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ORIGINAL RESEARCH

Diosgenin Ameliorates Cardiac Function following Myocardial Ischemia Through Angiogenic and Anti-Fibrotic Properties; An Experimental Study

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Abstract: **Introduction:** Angiogenesis through restoration of blood supply to the ischemic myocardium is a pivotal process that contributes to cardiac repair and leads to improvement of myocardial function. This study was conducted to evaluate cardioprotective effects of Diosgenin against myocardial infarction (MI) with focus on angiogenesis, myocardial fibrosis, and oxidative stress. **Methods:** 4 groups of male Wistar rats were considered for this study: (1) sham, (2) MI, (3) MI+Vehicle and (4) MI+Diosgenin. MI model was created by occluding left anterior descending (LAD) artery for 30 minutes and reperfusion was established for 14 days by opening this artery. Diosgenin (50 mg/kg) was given orally to the rats for 21 days (from 7 days before MI induction until the end of the 14-day reperfusion period). Cardiac injury markers including troponin I, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) were measured using enzyme-linked immunosorbent assay (ELISA), same as cardiac stress oxidative markers (superoxide dismutase (SOD), Malondialdehyde (MDA), reduced glutathione (GSH)). Echocardiography was used to measure heart function parameters and myocardial fibrosis was assessed via a specific tissue staining named Massons trichrome. Blood vessel staining kit was used to assess left ventricular angiogenesis. **Results:** Ischemia-reperfusion injury increased serum levels of troponin I, CK-MB and LDH, as well as cardiac malondialdehyde (MDA) and myocardial fibrosis. MI also decreased myocardial function (Ejection fraction (EF)% and Fractional shortening (FS)%) and Diosgenin treatment reversed these parameters. Capillary density as marker of angiogenesis significantly increased in all of MI groups. However, development of angiogenesis was significantly higher in Diosgenin group compared with MI group. **Conclusion:** Diosgenin exerts cardioprotective effects against ischemia-reperfusion injury by strengthening cardiac antioxidant defense and reducing deposition of collagen fibers. It seems that the strengthening of angiogenesis in heart tissue is one of the main mechanisms of Diosgenin to increase the heart's resistance against ischemia.

Keywords: Myocardial infarction; Reperfusion injury; Angiogenesis; Diosgenin

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1. Introduction

Myocardial infarction (MI), the most common cardiovascular disorder and one of the leading reasons of death around the world, is caused by coronary artery occlusion (1, 2). It has been reported that 620,000 cases of new coronary attacks and 295,000 cases of recurrent attack occur in the United States of America annually (3). Owing to low oxygen content in the infarcted region, MI confers massive death of cardiomyocytes, endothelial cells and fibroblasts, which in turn results in the induction of pathogenic inflammatory responses, collagen deposition and scar formation, and subsequently reduction of heart function (4, 5). Excessive generation of reactive oxygen species (ROS) in the ischemic myocardium after reperfusion leads to damages to the cell membrane and initiation of necrotic or apoptotic cell death (6).

Additionally, ROS hinders migration of endothelial progenitor cells (EPCs) to the sites of injury and initiation of angiogenic process in the ischemic heart (7). Therefore, suppression of oxidative stress can contribute to increasing angiogenesis and improvement of heart function after MI (8). Although many therapeutic strategies such as angiotensin II receptor antagonists, calcium channel blockers, and clinical reperfusion intervention have been used in recent years, their clinical use has been challenged due to many adverse effects and poor patient adherence to them (9). Supplementation of exogenous antioxidants and therapeutic agents that reinforce antioxidant defenses of the cardiomyocytes has been reported to be good for combating excessive produc-

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tion of ROS and oxidative stress following MI (10). Likewise, angiogenesis therapy has been proposed as one promising strategy to treat cardiovascular diseases such as MI (11).
Diosgenin is known as a steroidal saponin, found in some plants such as Dioscorea species and the Solanum that can act as anti-inflammatory, anti-tumor, anti-atherosclerotic and anti-oxidant agents (12-14). Likewise, Diosgenin participates in modulation of numerous physiological processes such as glucose and lipid metabolism, and inhibition of intracellular ROS generation (15-17). It has been recognized that a diet rich in Diosgenin reduces incidence of coronary artery disorders relevant to estrogen deficiencies in humans and animals (18, 19). Several previous studies have shown that diosgenin can contribute to prevention of lipid peroxidation, vascular calcification and aortic remodeling (20, 21). Thus, the present study was aimed to investigate the effect of Diosgenin on angiogenesis, oxidative stress, and myocardial fibrosis in a rat model of MI.

2. Methods

2.1. Study design and setting

This experimental study was conducted on 4 groups of male Wistar rats (1) sham, (2) MI, (3) MI+Vehicle and (4) MI+Diosgenin. MI modeling was created by occluding left anterior descending (LAD) artery for 30 minutes and reperfusion was established for 14 days by opening this artery. Diosgenin (50 mg/kg) was given orally to the rats for 21 days (from 7 days before MI induction until the end of the 14-day reperfusion period). Cardiac injury markers including troponin I, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) were measured using enzyme-linked immunosorbent assay (ELISA), same as cardiac stress oxidative markers (superoxide dismutase (SOD), Malondialdehyde (MDA), reduced glutathione (GSH)). Echocardiography was used to measure heart function parameters and myocardial fibrosis was assessed via a specific tissue staining named Masson's trichrome. Blood vessel staining kit was used to assess left ventricular angiogenesis.
The Ethics Committee of Iran University of Medical Sciences approved all experiments of this study (ethical code number: IR.IUMS.REC 1395.95-04-130-30007).

2.2. Chemicals

Diosgenin was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals were purchased from standard commercial suppliers.

2.3. Animals

Healthy adult male Wistar rats with an average weight between 250–300 gr were obtained from animal laboratory center, Iran University of Medical Sciences, Tehran, Iran. All animals were fed with rat's standard chow and had free access to drinking water. They were placed in a controlled temperature of 23–25°C and humidity of 55–70% on light–dark cycle (12h and 12h). One week prior to experiments, rats were acclimatized to animal lab conditions. Handling of animals was in consonance with the Guide for the Care and Use of Laboratory Animals of the Animal Care Committee of Iran University of Medical Science and the US National Institutes of Health guidelines for the care and use of laboratory animals (NIHPublicationNo.85–23, revised 1996).

2.4. Experimental design

48 rats were randomly divided into four groups: (1) sham operated group (n=12); these animals received all the surgical procedures except for the ligation of left anterior descending coronary artery (LAD), (2) MI group (n=12); rats were only subjected to 30 minutes of LAD ligation and then reperfusion was established for 14 days, (3) Vehicle group (n=12); animals underwent 30 minutes of LAD ligation and were treated with corn oil via gavage administration as the solvent of diosgenin for 14 consecutive days, and (4) MI + diosgenin group (n=12); animals were subjected to 30 minutes of LAD ligation and then treated with diosgenin (50 mg/kg) for 14 consecutive days (gavage administration).

2.5. Induction of acute MI model

The animals were weighed and a mixture of ketamine (60mg/kg) and xylazine (5mg/kg) was infused intraperitoneally for induction of anesthesia. Then they were fixed in supine position on the operating work surface. Body temperature of animals were monitored and controlled by thermal pad and heating lamp to ensure its maintenance at 37 °C during surgery. Animals were intubated and ventilated with room air through a rodent ventilator (tidal volume 2–3 mL, respiratory rate 65–70 per min). In the next step, a left thoracotomy at the fourth intercostal space was created to expose heart and incise pericardium in order to ligate LAD at 1–2 mm distal from tip of the left atrial appendix. A 6–0 silk suture slip knot was placed below its main branch. Then, both ends of the silk suture were passed through a small vinyl tube, pulled and tightened to induce ischemia for 30 minutes. ST segment elevation via lead II electrocardiography (ECG) recording and regional paleness of the myocardial surface after LAD ligation approved induction of ischemia.

2.6. Assessment of cardiac functional parameters

Transthoracic two dimensional (2D) guided M-mode echocardiography (HP Sonos 7500 System; Philips, Tampa, USA) was carried out 14 days after surgery to investigate cardiac function. A 10 MHz linear array transducer at 150 mm/s speed was used to perform the experiment. Animals were sedated with a mixture of ketamine and xylazine and after shaving the left side of the chest, they were placed on the operation desk in supine position to prepare clear images. In order to measure ejection fraction (EF), fractional shortening (FS), left ventricular internal diameter in diastole (LVIDd), and left ventricular internal diameter in

systole (LVIDs), parasternal 2D short-axis view at the level of papillary muscles was performed.

2.7. Assessment of myocardial enzymes

1 day after surgery and interventions, blood samples were collected and centrifuged at 3500 rpm for 5 min. In the next step, serum levels of CK-MB and LDH as markers of myocyte necrosis were determined using commercially available kits (CK-MB (116 079 H917), LDH (122 395 H917) Pars Azmoon, Tehran, Iran) by using auto analyzer (Roche Hitachi Modular DP Systems; Mannheim, Germany) following the manufacturer's instructions. Cardiac Troponin-I (cTn-I), biomarker of cardiac injury, detected by a specific ELISA Kit from Monobind Inc. according to the instructions (Lake Forest, California, USA).

2.8. Assessment of oxidative stress markers

The contents of reduced glutathione (GSH), the activity of superoxide dismutase (SOD) and Malondialdehyde (MDA) were measured by calorimetrically enzymatic assay kits (ZellBio GmbH, Ulm, Germany) in accordance with the manufacturer's instructions.

2.9. Assessment of interstitial fibrosis

In order to evaluate interstitial fibrosis at the end of the experimental period, Masson's trichrome staining was used. Briefly, heart tissues were fixed in 10% formalin for 24-48 hours. In the next step, paraffin embedded blocks were prepared from 4 hearts in each group, then 6-micron sections were prepared from these tissue blocks using microtome. After staining, these slices were evaluated under a light microscope. 5 slices were selected from each heart and 5 random fields were selected in each slice, and Images were taken using digital camera photograph. Finally, the ratio of the blue areas to the total surface of the left ventricle was calculated using Image J software (Image J, version 1.51).

2.10. Assessment of capillary density

At 14 days post MI, 6 m paraffin embedded sections were used for angiogenesis immunohistochemical staining. A blood vessel staining kit peroxidase system (Cat No. ECM 590, Millipore, USA & Canada) was used to evaluate capillary density (number/mm^2). The primary antibody was Anti-von Willebrand Factor, an endothelial cell marker. Vascular density was detected through counting the number of vessels within the peri-infarct area under a light microscope (×400). A digital camera was used to take photographs. An examiner blinded to experimental design carried out counting the vessels in 6 random fields within each section in 6 samples in each group. The number of vessels was reported as the number of vessels/mm^2.

2.11. Statistics analysis

Data were expressed as means ± standard error of the mean (S.E.M.). GraphPad Prism-5statistic software (LaJolla, CA, USA) was used for data analysis. Comparisons between three or more groups were performed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. P value less than 0.05 was deemed significant.

3. Results

3.1. Effect of diosgenin on cardiac injury marker

Figure 1 illustrates the effect of diosgenin on serum levels of LDH, CK-MB, and cTnI in the serum of different experimental groups at 24 hours after reperfusion. Statistical analysis showed significant elevation in LDH in MI (P<0.0001) and Vehicle-treated groups (P<0.0001) compared with sham animals. However, treatment with diosgenin (50 mg/kg) 7 days before induction of MI and 1 day after reperfusion markedly reduced levels of LDH compared to MI group at 24h after reperfusion (P=0.0008). With regard to CKMB, there was a significant rise in MI (P<0.0001), MI+vehicle (P<0.0001) and MI+diosgenin (P=0.0463) groups in comparation to Sham group. Same as LDH, diosgenin consumption decreased serum levels of CKMB compared to MI group (P=0.0019). Our results showed significant increase in cardiac troponin levels in both MI and MI+vehicle groups (P<0.0001) in comparison to sham animals. Treatment with diosgenin led to significant decrease of this marker compared to MI group (P=0.0016); however, there was significant difference between animals in diosgenin-treated group and sham animals (P=0.0384) in this regard.

3.2. Effect of diosgenin on cardiac function

As shown in figure 2, we noted a significant decrease in ejection fraction (EF%) in MI (P=0.0003) and MI+Vehicle-treated (P=0.0007) groups compared to sham operated animals. EF was markedly restored in the diosgenin-treated group compared with the MI group (P=0.0028). Fractional shortening (FS%) showed significant decrease in MI (P=0.0002) and MI+vehicle (P=0.0007) groups in comparison to sham group and treatment with diosgenin significantly refined FS compared to MI group (P=0.0184). Our results also showed that the left ventricle (LV) dimensions did not change during systole and diastole in all experimental groups.

3.3. Effect of diosgenin on cardiac oxidative stress markers

As shown in Figure 3, activity of SOD was markedly reduced in MI (P<0.0001) and Vehicle (P<0.0001) groups in comparison with sham group. Animals treated with diosgenin (50 mg/kg) for 7 days before induction of MI and 14 days after reperfusion exhibited significant increase in SOD activity compared with MI group (P<.0088). Content of GSH was significantly reduced in MI (P=0.0007) and vehicle (P=0.0011) groups by induction of LAD ligation, and treatment with diosgenin restored GSH content compared to MI group (P=0.0127).

On the other hand, induction of MI in animals significantly

elevated MDA levels compared with sham group (P<0.0001). MDA levels were markedly reduced in the diosgenin-treated group compared with MI group (P=0.0014).

3.4. Effect of diosgenin on myocardial fibrosis

Our results showed that there were more collagen deposition in the heart tissues of the animals subjected to MI compared to sham group, and treatment with diosgenin decreased collagen deposition (Figure 4). Quantitative analysis also showed that induction of MI increased fibrosis compared with sham group (P<0.0001 for MI and vehicle, P=0.0095 for diosgenin). Diosgenin decreased fibrosis formation compared to MI (P=0.0285) and MI+vehicle (P=0.0176) groups, however there were still significant myocardial fibrosis in the diosgenin-treated group compared with sham group.

3.5. Effect of diosgenin on capillary density

Myocardial sections stained with Anti-von Willebrand Factor antibody showed that there was elevation in the capillary density in all rats with MI (P=0.0443 for MI, P=0.0269 for vehicle, and P<0.0001 for diosgenin compared to sham group). The MI animals treated with diosgenin (50 mg/kg) 7 days before induction of MI and 14 days after reperfusion showed a higher amount of capillary density compared with the MI group (P=0.0100) (Figure 5).

4. Discussion

Cardioprotection means maintaining the function of the heart against harmful stressors to the heart. In fact, any mechanism that leads to the reduction of cardiac tissue damage in pathological conditions can have cardioprotective effects. So strategies such as strengthening the antioxidant system or preventing cardiac remodeling will have cardioprotective effects. In this work, we provided new aspects of cardioprotective effects of diosgenin against MI. First, our findings showed that diosgenin could contribute to preservation of cardiac function through reduction of oxidative stress markers and reinforcement of endogenous antioxidant system. Second, diosgenin could reduce myocardial fibrosis and stimulate angiogenesis.

Creatine kinase (CK) is an enzyme that contributes to the phosphorylation of creatine. Three sub-members of this enzyme have been recognized in the brain (CK-BB), heart (CK-MB) and muscle (CK-MM). Elevated serum CK-MB activity is an indicator for diagnosis of MI (22). On the other hand, lactate dehydrogenase (LDH) release from damaged tissue is an indicator of cell membrane damage. In keeping with previous studies, our findings showed that cardiac injury markers such as LDH, CK-MB, and cTnI increased in MI and were reduced by diosgenin. In contrast with our findings, it has been shown that only oral administration of diosgenin could not reduce cardiac marker enzymes such as LDH and CK-MB, while its combination with exercise training markedly decreased these enzymes. It should be noted that the dose of diosgenin in our study (50 mg/kg) was very higher than the above-mentioned study (15 mg/kg) (23). Diosgenin has been reported to exert cardioprotective effects against doxorubicin-induced cardiotoxicity through restoring activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as reducing levels of thiobarbituric acid relative substances (TBARS) (15). Likewise, another study showed that diosgenin exerts anti-lipoperoxidative and membrane stabilizing effects in coping with myocardial infarction (24). These are in consonance with our findings, which demonstrated that induction of myocardial damages by LAD ligation resulted in overwhelming endogenous antioxidant system and increasing lipid peroxidation that were reversed by treatment with 50 mg/kg concentration of diosgenin. Additionally, other studies confirmed protective effects of diosgenin against ischemia/ reperfusion injuries by restoring activities of antioxidant enzymes such as catalase (25).

Some previous studies have shown that diosgenin had cardioprotective effects against myocardial ischemia reperfusion injury by inhibiting acute inflammatory responses and improving cardiac function parameters such as left ventricular end-diastolic pressure (26, 27). In keeping with previous reports, our observations showed that treatment with 50 mg/kg concentration of diosgenin during 7 days before induction of MI and 14 days after MI led to restoration of EF and FS, subsequently improving heart function. Thus, it seems that diosgenin by reducing oxidative damages may be able to preserve myocardial tissue against damages, and prevent the expansion of fibrotic area and myocardial dysfunction. Furthermore, our results indicated that diosgenin reduced collagen deposition and subsequently, myocardial fibrosis in the MI rats. In agreement with our observation in Masson's trichrome staining, Zhou et al. found that diosgenin was able to reduce cardiac fibrosis by suppressing angiotensin II-induced extracellular matrix remodeling in cardiac fibroblasts via modulation of the TGF-1/Smad3 signaling pathway (28). Therefore based on these findings, diosgenin prevents left ventricular collagen deposition and formation of fibrosis, probably via TGF- dependent mechanism and preventing maladaptive left ventricular remodeling. Diosgenin preserved heart tissue integrity via decreasing left ventricular remodeling, thereby improving left ventricular function at day 14 after induction of MI.

To provide greater insights regarding mechanisms through which diosgenin exerts cardioprotective effects against MI, we further evaluated its effects on angiogenesis process. Angiogenesis is defined as the formation of new capillary blood vessels at capillary level that contributes to restoring blood supply to the affected area. Angiogenesis is important for survival of cells during wound healing, MI, cancer, inflammation, fractures, and burns (29). As a result of vessel permeability owing to degradation of basement membrane and proteolysis of the surrounding extracellular matrix, the migration and proliferation of endothelial cells leads to formation of new capillary blood vessels to ensure sufficient oxy-

gen and nutrient supplementation to the desired tissue (30). Our findings showed that LAD ligation for 30 min followed by 14 days of reperfusion resulted in increased left ventricular angiogenesis. Angiogenesis with diosgenin treatment was stronger compared to MI and vehicle groups. This is in accordance with another study that has shown that diosgenin induced angiogenesis via a hypoxia-inducible factor-1-dependent mechanism and activation of p38 mitogen-activated protein kinase and Akt signaling pathways in osteoblasts (31). Thus, it seems that diosgenin could increase new blood formation in the peri-infarcted area via Akt-dependent signaling, in turn increasing blood supply to the area at risk and preserving function of cardiomyocytes and preventing cell loss. Through this mechanism, it may also cause improvement of myocardial function as seen in the echocardiography. Angiogenic and anti-remodeling properties of diosgenin must be addressed more mechanistically and investigated in the future studies.

5. Limitations

In this study, we could not assess effects of diosgenin on angiogenic factors such as Vascular endothelial growth factor (VEGF) via western blot analysis and molecular mechanism of the anti-fibrotic properties of the diosgenin because of the financial limitations. The authors suggest more basic studies in order to more precisely understand the mechanisms of cardioprotective effects of diosgenin.

6. Conclusions

Collectively, our observations showed that treatment with diosgenin (50 mg/kg) 7 days before induction of MI and 14 days after reperfusion can result in cardioprotection in the rat model of MI. These protective effects can be linked to suppression of oxidative stress and myocardial fibrosis as well as reinforcement of endogenous antioxidant system, improvement of cardiac function, and increasing angiogenesis.

7. Declarations

7.1. Acknowledgments

This study was financially supported by research affairs of Iran University of Medical Sciences (Tehran, Iran, grant number: 95-04-130-30007).

7.2. Author Contribution

Y.A: Study design, execution, data analysis, manuscript drafting, and critical discussion. K.R: Data collection and analysis, manuscript writing and drafting. M.S.P and A.M: performing experiments and writing draft.

7.3. Funding

The study was supported by Iran University of Medical Sciences, Tehran, Iran.

7.4. Declarations of interest

Authors have no conflict of interest to declare.

7.5. Data Availability

Data of the study will be provided if anyone needs them.

7.6. Using artificial intelligence chatbots

None.

References

1. Pletsch-Borba L, Grafetstätter M, Hüsing A, Maldonado SG, Kloss M, Groß M-L, et al. Biomarkers of vascular injury in relation to myocardial infarction risk: A population-based study. Sci Rep. 2019; 9(1):3004.
2. Choopani S, Imani A, Faghihi M, Askari S, Edalatyzadeh Z. Chronic Sleep Deprevation And Ventricular Arrhythmias: Effect Of Symphatic Nervous System. J Cell Mol Anesth. 2016;1(2):e149508.
3. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. Executive summary: heart disease and stroke statistics—2014 update: a report from the American Heart Association. Circulation. 2014;129(3):399-410.
4. Janicki JS, Brower GL, Gardner JD, Chancey AL, Stewart JA. The dynamic interaction between matrix metalloproteinase activity and adverse myocardial remodeling. Heart fail rev. 2004; 9(1):33-42.
5. Rakhshan K, Azizi Y, Naderi N, Afousi AG, Aboutaleb N. ELABELA (ELA) peptide exerts cardioprotection against myocardial infarction by targeting oxidative stress and the improvement of heart function. Int J Pept Res Ther. 2019;25(2):613-21.
6. Saporito F, Baugh LM, Rossi S, Bonferoni MC, Perotti C, Sandri G, et al. In Situ Gelling Scaffolds Loaded with Platelet Growth Factors to Improve Cardiomyocyte Survival after Ischemia. Acs biomater sci eng. 2018; 5(1):329-38.
7. Cheng Y, Jiang S, Hu R, Lv L. Potential mechanism for endothelial progenitor cell therapy in acute myocardial infarction: Activation of VEGF-PI3K/Akte-NOS pathway. Ann Clin Lab Sci. 2013; 43(4):395-401.
8. Jin P, Li T, Li X, Shen X, Zhao Y. Suppression of oxidative stress in endothelial progenitor cells promotes angiogenesis and improves cardiac function following myocardial infarction in diabetic mice. Exp Ther Med. 2016; 11(6):2163-70.
9. Swieczkowski D, Mogielnicki M, Cwalina N, Zuk G, Pisowodzka I, Ciecwierz D, et al. Medication adherence in patients after percutaneous coronary intervention due to acute myocardial infarction: From research to clinical implications. Cardiol J. 2016; 23(5):483-90.
10. Souri F, Rakhshan K, Erfani S, Azizi Y, Maleki SN, Aboutaleb N. Natural lavender oil (Lavandula angustifolia) exerts cardioprotective effects against myocardial in-

farction by targeting inflammation and oxidative stress. Inflammopharmacology. 2019;27(4):799-807.
11. Lee DJ, Cavasin MA, Rocker AJ, Soranno DE, Meng X, Shandas R, et al. An injectable sulfonated reversible thermal gel for therapeutic angiogenesis to protect cardiac function after a myocardial infarction. J Biol Eng. 2019; 13(1):6.
12. Hirai S, Uemura T, Mizoguchi N, Lee JY, Taketani K, Nakano Y, et al. Diosgenin attenuates inflammatory changes in the interaction between adipocytes and macrophages. Mol Nutr Food Res. 2010; 54(6):797-804.
13. Moalic S, Liagre B, Corbière C, Bianchi A, Dauça M, Bordji K, et al. A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells. FEBS letters. 2001;506(3):225-30.
14. Sethi G, Shanmugam MK, Warrier S, Merarchi M, Arfuso F, Kumar AP, et al. Pro-apoptotic and anti-cancer properties of diosgenin: A comprehensive and critical review. Nutrients. 2018;10(5):645.
15. Chen C-T, Wang Z-H, Hsu C-C, Lin H-H, Chen J-H. In vivo protective effects of diosgenin against doxorubicin-induced cardiotoxicity. Nutrients. 2015;7(6):4938-54.
16. McAnuff MA, Omoruyi FO, Morrison EY, Asemota HN. Plasma and liver lipid distributions in streptozotocin-induced diabetic rats fed sapogenin extract of the Jamaican bitter yam (Dioscorea polygonoides). Nutr Res. 2002; 22(12):1427-34.
17. McAnuff MA, Harding WW, Omoruyi FO, Jacobs H, Morrison EY, Asemota HN. Hypoglycemic effects of steroidal sapogenins isolated from Jamaican bitter yam, Dioscorea polygonoides. Food Chem Toxicol. 2005;43(11):1667-72.
18. Esfandiarei M, Lam JT, Yazdi SA, Kariminia A, Dorado JN, Kuzeljevic B, et al. Diosgenin modulates vascular smooth muscle cell function by regulating cell viability, migration, and calcium homeostasis. J Pharmacol Exp Ther. 2011;336(3):925-39.
19. Manivannan J, Shanthakumar J, Silambarasan T, Balamurugan E, Raja B. Diosgenin, a steroidal saponin, prevents hypertension, cardiac remodeling and oxidative stress in adenine induced chronic renal failure rats. RSC Adv. 2015; 5(25):19337-44.
20. Pari L, Monisha P, Jalaludeen AM. Beneficial role of diosgenin on oxidative stress in aorta of streptozotocin induced diabetic rats. Eur J Pharmacol. 2012;691(1-3):143-50.
21. Manivannan J, Barathkumar T, Sivasubramanian J, Arunagiri P, Raja B, Balamurugan E. Diosgenin attenuates vascular calcification in chronic renal failure rats. Mol Cell Biochem. 2013;378(1-2):9-18.
22. Puleo PR, Guadagno PA, Roberts R, Scheel MV, Marian AJ, Churchill D, et al. Early diagnosis of acute myocardial infarction based on assay for subforms of creatine kinase-MB. Circulation. 1990;82(3):759-64.
23. Salimeh A, Mohammadi M, Mohaddes G, Badalzadeh R. Protective effect of diosgenin and exercise training on biochemical and ECG alteration in isoproterenol-induced myocardial infarction in rats. Iran J Basic Med Sci. 2011; 14(3): 264-274.
24. Jayachandran K, Vasanthi HR, Rajamanickam G. Antilipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction. Mol Cell Biochem. 2009;327(1-2):203-10.
25. Oyelaja-Akinsipo OB, Dare EO, Katare DP. Protective role of diosgenin against hyperglycaemia-mediated cerebral ischemic brain injury in zebrafish model of type II diabetes mellitus. Heliyon. 2020;6(1):e03296.
26. Wang H-W, Liu H-J, Cao H, Qiao Z-Y, Xu Y-W. Diosgenin protects rats from myocardial inflammatory injury induced by ischemia-reperfusion. Medical science monitor: Int J Exp Clin Res. 2018; 24:246-253.
27. Ebrahimi H, Badalzadeh R, Mohammadi M, Yousefi B. Diosgenin attenuates inflammatory response induced by myocardial reperfusion injury: role of mitochondrial ATP-sensitive potassium channels. J Physiol Biochem. 2014;70(2):425-32.
28. Zhou HT, Yu XF, Zhou GM. Diosgenin inhibits angiotensin II-induced extracellular matrix remodeling in cardiac fibroblasts through regulating the TGF-1/Smad3 signaling pathway. Mol Med Rep. 2017; 15(5):2823-8.
29. Badimon L, Borrell M. Microvasculature recovery by angiogenesis after myocardial infarction. Curr Pharm Des. 2018;24(25):2967-73.
30. Ferrara N, Gerber H-P. The role of vascular endothelial growth factor in angiogenesis. Acta haematol. 2001;106(4):148-56.
31. Yen ML, Su JL, Chien CL, Tseng KW, Yang CY, Chen WF, et al. Diosgenin induces hypoxia-inducible factor-1 activation and angiogenesis through estrogen receptor-related phosphatidylinositol 3-kinase/Akt and p38 mitogen-activated protein kinase pathways in osteoblasts. Mol pharmacol. 2005;68(4):1061-73.

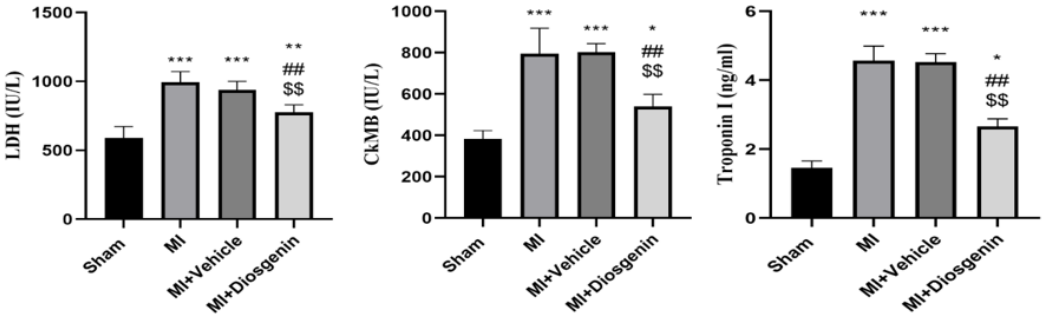

Figure 1: The level of cardiac injury markers (n=4 per group). A) LDH: Lactate dehydrogenase (IU/L), B) CK-MB: creatine kinase myocardial band (IU/L), and C) Troponin I (ng/ml). MI: myocardial infarction. Data are presented as means ± standard error of the mean (S.E.M.). *p< 0.05, **p< 0.01, and ***p< 0.001 vs. Sham, ## p< 0.01 vs. MI, and $$ p< 0.01 vs. MI + Vehicle.

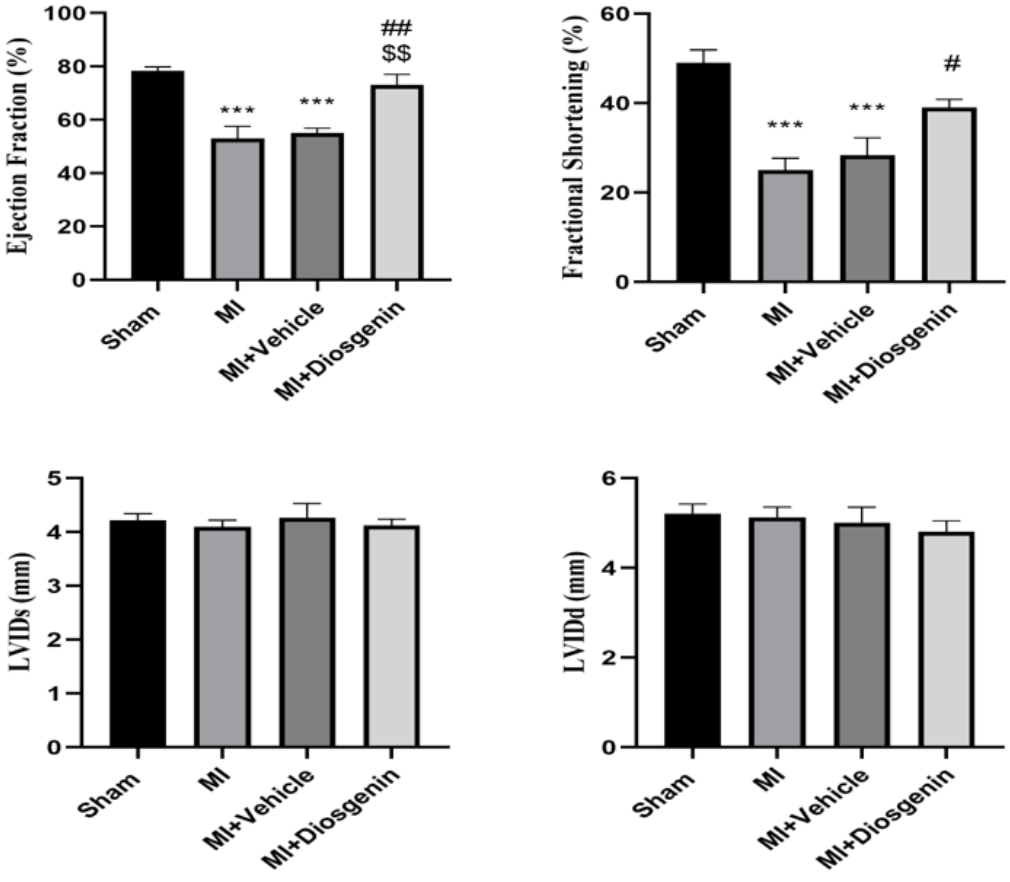

Figure 2: Cardiac function (n=5). LVIDs: left ventricular internal diameter in systole, LVIDd: Left ventricular internal diameter in diastole, MI: myocardial infarction. Data are presented as means ± standard error of the mean (S.E.M.). ***p< 0.001 vs. Sham, # p< 0.05 and ## p< 0.01vs. MI, and $$ p< 0.01 vs. MI + Vehicle.

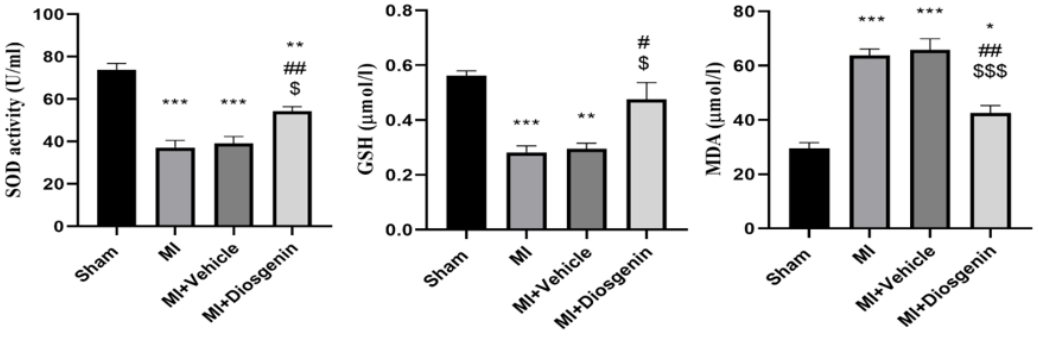

Figure 3: Oxidative stress markers in cardiac tissue (n=4). Left) SOD: Superoxide dismutase, Middle) GSH; Glutathione, and Right) MDA; Malondialdehyde. MI: myocardial infarction. Data are presented as means ± standard error of the mean (S.E.M.). **p< 0.01 and ***p< 0. 001 vs. Sham, # p< 0.05 and ## p< 0.01 vs. MI, and $ p< 0.05 and $$$ p< 0. 001 vs. MI + Vehicle.

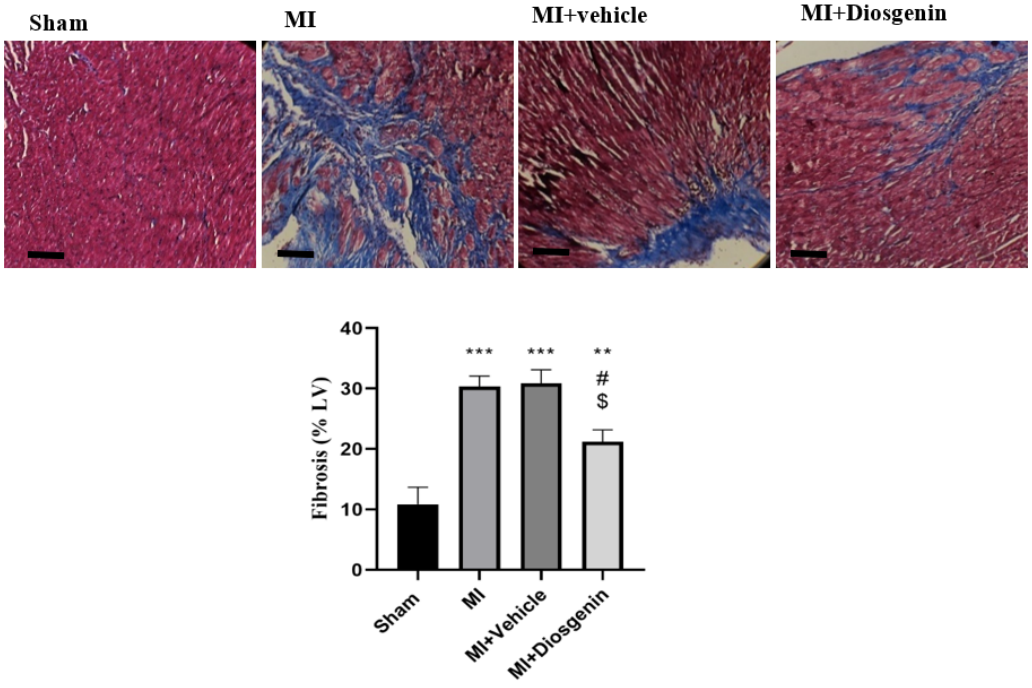

Figure 4: 6-micrometer cardiac sections stained with Masson's trichrome stain (n=4). Left) Masson's trichrome stained specimens, 400x magnification. Right) statistical analysis of fibrosis. Data are presented as means ± standard error of the mean (S.E.M.). **p< 0.01 and ***p< 0.001 vs. Sham, # p< 0.05 vs. MI, and $ p< 0.05 vs. MI + Vehicle. Bar: 100 micrometers.

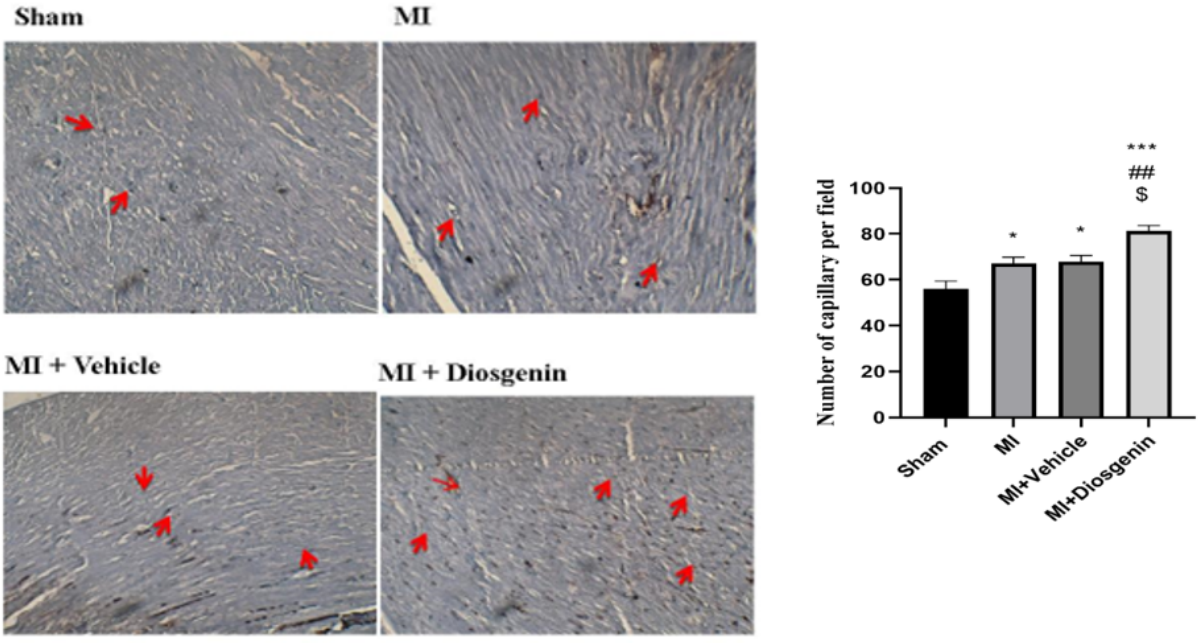

Figure 5: Immunohistochemical staining (left) and statistical analysis of neovascularization (right) at day 14 after myocardial infarction (n=4). Images demonstrated capillary density stained with Anti-von Willebrand factor antibody in different experimental groups. 400x magnification. Red arrows show microvessels. Data are presented as means ± standard error of the mean (S.E.M.). *p< 0.05 and ***p< 0. 001 vs. Sham, ## p< 0.01 vs. MI, and $ p< 0. 05 vs. MI + Vehicle.