplitude (mV)	**0.73±0.03**	**0.38±0.011[a]**	**0.47±0.02[a&b]**

Data were expressed as mean ± SD. MI, myocardial infarction. [a]P<0.05 in comparison with control group.
[b]P<0.05 in comparison with MI group.

Changes in cardiac enzymes in different studied groups: (Table-3)
There was a significant increase in serum levels of both CK-MB and LDH in MI group in comparison with the control group. In MI pretreated with vitamin D3, a significant reduction in cardiac enzymes was recorded in comparison with the MI group.

Changes in kidney function in different studied groups: (Table-3)
In MI group, a significant increase was recorded in serum creatinine and urine (total protein and albumin), with a significant decrease in urine creatinine level and creatinine clearance in comparison with control group. In MI pretreated with vitamin D3, a significant reduction was reported in serum creatinine and urine (total protein and albumin), with a significant elevation in urine creatinine level and creatinine clearance in comparison with MIgroup.

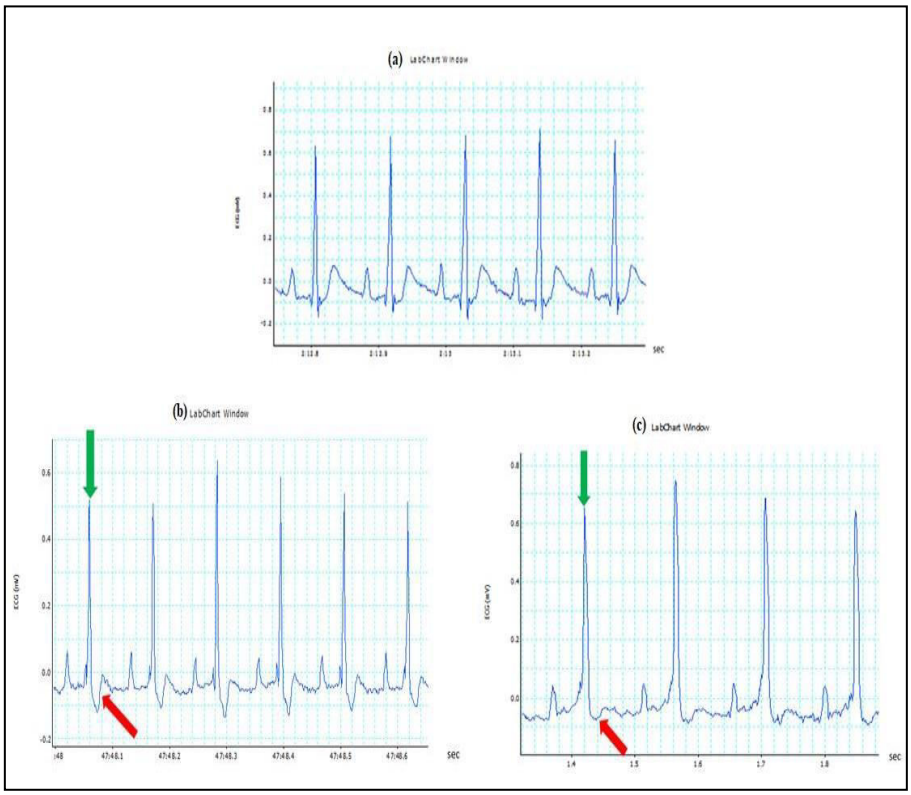

Fig. 1 ECG recording for a rat from (a) Control group showing normal ECG. (b) MI group showing both decreased R-wave amplitude (green arrow) and increased ST- segment depression (red arrow). (c) MI pretreated with vitamin D showing increase in R-wave amplitude (green arrow) and decrease in ST- segment depression (red arrow)

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Table-3: Biochemical changes in different studied groups (Number of rats in each group= 9)

Groups Parameters	Control	MI	MI pretreated with vitamin D3
Serum CK-MB (ng/ml)	**2.43±0.71**	**61.74±4.43[a]**	**33.60±1.95[a&b]**
Serum LDH (IU/L)	**189.42±6.09**	**275.13±2.79[a]**	**244.10±2.20[a&b]**
Serum creatinine (mg/dl)	**0.81±0.07**	**5.54±0.41[a]**	**2.81±0.22[a&b]**
Urine creatinine (mg/dl)	**56.61±2.25**	**22.11±1.96[a]**	**34.33±3.58[a&b]**
Urine total protein (g/dl)	**0.33±0.03**	**2.7±0.08[a]**	**1.13±0.14[a&b]**
Urine albumin (g/dl)	**0.25±0.05**	**2.01±0.1[a]**	**0.7±0.06[a&b]**
Creatinine clearance (ml/min)	**0.28±0.049**	**0.0048±0.0012[a]**	**0.024±0.004[a&b]**
Serum MDA (ng/ml)	**10.39±1.95**	**34.78±3.60[a]**	**21.72±3.09[a&b]**
Serum GSH (ng/ml)	**20.39±1.95**	**2.06±0.24[a]**	**5.81±0.41[a&b]**
Serum IL-6 (pg/ml)	**1.37±0.28**	**14±2.73[a]**	**3.77±0.56[a&b]**
Serum TNF-α (pg/ml)	**1.58±0.32**	**20.88±5.06[a]**	**5.97±1.38[a&b]**
Serum 1, 25(OH)2D (pg/ml)	**125.78±4.63**	**66.33±6.75[a]**	**85.78±7.64[a&b]**

Data were expressed as mean±SD. [a]P<0.05 in comparison with control group. [b]P<0.05 in comparison with MI group. MI, myocardial infarction; CK-MB, creatine kinase myoglobin binding; LDH, lactate dehydrogenase; MDA, malondialdehyde; GSH, reduced glutathione; IL-6, interlukin-6; TNF-α, tumor necrosis factor alpha; 1,25(OH)2D, 1,25-dihydroxy vitaminD.

Changes in serum oxidative stress and inflammatory markers in different studied groups: (Table-3)

There was a significant increase in serum levels of MDA, IL-6 and TNF-□□ with a significant decrease in serum GSH in MI group in comparison with the control group. In MI pretreated with vitamin D3, there was a significant reduction in serum levels of MDA, IL-6 and TNF-□□ with a significant rise in serum GSH in comparison with the MI group.

Changes in serum 1, 25(OH)2D in different studied groups: (Table-3)

There was a significant decrease in serum levels of 1, 25(OH) $_2$ D in MI group in comparison with the control group. In MI pretreated with vitamin D3, a significant increase in its level was recorded in comparison with the MI group.

Pearson's correlation coefficient (r) between serum 1, 25(OH)2 D and some studied parameters within MI group: (Table-4)

Within MI group, a significant negative correlation was detected between serum 1, 25$(OH)_2$D and each of serum CK-MB, serum LDH, urine total protein, urine albumin, serum MDA, serum IL-6 and serum TNF-□, but it was positively correlated with each of creatinine clearance and serum GSH.

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Table-4: Pearson's correlation coefficient (r) between serum 1, 25(OH)2D and some studied parameters within MI group (Number of rats in each group= 9)

Parameters **Correlations**		**Serum CK-MB**	**Serum LDH**	**Creatinine clearance**	**Urine total protein**	**Urine albumin**	**Serum MDA**	**Serum GSH**	**Serum IL-6**	**Serum TNF-α**
Serum 1, 25(OH)2D	**r value**	**-0.924**	**-0.839**	**0.837**	**-0.956**	**-0.903**	**-0.867**	**0.866**	**-0.941**	**-0.987**
	P value	**<0.001**	**< 0.01**	**<0.01**	**<0.001**	**<0.001**	**<0.01**	**<0.01**	**<0.001**	**<0.001**

1,25(OH)2D, 1,25-dihydroxy vitamin D; CK-MB, creatine kinase myoglobin binding; LDH, lactate dehydrogenase; MDA, malondialdehyde; GSH, reduced glutathione; IL-6, interlukin-6; TNF-α, tumor necrosis factor alpha.

Histopathological examination of the heart in different studied groups: (Fig. 2)
In control group (Fig. 2a), there was normal architecture of cardiac wall with branching and anastomosing myofibers bounded with endomysium, cardiomyocytes have central oval, euchromatic nuclei and flat nuclei of fibroblasts. In MI group (Fig. 2b), structural changes detected include pyknotic (apoptotic) nuclei, vascular changes and inflammation. In MI pretreated with vitamin D3 group (Fig. 2c), observed changes include pyknotic (apoptotic) nuclei and interstitial edema.

Histopathological examination of the kidney in different studied groups: (Fig. 3)
In control group (Fig. 3a), there was normal glomeruli and tubules. In MI group (Fig. 3b), structural changes noticed include tubular necrosis and thyroidization. In MI pretreated with vitamin D3 group (Fig. 3c), observed structural changes include mild tubular necrosis and thyroidization.

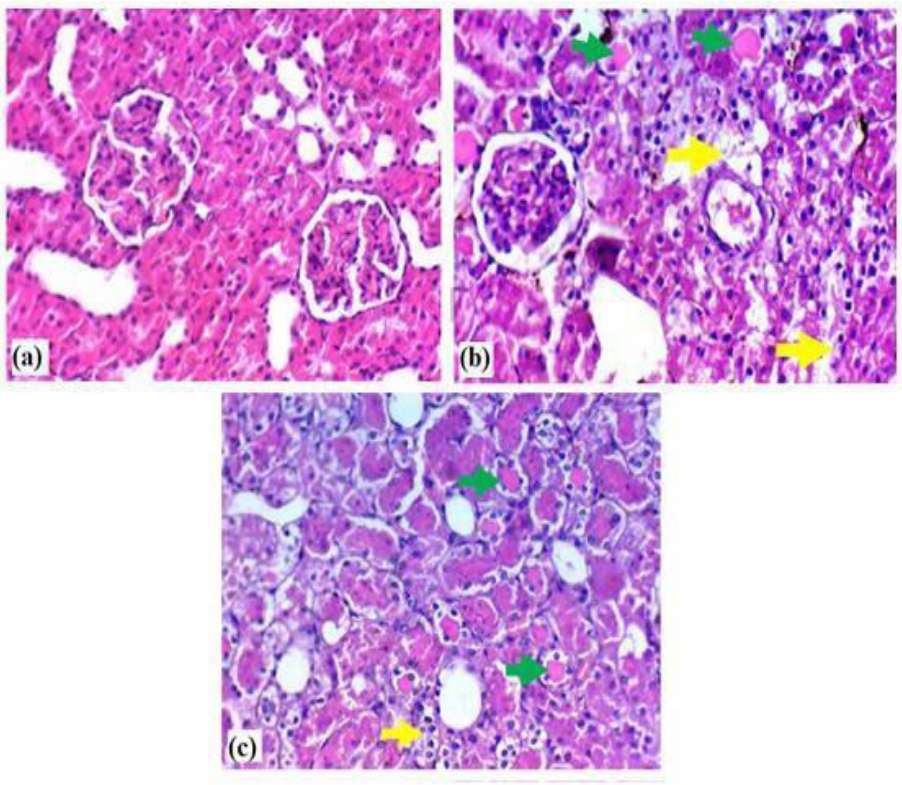
Immunohistochemical study of the heart in different studied groups: (Fig. 4)
Cardiac Bax immunostaining was negative in control group (Fig. 4a), but it was marked in MI group (Fig. 4b), and moderate in MI pretreated with vitamin D3 group (Fig. 4c).

Immunohistochemical study of the kidney in different studied groups: (Fig. 5) Renal
cytoplasmic Bax immunostaining was negative in control group (Fig. 5a), butitwasmarkedinMIgroup(Fig.5b),andmoderateinMIpretreatedwith vitamin D3 group (Fig. 5c).

4. DISCUSSION

In this study, occurrence of myocardial infarction was confirmed by the significant depression in S-T segment and the significant decrease in R wave amplitude in MI group when compared with the control group which was in agreement with Klein et al. [23] and Yang et al. [58]. Klein et al. [23] explained the S-T segment depression by the difference in electrical potential between normal and ischemic zone that lead to injury currents flowing

from the depolarized ischemic regions to normal regions. Yang et al. [58] owed the depression of R wave amplitude to myocardial edema after myocardial infarction and they



reported that R wave amplitude carries information about infarctionsize.

Fig. 2 Photomicrographs of histopathological examination of the heart in different studied groups (H&E x400) (a) Control group showed normal architecture of cardiac wall with branching and anastomosing myofibers bounded with endomysium, cardiomyocytes have central oval, euchromatic nuclei (black arrow) and flat nuclei of fibroblasts (green arrow). (b) MI group showed pyknotic (apoptotic) nuclei (black arrow), vascular changes (green arrow) and inflammation (yellow arrow). (c) MI pretreated with vitamin D3 group showed pyknotic (apoptotic) nuclei (green arrow) and interstitial edema (blackarrow)

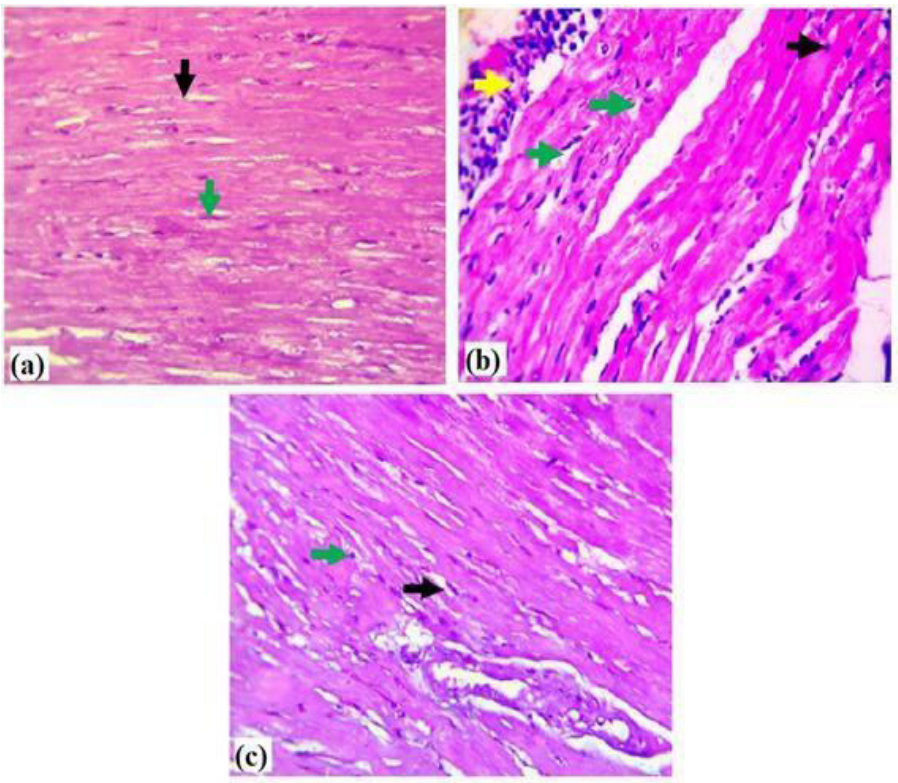
Also, damage in cardiac muscle was assessed by the increase in serum cardiac enzymes (CK-MB and LDH) in MI group in comparison with the control group, which was in line with Rani et al. **[45]** and Fan et al. **[15]** who referred that to the leak out of these enzymes from damaged cardiac muscle.

In addition, serum levels of pro-inflammatory cytokines (TNF-α and IL-6) were increased in the MI group when compared with the control group which declared presence of inflammation which may have a role in the pathogenesis of MI. This was supported by Bulboacă et al. **[6]** who reported that, ischemia of myocardial tissue activated cardiomyocytes and local myocardial mononuclear macrophages in the infarcted zone to secrete large amounts of TNF-α and IL-6.

Also, oxidative stress in MI group was detected as there was a significant increase in serum level of MDA with a significant decrease in GSH serum level when compared with the control group which was in accordance with Chen et al.

[7] who owed this to the effect of ISO which increased oxygen free radicals generations and lipid peroxidation of cell membrane that was reflected by the increase in MDA, while, GSH which is involved in cellular protection from deleterious effects of oxygen free radicals was decreased.

Fig. 3 Photomicrograph of histopathological examination of the kidney in different studied groups (H&E x400) (a) Control group showed normal glomeruli and tubules. (b) MI group showed tubular necrosis (yellow arrow) and thyroidization (green arrow). (c) MI pretreated with vitamin D3 group showed mild tubular necrosis (yellow arrow) and thyroidization (green arrow)



In MI group, histopathological examination of heart showed pyknotic nucleus, vascular and inflammatory changes which confirmed occurrence of MI and this was in line with Tian et al. **[52]** and Nabofa et al. **[37]** who found that cardiac ischemia caused irreversible condensation of chromatin in the nucleus (pyknotic nucleus) of a cell undergoing apoptosis, increased vascular permeability in the myocardial interstitium which lead to intercellular edema and inflammatory response with an initial invasion ofneutrophils.

Immunohistochemistry of the heart in MI group showed marked Bax immunostaining which was supported by Zhang et al. **[60]**, Sun et al. **[50]** and Gupta et al. **[16]** who reported that Bax is a pro-apoptotic factor that participates in the mitochondrial pathway of apoptosis as over expression of Bax protein (also known as pore-forming protein) forms pores in the outer mitochondrial membrane after activation, resulting in loss of membrane integrity and release of cytochrome c.

In this study, kidney functions were studied by estimating serum level of creatinine, urine (creatinine, total protein and albumin) and the creatinine clearance. In MI group, there was a significant increase in both serum level of creatinine, and urinary level of total protein and albumin, with a significant decrease in urinary level of creatinine and creatinine clearance which confirmed deterioration in kidney functions and development of acute kidney injury.

These resultsweresupportedbyShachametal.**[49],**Liuetal.**[27]**andMansfieldetal.
[33] who referred this to prerenal hypoperfusion which activated the renin angiotensin aldosterone system, sympathetic nervous system, and vasopressin secretion leading to fluid retention and increased prerenal azotemia. Parr et al. **[43]** declared that AKI was a risk factor for the subsequent proteinuria. Also, Hsu etal.
[17] confirmed that proteinuria after AKI was accompanied by loss of kidney function.

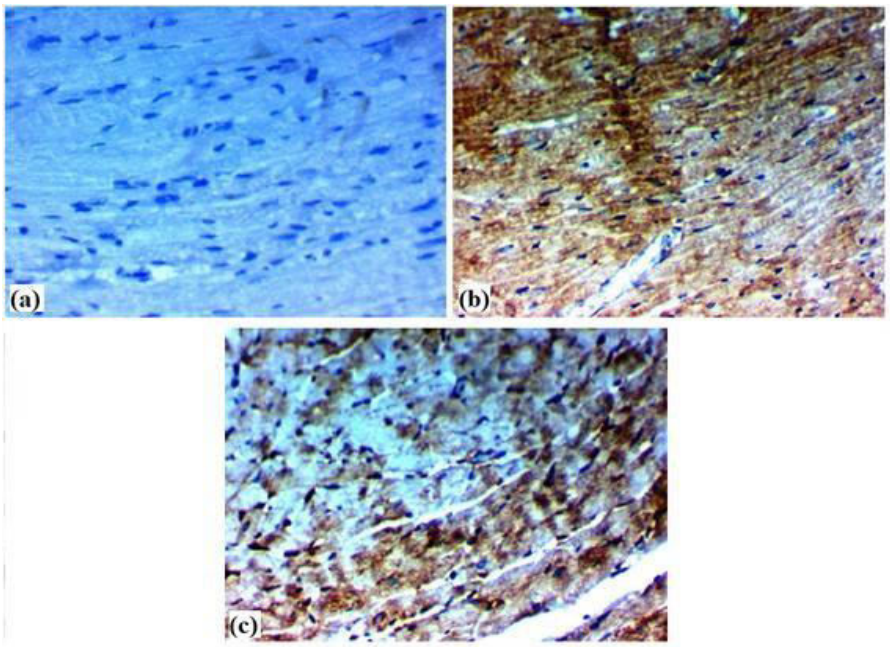


Fig. 4 Immunohistochemical study of the heart in different studied groups (IHC x400) (a) Control group showed negative Bax immunostaining. (b) MI group showed marked Bax immunostaining. (c) MI pretreated with vitamin D3 group showed moderate Bax immunostaining

Histopathological examination of renal tissue in MI group showed tubular necrosis and thyroidization (atrophic tubules containing proteinaceous casts) which suggested presence of acute kidney injury as supported by Ozbilgin et al. **[39]** and Jochmans et al. **[21]** who reported proximal and distal tubular damage (dilation, atrophy, loss of brush border and necrosis) and thyroidization as acute kidney injuryfeatures.

Immunohistochemical study of renal tissue in MI group revealed marked Bax immunostaining which declared presence of apoptotic changes in kidney tissue which was in agreement with Parikh et al. **[42]** and Zhou et al. **[61]** who realized that renal ischemia induced marked neutrophil infiltrations in kidney tissues which stimulated cascade of apoptosis that lead to renal tubular cell death.

In MI group, there was a significant decrease in serum level of 1, $25(OH)_2D$ when compared with the control group which may be due to AKI. This result was supported by Chen et al. **[7]**, Vijayan et al. **[54]** and Palmeira et al. **[40]** who reported the association between hypovitaminosis D and acute MI. Several mechanisms could explain the decrease in serum level of 1, $25(OH)_2D$ in AKI including that the kidneys generate the majority of 1α-hydroxylase CYP27B1 (is mainly located in the inner mitochondrial membrane of renal proximal tubule epithelium), and is easily injured in AKI [30]. Also, fibroblast growth factor 23 (FGF23), a bone derived hormone reduces circulating 1, $25(OH)_2D$ levels, is increased in AKI **[9]**.

Moreover, AKI is accompanied by a decrease in serum level of vitamin D binding protein **[25]**. On studying correlation between 1, $25(OH)_2D$ and different studied parameters in MI

group, there was a significant negative correlation between its serum level and both; serum level of (CK-MB, LDH, MDA, TNF-α and IL-6) and urine level of (total protein and albumin), but a significant positive correlation was detected between serum 1, 25$(OH)_2$D and both creatinine clearance and serum GSH. These results may declare that vitamin D could play a role in the pathogenesis of acute kidney injury occurred with acute myocardial infarction and this was partly supported by Xu et al. **[57]** and de Almeida et al. **[10]**. Thus, the possible protective effect of vitamin D3 supplementation on both cardiac and renal functions in acute MI was assessed in thisstudy.

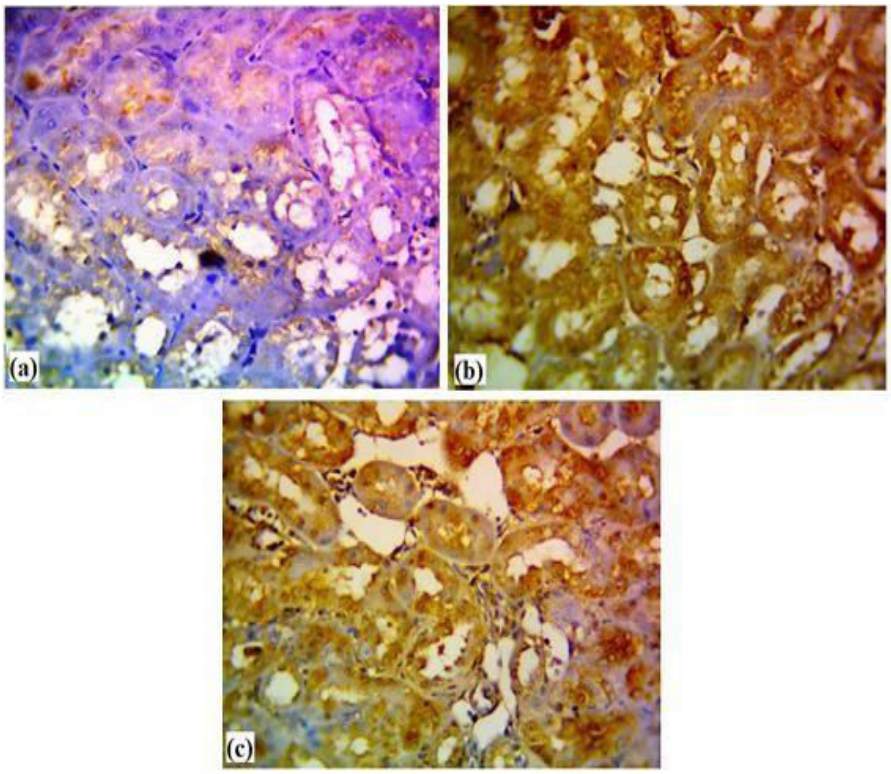


Fig. 5 Immunohistochemical study of the kidney in different studied groups (IHC x400). (a) Control group showed negative Bax immunostaining. (b) MI group showed marked cytoplasmic Bax immunostaining (c) MI pretreated with vitamin D3 group showed moderate cytoplasmic Bax immunostaining

In MI pretreated with vitamin D3 group, there was a significant improvement in ECG changes (decrease in S-T segment depression and increase in R wave amplitude) with a significant decrease in serum cardiac enzymes (CK-MB and LDH) when compared with MI group which suggested a cardioprotective effect of vitamin D3 as supported by El-Gohary, Allam **[13].** In contrary**,** Pandit et al. **[41]** found no association between vitamin D levels and left ventricular diastolic performance. The discrepancy may be due to difference in species, dose of vitamin D and duration of study.

Also, in MI pretreated with vitamin D3 group, there was a significant decrease in pro-inflammatory markers (TNF-α and IL-6) together with improved oxidative state (decrease in serum MDA level and increase in GSH serum level) which suggested that vitamin D3 has anti-inflammatory and antioxidant properties as reported by Saeedian Kia et al**. [47],** Manousaki et al. **[31]**, Milazzo et al. **[36]** and Sepidarkish et al. **[48]**. Manousaki et al. **[31]** stated that vitamin D suppressed cyclo-oxygenase expressions, and stimulated 15-hydroxyprostaglandin dehydrogenasewhichinitiatedprostaglandincatabolism.Also,SaeedianKiaetal.

[47] reported that vitamin D increased the expression of mitogen-activated protein kinase phosphatase-5 which caused dephosphorylation and inactivation of the p38 stress-induced kinase, resulting in a decrease in the production of pro- inflammatory cytokines.
Moreover, Milazzo et al. **[36]** declared that vitamin D inhibited nuclear factor- κB signaling and this decreased level of pro-inflammatory cytokines. Contrarily, Witham et al. **[55]** showed no reduction in blood level of inflammatory cytokines after 8 weeks of vitamin D supplementation. This contradiction is probably due to the difference in duration and type of the study. Sepidarkish et al. **[48]** owed the antioxidant effect of vitamin D to suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which is a major resource of reactive oxygen species.
Histopathological and immunohistochemical examination of the heart in MI pretreated with vitamin D3 group showed some improvement as noticed by the decrease in inflammation and the moderate Bax immunostaining which was supported by Tabasi et al. **[51]**, Kroner Jde et al. **[24]** and Barbarawi et al. **[5]**. Tabasi et al. **[51]** reported that vitamin D had anti-apoptotic activity in systemic lupus erythematosus by increasing number of regulatory T cells which suppressed immune response.
On assessment of kidney functions and its histopathological examination in MI pretreated with vitamin D3 group, there was a significant decrease in both; serum level of creatinine and urine levels of (total protein and albumin), but there was a significant increase in urine creatinine level and creatinine clearance accompanied by a decrease in necrosis of renal tissue on histopathological examination with moderate Bax immunostaining on immunohistochemical study of renal tissue which reflected improvement in kidney structure and function that was in agreementwithHuretal.**[19]**,Jeanetal.**[20]**andHuen,Cantley**[18]**.Huretal.
[19] found that vitamin D mediated protection against gentamicin induced acute kidney injury and they owed this to the negative regulatory effect of vitamin D on renin angiotensin aldosterone system by suppressing renin expression.
Limitations of this study included that it was done on rats; thus, the results of this study may not be applicable to human. Also, the number of rats in each group was small. Moreover, this study was of short duration and long-term therapy was not considered.
In conclusion, vitamin D3 has a protective effect on heart and kidney functions in the rat model of myocardial infarction and this beneficial effect could be related to its anti-inflammatory, antioxidant and anti-apoptotic actions.

Compliance with ethical standards
Conflict of interest: The authors declare that there are no conflicts of interest.

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