

Cardioprotective Effects of Nano-Vitamin D on Isoprenaline-Induced Myocardial Infarction Rat Model

Omyma Galal^a, Ahmed Mostafa^b, Haytham Mohamed^c, Ahmed RH Ahmed^d,
Marwa S. Hashim^e, Nagwa Mohamed^{b*}

^a Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

^b Department of Medical Physiology, Faculty of Medicine, Sohag University, Sohag, Egypt.

^c Department of Medical Physiology, Faculty of Medicine, South Valley University, Qena, Egypt.

^d Department of Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt.

^e Department of Biochemistry, Faculty of Medicine, Sohag University, Sohag, Egypt.

Abstract

Background: Cardiovascular disease (CVD) is a public health problem accounting for 17.9 million deaths worldwide in 2019. Vitamin D is a fat-soluble vitamin that has various cardioprotective actions and its deficiency is associated with a variety of CVDs. Nano systems for vitamin D may overcome the variable oral bioavailability, poor water solubility and chemical degradation of vitamin D.

Objectives: The potential cardioprotective effect of oral vitamin D and vitamin D nanoparticles was evaluated on isoprenaline induced myocardial infarction (MI) rat model.

Materials and method: the study evaluated the effect of vitamin D and vitamin D nanoparticles on MI rate models. MI induced by isoprenaline 100 mg/ kg on the last two days of the 30 day treatment period. We analyzed cardiac injury, lipid peroxidation markers and lipid profile.

Results: isoprenaline treated rats show marked elevation in cardiac troponin-I (cTn-I), and malondialdehyde (MDA), (p value <0.0001). Oral vitamin D reduced cTn-I and MDA levels and improved lipid profile. Vitamin D nanoparticles enhance the cardioprotective effect of conventional vitamin D.

Conclusion: vitamin D nanoparticles have a more efficient cardioprotective effect against isoprenaline induced MI in rats compared to oral conventional vitamin D.

Key words: myocardial infarction, isoprenaline, vitamin D, vitamin D nanoparticles.

DOI: 10.21608/svuijm.2022.131434.1301

*Correspondence: nagwaabdelmawgood@gmail.com

Received: 5 March,2022.

Revised: 20 March,2022.

Accepted: 7 April, 2022.

Cite this article as: Omyma Galal, Ahmed Mostafa, Haytham Mohamed, Ahmed RH Ahmed, Marwa S. Hashim, Nagwa Mohamed. (2022). Cardioprotective Effects of Nano-Vitamin D on Isoprenaline-Induced Myocardial Infarction Rat Model. *SVU-International Journal of Medical Sciences*. Vol.5, Issue 2, pp: 136-151

Introduction

CVD is the main cause of mortality in the world and causes ~17.9 million deaths in 2019, which represents about 32% of total losses per year worldwide (Bhatia et al., 2021). Ischemic heart disease (IHD) is increasing in frequency accounting for 20% of all deaths in the United States and Europe (Keller et al., 2019). Hypercholesterolemia, hypertension, obesity, and diabetes are the key risk factors for IHD. Increased dietary saturated fat and trans-fat consumption raises plasma low-density lipoprotein cholesterol (LDL-C), which is a major risk factor for IHD. Inflammation, oxidative stress, endothelial dysfunction, and insulin sensitivity are among the other risk factors (Hensrud and Heimbürger, 2020).

The acute presentation of IHD, myocardial infarction (MI), is a life-threatening health condition. MI is the leading cause of death worldwide, with an increasing frequency in developing countries as well (Contessotto and Pandit, 2021). Increased risks of MI have been associated with aging, diabetes, atherosclerotic vascular disease, hypertension, smoking, and drinking alcohol. A healthy lifestyle as physical activity and antioxidant rich diet decrease the incidence of MI (Abdelhalim et al., 2021). MI is associated with sudden death of up to a billion cardiomyocytes that triggers an inflammatory response that plays a crucial role in the pathogenesis and repair of MI (Frangogiannis, 2018).

Isoproterenol (ISO) is a synthetic non-selective β -agonist that has positive chronotropic and inotropic effects at low doses while depleting cardiomyocyte energy reserves at high doses. ISO-induced MI is a widely accepted mouse model that demonstrates various structural abnormalities like those observed in MI of human beings. MI mouse model can be used to evaluate the effects of new cardioprotective agents (Ansari et al., 2019). Injected isoprenaline undergoes auto-oxidation, resulting in the production of free radicals, which drive lipid peroxidation, causing damage of the myocardial cell membrane. Myocardial tissue damage is mediated by inflammatory processes through the release of proteolytic enzymes (Saparov et al., 2017).

Vitamin D is a fat-soluble vitamin. It has two primary forms: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). The difference in the chemical structure between the two forms of vitamin D does not affect the metabolism or clinical responses once activated within the body apart from differences in toxicity as indicated by experimental studies. The vitamin D receptor is found in almost all tissues and cells of the body (Sosa-Henríquez, 2020).

Vitamin D has pleiotropic cardiovascular effects by activating its nuclear receptor in cardiomyocytes and vascular endothelial cells. Vitamin D regulates the renin-angiotensin-aldosterone system, adiposity, energy

expenditure, and pancreatic cell activity. Vitamin D deficiency can contribute to coronary risk factors, vascular dysfunction, arterial stiffening, and hyperlipidemia. It also predisposes to hypertension, diabetes mellitus and the metabolic syndrome, left ventricular hypertrophy, CHF, stroke, peripheral arterial disease, and chronic vascular inflammation (**Libby et al., 2021**).

Nanoscale drug delivery systems (NDDS) have been considered one of the most overwhelming advancements in today's pharmaceutical and nutraceutical business. These technologies provide enhanced bioavailability and targeting at the cellular and even subcellular level, opening new avenues for disease prevention and treatment, as well as maintaining health (**Antal and Ardelean, 2021**).

From the pharmaceutical point of view, vitamin D has variable oral bioavailability, poor water solubility and chemical degradation upon exposure to light, oxygen, or elevated temperature. These hinder the clinical application as well as food and beverage fortification with vitamin D (**Guttoff et al., 2015**). The nano system for vitamin D overcomes these challenges due to its ability to enhance vitamin D bioavailability and maximize its therapeutic potential.

Material and methods

Experimental animals

Forty adult male Westar albino rats obtained and housed in polycarbonate cages (20cm ×30cm×40cm) in Qena Faculty of Medicine, Egypt. The rats were apparently healthy, weighing 170–220g. They were allowed for free access to water and standard pellet diet and kept under standard conditions of temperature and humidity with 12/12 h light/dark cycles. Animal ethical considerations were completely fulfilled according to the Research Ethics Committee of Qena Faculty of Medicine, South Valley University guidelines. After acclimatization for one-week rats will be randomly allocated into four groups (n =10) according to the experimental design.

Experimental design

Rats were randomly allocated into four groups (n =10).

Group I: Normal rats (Control)

Group II: Isoprenaline injected rats (myocardial infarction model group)

Group III: Isoprenaline-injected rats pretreated with conventional vit D (treatment group with conventional vitamin D)

Group IV: isoprenaline-injected rats pretreated with vitamin D nanoparticles (treatment group with vitamin D nanoparticles).

Group I and II received a vehicle of vitamin D (0.8 ml of olive oil orally/day) for 4 weeks. Group I

received a vehicle of ISO (normal saline; s.c. injection single dose / day for two successive doses at 24 hrs interval) on the last two days of the experiment. Groups III received vitamin D in a dose 1 µg (40 IU/ kg), orally/day). Group IV received vitamin D nanoparticles; each 9 µg of nano-vitamin D powder dissolved in 1 ml distilled deionized water produced (3.240 IU/ml). The dose of vitamin D loaded nanoparticles administered calculated so that each rat received a daily oral dose of 40 IU/kg (El-Sherbiny et al., 2018). The duration of the experiment will be 4 weeks (Abood and Elshal, 2015).

For induction of myocardial infarction rats in groups II, III and IV received isoprenaline hydrochloride dissolved in saline (100mg/kg; s.c. injection single dose / day for two successive doses at 24hrs interval) on the last 2 days of the treatment period (Sharma et al., 2018). Twenty-four hours after the last treatment, overnight fasted rats weighed, anesthetized with inhalational anesthetic isoflurane 100 %, sacrificed, a midline abdominal incision made for collection of blood specimens from the abdominal aorta. The Blood centrifuged at 5000 rpm for 15 minutes. Separated serum was stored at -20°C for later biochemical analysis. The hearts were weighted, divided into two halves. One half of heart tissues perfused with a phosphate buffered saline solution (PBS), pH 7.4, containing 0.16 mg heparin / ml to remove any red blood cells and clots and preserved in liquid

nitrogen for subsequent homogenization (Abood and Elshal, 2015).

Drugs and chemicals

Vitamin D: purchased from Sigma Aldrich USA and prepared, 1000 IU/ ml dissolved in commercial olive oil and mixed using ultrasound cleaner at chemical laboratory at Sohag Faculty of science, Sohag University, to obtain a concentration of 50 IU/ml.

Nano-Vitamin D: Brand: NT-VDNP, purchased from Nanotech for Photo Electronics, Dreamland, Gate 3, What Road, 6th October, Cairo, Egypt. Each 9 µg of nano-vitamin D powder dissolved in 1 ml distilled deionized water produce (3.240 IU/ml) (El-Sherbiny et al., 2018). The drug was tested. Size & Shape: TEM was performed on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively.

Isoprenalin 98 %: purchased from Pharma Quanao Chemical Co., Ltd

Isoflurane 100 %: purchased from Pharco-Pharmaceuticals.

Cardiac- Specific Troponin-I (cTn-I) ELISA kits from PerkinElmer

Lipid profile: HDL cholesterol - Precipitant, Cholesterol – Liquizyme and Triglyceride-Liquizyme from SPECTRUM

Lipid Peroxide (Malonaldehyde) from BIODIAGNOSTIC.

Determination of cardiac troponin-I (cTn-I)

cTn-I concentration was estimated using (cTn-I) ELISA kits from PerkinElmer, Catalog Number:10604, for the quantitative determination of cTn-I in serum according to the manufacturer's protocol.

Determination of lipid peroxidation as malondialdehyde (MDA)

The extent of lipid peroxidation in the heart was estimated colorimetrically measuring MDA, which is one of the end products of lipid peroxidation (Su et al., 2022). The tissues were homogenized in 5 – 10 ml cold buffer (50 mM potassium phosphate, pH 7.5.) per gram tissue, and then centrifuged at 4000 r.p.m for 15 minutes. The supernatant was taken for assay. Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product, the absorbance of the resultant pink product can be measured at 534 nm. MDA calculated according to the formula

$$\text{MDA in Tissue} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \frac{10}{\text{g. tissue used}} = \text{nmol / g.tissue}$$

Determination of lipid profile

Serum levels of cholesterol, HDL-C, TGs were assayed using kits from SPECTRUM following manufacturer's protocols. LDL cholesterol was

calculated according to the traditional Friedewald equation which postulated that; LDL-C equal total cholesterol minus HDL cholesterol minus TGs divided by five.

Statistical analysis

Data were analyzed using GraphPad Prism® software version 9 (GraphPad Software, San Diego, CA) and presented as means ± SD or median ± range. Significance was determined by One-Way ANOVA followed by Tukey-Kramer's test for parametric numerical variables, and Kruskal-Wallis test for non-parametric numerical variables. P value < 0.05 was considered statistically significant.

Results

Effect of vitamin D and vitamin D nanoparticles on serum cTn-I

There was a significant difference between the 4 groups as regards serum cTn-I levels (P<0.0001). Group II showed marked increase in serum cTn-I level (P <0.0001). Vitamin D treated group (group III) showed improvement in serum cTn-I level by 64% when compared to MI model group, but still higher than the normal control group (P= 0.0008). vitamin D nanoparticles treated group (group IV) showed marked reduction in serum cTn-I level by 82% and 50% when compared to MI model group and conventional vitamin D treated group respectively, with no statistically significant difference when compared to the normal control group, (Fig.1).

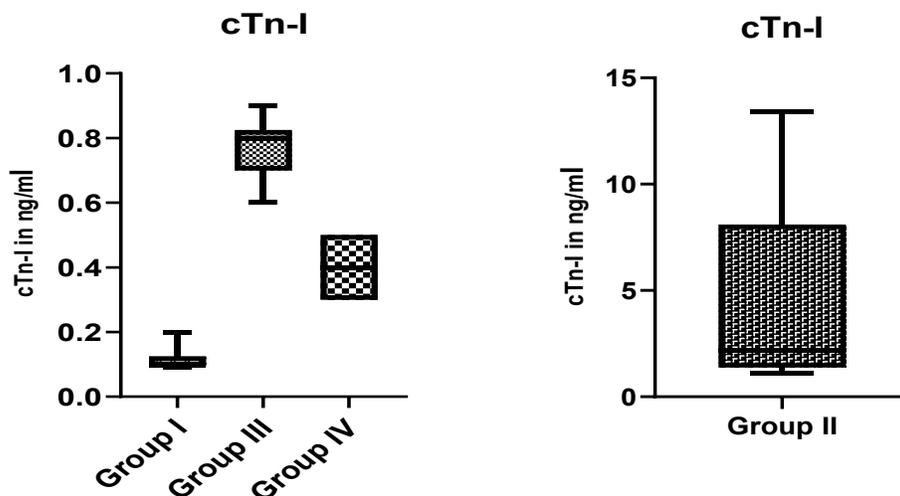


Fig.1. cTn-I serum level difference among the studied groups: data are expressed as median and 2.5 and 97.5 percentile, n = 10. Kruskal Wallis test was used to test the difference between multiple groups.

Effect of vitamin D and vitamin D nanoparticles on lipid peroxidation product MDA

There was a statistically significant difference between the four groups regarding tissue MDA level ($P < 0.0001$). MDA tissue level increased markedly in MI model group (Group II) when compared to the three other groups. Treatment with vitamin D in (group III) reduced MDA level by 63 % when compared to MI model group but still higher than MDA level in normal control group ($P = 0.0078$). MDA level in vitamin D nanoparticles treated group (Group IV) showed marked improvement by 86 % and 62 % when compared to group II and group III respectively, without statistically significant difference when compared with the normal control group, (Fig.2).

Effect of vitamin D and vitamin D nanoparticles on lipid profile

The serum values of total cholesterol, triglycerides and LDL cholesterol were significantly higher in MI model group compared to normal control group ($P < 0.0001$). However, the total cholesterol, triglycerides and LDL cholesterol were markedly improved in oral vitamin D-treated group by 43%, 30% and 64% respectively compared to MI model group, ($P < 0.0001$), and with no statistically significant difference when compared to normal control group. serum lipid profile of Vitamin D nanoparticles-treated rats was lower (by 21%, 22%, and 64% respectively) than that observed in conventional oral vitamin D-treated rats ($P < 0.0001$), (Fig.3).

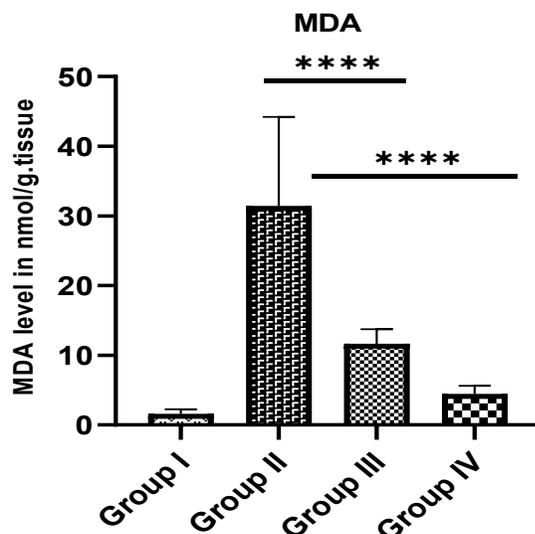


Fig.2. The MDA difference among studied groups. Data are presented as mean \pm SD, $n=10$. ANOVA was used to test the difference between multiple groups. Tukey's correction was used for post hoc analysis.

Regarding HDL, MI model group showed marked decrease in serum HDL by 34% compared to normal control group ($P < 0.0001$), but this decrease was markedly restored by conventional vitamin D treatment, where HDL level increased by 43 % compared to MI model group ($P < 0.0001$), without statistically significant difference compared to normal control group. Vitamin D nanoparticles-treated rats showed more increase by 0.33-fold in serum HDL levels compared with conventional vitamin D-treated group ($P < 0.0001$), (**Fig.4**).

Discussion

Ischemic heart disease (IHD) is becoming more common, accounting for 20% of all deaths in the United States and Europe (**Keller et al., 2019**), accounting for half of all CVD-associated morbidity and mortality. IHD is mostly caused by acute MI and, to a lesser extent, angina according to the Global Burden of Disease project. Because of overall population growth and population aging, the worldwide burden of IHD has grown by 29 million disability-adjusted life-years (DALYs) representing a 29% increase (**Gaziano and Gaziano, 2016**).

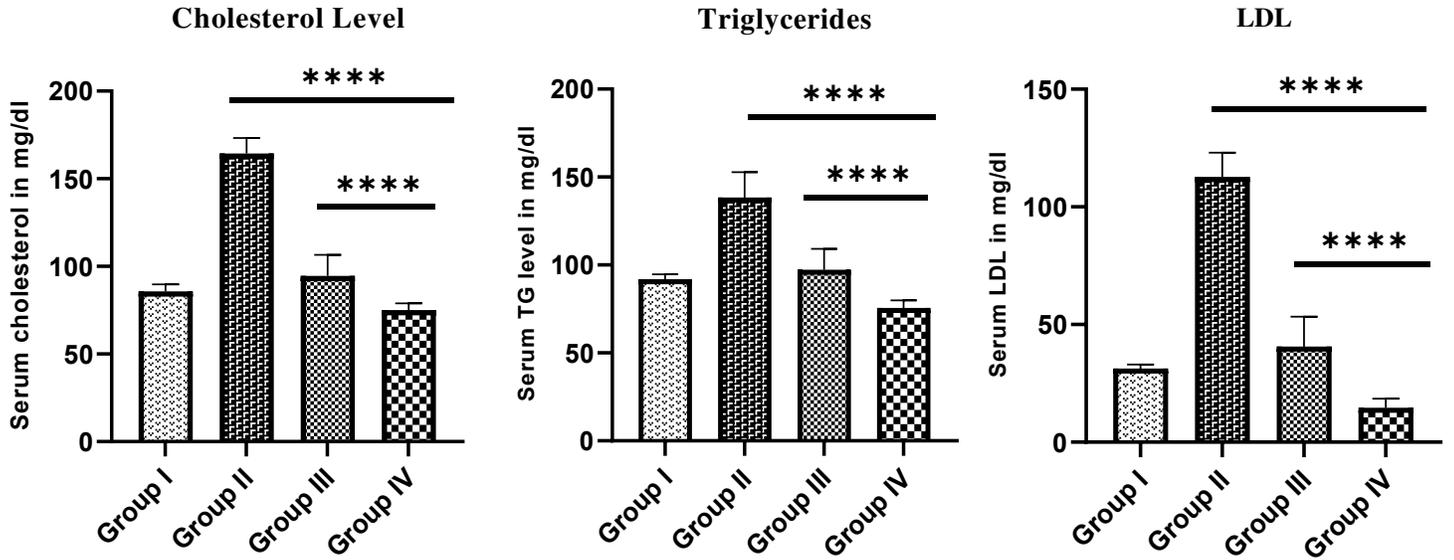


Fig.3. Total cholesterol, triglycerides, and LDL cholesterol difference among the studied groups. Data are presented as mean \pm SD, n = 10. ANOVA was used to test the difference between multiple groups. Tukey's correction was used for post hoc analysis.

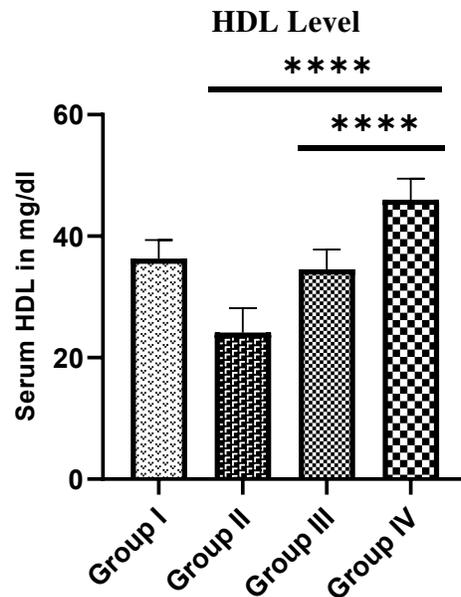


Fig.4. HDL cholesterol difference among the studied groups, Data are presented as mean \pm SD, n = 10. ANOVA was used to test the difference between multiple groups. Tukey's correction was used for post hoc analysis.

ISO is a potent β -adrenergic receptor agonist that is metabolized by the enzyme catechol-O-methyltransferase in the human body. Metabolic products of high doses of ISO produce toxic cardiac effects by producing too many free radicals, causing intracellular Ca^{2+} overload, and causing mitochondrial dysfunction due to a lack of blood flow, resulting in severe infarct necrotic cardiac cells (Wong et al., 2017).

ISO-induced cardiac damage enhances the permeability of the cardiac cell membranes, with release of the cytosolic enzymes in the bloodstream. One of these enzymes is cTn-I, which can produce ATP, a phosphate energy storage form. Troponin I is the regulatory subunit of the troponin complex, which is connected to the actin thin filament in muscle cells. It forms a protein complex with troponin T and troponin C in both skeletal and cardiac muscle, and the complex regulates muscle contraction (Rifai, 2017).

cTn-I is the ideal biomarker for diagnosis of acute myocardial injury (AMI) due to its specificity, selectivity, and sensitivity reaching detectable level within 4 hours of initiation of AMI. It's recognized by the European Society of Cardiology as the gold standard biomarker for AMI detection (Clerico et al., 2020).

In this study, marked elevation in cTn-I level was observed in ISO-treated

rats (Group II) in comparison with the control group (Group I) indicating successful induction of MI as explained in figure 1. These results come in agreement with many previous studies (Emran et al., 2021), (Attia et al., 2021), (Liu et al., 2021) who found the same result.

Vitamin D has an important role in cardiovascular disorders (Janjusevic et al., 2022). It prevents atherosclerosis and endothelial dysfunction (Norman and Powell, 2014). VDR signaling enhances NO production while suppressing cyclooxygenase-2, thromboxane-prostanoid receptor expression and endothelial adhesion molecules (Andrukova et al., 2014). Vitamin D may also protect endothelial cells by reducing stress and oxidative stress in the endoplasmic reticulum (Haas et al., 2016). In the heart, VDR activation lowers cardiac hypertrophy and regulating matrix metalloproteinases and tissue inhibitors of metalloproteinases expression. VDR activation has a variety of consequences on myocardial calcium flux, which may have an impact on cardiac electrophysiology and contractility (Pilz et al., 2010).

Administration of vitamin D in ISO – treated rats (Group III) showed a profound cardio-protection, with reduction of serum cTn-I level in comparison with MI model (Group II) as reported by (El-Gohary and Allam, 2017), and (Abood and Elshal, 2015).

Awad et al also reported the beneficial effect of vitamin D administration in reducing cTn-I level in doxorubicin induced cardiotoxicity in rats (Awad et al., 2021).

NDDS have developed to deliver drugs to target sites effectively, avoiding drug degradation and preserving drug physicochemical properties. NDDS control drug behaviors on tissues, cells, and organelles, and can be applied to break through multi-stage barriers and manipulate drug metabolism (Wang et al., 2021). Herein, we demonstrated that vitamin D nano formulation enhanced its cardioprotective effect in ISO-induced MI rat model.

To the best of our knowledge this is the first study about nano vitamin D cardio protection, interestingly marked improvement in cTn-I level in vitamin D nanoparticles treated group (Group IV) by 82% and 50% in comparison with Group II and Group III respectively.

MI is a type of ischemic heart disease that occurs when a coronary artery is blocked. Oxidative stress and inflammation play a key role in the etiopathogenesis of plaque formation, instability, and consequent formation of the thrombus (Shahzad et al., 2019). Glycolipids, phospholipids, and cholesterol are polyunsaturated lipids that are important components of plasma membranes, helping to maintain cell shape and control cell activity. When oxidative stress breaks out, reactive oxygen species (ROS) attack polyunsaturated fatty acids of

polyunsaturated lipids leading to lipid peroxidation (Gaschler and Stockwell, 2017; Su et al., 2022).

Lipid peroxidation causes increased cell membrane permeability, DNA mutation, and even cell death, resulting in aging, illnesses, and other physiopathology effects (Ito et al., 2019; Su et al., 2019). MDA is one of the end products of lipid peroxidation, created by increased ROS and is an essential indication of membrane damage and bodily aging. MDA level is closely linked to leukemia, cardiovascular, cerebrovascular diseases, and cancer (Tajika et al., 2012; Zhang et al., 2020a).

Our results in this study revealed marked elevation in MDA level in cardiac tissue homogenate in ISO treated rats denoting oxidative stress and cell membrane damage. This result in accordance with (Hossini et al., 2022), (Abdelhalim et al., 2021), (Emran et al., 2021), who found a marked elevation of MDA in Iso induced MI. Also, our results come in line with previous human study that investigated the MDA level in MI patients, the study reported higher MDA serum level in complicated cases with arrhythmia and in the dead group (Yin et al., 2019).

On administration of vitamin D for 4 weeks before induction of infarct like lesion, our results showed marked amelioration of oxidative stress as evidenced by reduction in MDA level in tissue homogenate in group III compared with group II. These findings are in line

with previous studies which reported marked reduction of MDA in vitamin D treated rats (El-Gohary and Allam, 2017), (Abood and Elshal, 2015). Also reported by the study of Moradi et al who reported the beneficial effect of vitamin D in mitigating the pressure overload-induced cardiac hypertrophy via Inhibition of GTPase Rac1 expression (Moradi et al., 2021).

On administration of vitamin D nanoparticles for 4 weeks prior to induction of MI, very significant improvement of MDA in group IV by 86 % and 62 % compared with group II and group III respectively.

Dyslipidemia is an important factor in the development of CVD because it promotes the formation of atherosclerosis, which resulted in the development of IHD, hypertension, and stroke. It is also an important risk factor for acute MI and contributes to serious disease burden. Dyslipidemia patients who have experienced acute MI have higher risks of cardiovascular events and heavier burden of disease. CVD is characterized by a variety of abnormalities of serum lipids, increased TG, total cholesterol, LDL-Cholesterol and decreased HDL- cholesterol (Crismaru et al., 2020; Zhang et al., 2020b).

Lipid peroxidation plays an important role in lipoprotein modification with subsequent development of atherosclerosis. Lipid peroxidation is the cause of ISO-induced cardiac toxicity thus ISO induced

myocardial necrosis has been shown to elevate plasma TC, TG, LDL-C, and decrease HDL-C levels (Mopuri et al., 2017).

The current study results showed marked elevation in TC, TG, LDL-Cholesterol with reduction in HDL-C in ISO treated rats as reported by Mopuri and his colleagues (Mopuri et al., 2017) and (Kariyappa et al., 2019).

Vitamin D administration before induction of MI improves serum TC, TG, LDL-C, and HDL-C. These results of the current study are in line with the effect of vitamin D administration on lipid profile in nonalcoholic fatty liver disease (NAFLD) (El-Sherbiny et al., 2018). Vitamin D administration in western diet-fed obese rats improved lipid profile as regard TG, LDL-C, and HDL-C in accordance with the results of the current study while TC level increased which is against our results (Cordeiro et al., 2021). Also, our results in the current study agree with the meta-analysis of Morvaridzadeh et al, as they reported the beneficial effect of vitamin D supplementation on the TC, TG, HDL-C (Morvaridzadeh et al., 2021). Zhang et al in their meta-analysis of the effect of vitamin D on lipide profile in postmenopausal women had reported that vitamin D has beneficial effect on TG, while clinically negligible effect on lowering LDL-C and increase HDL-C (Zhang et al., 2022).

Vitamin D nanoparticles administration for four weeks prior to induction of MI exhibited marked

enhancement of the effects of vitamin D in improving lipid profile as reported by EL-Sherbiny and his colleagues on their experiment of the effect of nano-formulation of vitamin D in NAFLD (El-Sherbiny et al., 2018).

Conclusion

To the best of our knowledge, this is the first study about the cardioprotective effects of Nano-vitamin D on ISO induced MI.

Nano-vitamin D administration in ISO-induced MI (Group IV) has very significant cardioprotective effects as evidenced by reduction in cTn-I, decreasing lipid peroxidation product MDA and improving lipid profile compared to the conventional vitamin D.

References

- **Abdelhalim AT, Mahmoud SA, Nur NM, Shaban MA, Mansour S, Ibrahim S. (2021).** Cardioprotective Effects of Gallic Acid on an Isoprenaline-Induced Myocardial Infarction Rat Model. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 11:174.
- **Abood AM, Elshal M. (2015).** VDR stimulation improves outcome of isoprenaline-induced myocardial infarction in rats via down-regulation of cardiac inos gene expression. *26:755-764.*
- **Andrukhova O, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, et al. (2014).** Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. *Molecular Endocrinology*, 28:53-64.
- **Ansari MA, Iqbal A, Ekbbal R, Haque SE. (2019).** Effects of nimodipine, vinpocetine and their combination on isoproterenol-induced myocardial infarction in rats. *Biomedicine & Pharmacotherapy*, 109:1372-1380.
- **Antal DS, Ardelean F. (2021).** Chapter 16 - Challenges in the nanoscale delivery systems development in the pharmaceutical and nutraceutical markets. In: de Oliveira MR, editor. *Mitochondrial Dysfunction and Nanotherapeutics: Academic Press*, p 441-458.
- **Attia AAE-SA, Elfadeel A, Motawie S, Hassan MSI. (2021).** A Study of The Cardio-Protective Effect of Pioglitazone on Isoprenaline-Induced Myocardial Infarction in Male Albino Rats. *The Egyptian Journal of Hospital Medicine*, 85:2816-2822.
- **Awad HH, El-Derany MO, Mantawy EM, Michel HE, Mona M, El-Din RAS, et al. (2021).** Comparative study on beneficial effects of vitamins B and D in attenuating doxorubicin induced cardiotoxicity in rats: Emphasis on calcium homeostasis. *Biomedicine & Pharmacotherapy*, 140:111679.

- **Bhatia M, Dixit P, Kumar M, Dwivedi KL. (2021).** Impending epidemic of cardiovascular diseases among lower socioeconomic groups in India. *The Lancet Healthy Longevity*, 2: e314-e315.
- **Clerico A, Zaninotto M, Padoan A, Ndreu R, Musetti V, Masotti S, et al. (2020).** Harmonization of two hs-cTnI methods based on recalibration of measured quality control and clinical samples. *Clinica Chimica Acta* 510:150-156.
- **Contessotto Pand Pandit A. (2021).** Therapies to prevent post-infarction remodelling: from repair to regeneration. *Biomaterials*, 120906.
- **Cordeiro MM, Biscaia PB, Brunoski J, Ribeiro RA, Franco GCN, Scomparin DX. (2021).** Vitamin D supplementation decreases visceral adiposity and normalizes leptinemia and circulating TNF- α levels in western diet-fed obese rats. *Life Sciences*, 278:119550.
- **Crismaru I, Stoian AP, Bratu OG, Gaman M-A, Stanescu AMA, Bacalbasa N, Diaconu CC. (2020).** Low-density lipoprotein cholesterol lowering treatment: the current approach. *Lipids in health and disease*, 19:1-10.
- **El-Gohary OA, Allam MM. (2017).** Effect of vitamin D on isoprenaline-induced myocardial infarction in rats: possible role of peroxisome proliferator-activated receptor- γ . *Canadian journal of physiology and pharmacology*, 95:641-646.
- **El-Sherbiny M, Eldosoky M, El-Shafey M, Othman G, Elkattawy HA, Bedir T, et al. (2018).** Vitamin D nanoemulsion enhances hepatoprotective effect of conventional vitamin D in rats fed with a high-fat diet. *Chemico-Biological Interactions*, 288:65-75.
- **Emran T, Chowdhury NI, Sarker M, Bepari AK, Hossain M, Rahman GS, Reza HM. (2021).** L-carnitine protects cardiac damage by reducing oxidative stress and inflammatory response via inhibition of tumor necrosis factor-alpha and interleukin-1beta against isoproterenol-induced myocardial infarction. *Biomedicine & Pharmacotherapy*, 143:112139.
- **Frangogiannis NG. (2018).** Cell biological mechanisms in regulation of the post-infarction inflammatory response. *Current Opinion in Physiology*, 1:7-13.
- **Gaschler MM, Stockwell BR. (2017).** Lipid peroxidation in cell death. *Biochemical and biophysical research communications*, 482:419-425.
- **Gaziano TA, Gaziano JM. (2016).** Global Evolving Epidemiology, *Natural History*,

- and Treatment Trends of Myocardial Infarction. Any screen Any time Anywhere:11.
- **Guttoff M, Saberi AH, McClements DJ. (2015).** Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. *Food chemistry* 171:117-122.
 - **Haas MJ, Jafri M, Wehmeier KR, Onstead-Haas LM, Mooradian AD. (2016).** Inhibition of endoplasmic reticulum stress and oxidative stress by vitamin D in endothelial cells. *Free Radical Biology and Medicine*, 99:1-10.
 - **Hensrud D, Heimburger D. (2020).** Nutrition's interface with health and disease. *Goldman-Cecil Medicine 26th ed* Philadelphia, PA: Elsevier.
 - **Hossini A, Rajabian A, Sobhanifar M-A, Alavi MS, Taghipour Z, Hasanpour M, et al. (2022).** Attenuation of isoprenaline-induced myocardial infarction by *Rheum turkestanicum*. *Biomedicine & Pharmacotherapy* ,148:112775.
 - **Ito F, Sono Y, Ito T. (2019).** Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*, 8:72.
 - **Janjusevic M, Gagno G, Fluca AL, Padoan L, Beltrami AP, Sinagra G, et al. (2022).** The peculiar role of vitamin D in the pathophysiology of cardiovascular and neurodegenerative diseases. *Life Sciences*, 289:120193.
 - **Kariyappa AS, Ramachandregowda S, Anand S, Dundaiah B, Gopinath MM, Tekupalli R. (2019).** *Decalepishamiltonii* and its bioactive compounds protects isoproterenol-induced myocardial oxidative stress in rats. *Pharmacognosy Magazine*, 15:320.
 - **Keller K, Hobohm L, Münzel T, Ostad MA. 2019.** Sex-specific differences regarding seasonal variations of incidence and mortality in patients with myocardial infarction in Germany. *International journal of cardiology* 287:132-138.
 - **Libby P, Bonow RO, Mann DL, Tomaselli GF, Bhatt D, Solomon SD, et al. (2021).** *Braunwald's Heart Disease-E-Book: A Textbook of Cardiovascular Medicine*, Elsevier Health Sciences.
 - **Liu B, Zhang Y, Wu Q, Wang L, Hu B. (2021).** Alleviation of isoprenaline hydrochloride induced myocardial ischemia injury by brucine through the inhibition of Na⁺/K⁺-ATPase.

- Experimental Gerontology ,149:111332.
- **Mopuri R, Pulaganti M, Kareem MA, Islam MS, KR DG, Irene M, et al. (2017).** Molecular assessment of protective effect of Vitex negundo in ISO induced myocardial infarction in rats. *Biomedicine & Pharmacotherapy* , 92:249-253.
 - **Moradi A, Maroofi A, Hemati M, Hashemzade T, Alborzi N, Safari F. (2021).** Inhibition of GTPase Rac1 expression by vitamin D mitigates pressure overload-induced cardiac hypertrophy. *IJC Heart & Vasculature*, 37:100922.
 - **Morvaridzadeh M, Agah S, Alibakhshi P, Heydari H, Hoseini AS, Palmowski A, Toupchian O, et al. (2021).** Effects of Calcium and Vitamin D Co-supplementation on the Lipid Profile: A Systematic Review and Meta-analysis. *Clinical Therapeutics*, 43:274-296.
 - **Norman P, Powell J. (2014).** Vitamin D and cardiovascular disease. *Circulation research*, 114:379-393.
 - **Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W.(2010).** Vitamin D deficiency and myocardial diseases. *Molecular nutrition & food research* , 54:1103-1113.
 - **Rifai N. (2017).** Tietz Textbook of Clinical Chemistry and Molecular Diagnostics-E-Book: Elsevier Health Sciences.
 - **Saparov A, Ogay V, Nurgozhin T, Chen WC, Mansurov N, Issabekova A, et al. (2017).** Role of the immune system in cardiac tissue damage and repair following myocardial infarction. *Inflammation Research*, 66:739-751.
 - **Shahzad S, Mateen S, Hasan A, Moin S. (2019).** GRACE score of myocardial infarction patients correlates with oxidative stress index, hsCRP and inflammation. *Immunobiology* 224:433-439.
 - **Sharma AK, Kumar A, Taneja G, Nagaich U, Deep A, Datusalia AK, et al. (2018).** Combined and individual strategy of exercise generated preconditioning and low dose copper nanoparticles serve as superlative approach to ameliorate ISO-induced myocardial infarction in rats. *Pharmacological Reports*, 70:789-795.
 - **Sosa-Henríquez M. (2020).** Cholecalciferol and calcifediol for vitamin D supplementation. *Osteoporosis International* ,31:391-392.
 - **Su D, Wang X, Zhang W, Li P, Tang B. (2022).** Fluorescence imaging for visualizing the bioactive molecules of lipid peroxidation within biological

- systems. *TrAC Trends in Analytical Chemistry*, 146:116484.
- **Su L-J, Zhang J-H, Gomez H, Murugan R, Hong X, Xu D, et al. (2019).** Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative medicine and cellular longevity*, 2019.
 - **Tajika K, Okamatsu K, Takano M, Inami S, Yamamoto M, Murakami D, et al. (2012).** Malondialdehyde-modified low-density lipoprotein is a useful marker to identify patients with vulnerable plaque. *Circulation Journal*, CJ-12-0183.
 - **Wang J, Ni Q, Wang Y, Zhang Y, He H, Gao D, et al. (2021).** Nanoscale drug delivery systems for controllable drug behaviors by multi-stage barrier penetration. *Journal of Controlled Release*, 331:282-295.
 - **Wong ZW, Thanikachalam PV, Ramamurthy S. (2017).** Molecular understanding of the protective role of natural products on isoproterenol-induced myocardial infarction: A review. *Biomedicine & Pharmacotherapy*, 94:1145-1166.
 - **Yin Y, Han W, Cao Y. (2019).** Association between activities of SOD, MDA and Na⁺-K⁺-ATPase in peripheral blood of patients with acute myocardial infarction and the complication of varying degrees of arrhythmia. *Hellenic Journal of Cardiology*, 60:366-371.
 - **Zhang J, Yang Z, Zhang S, Xie Z, Han S, Wang L, et al. (2020a).** Investigation of endogenous malondialdehyde through fluorescent probe MDA-6 during oxidative stress. *Analytica Chimica Acta*, 1116:9-15.
 - **Zhang M, Chen P, Li H. (2020b).** PCV19 Economic Burden of Disease on Patients with ACUTE Myocardial Infarction Combining Dyslipidemia in China. *Value in Health Regional Issues*, 22: S29.
 - **Zhang W, Yi J, Liu D, Wang Y, Jamilian P, Gaman M-A, et al. (2022).** The effect of vitamin D on the lipid profile as a risk factor for coronary heart disease in postmenopausal women: a meta-analysis and systematic review of randomized controlled trials. *Experimental Gerontology*, 161:111709.