Available online at http://jbcp.shahed.ac.ir/

Journal of Basic and Clinical
PATHOPHYSIOLOGY

Volume 4, Number 1, Autumn-Winter 2015-2016

Diosgenin attenuates cardiac oxidative stress in streptozotocin- induced diabetic rat

Jafar Golshahi, Farshad Roghani-Dehkordi*

Department of Cardiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Article info
Received: 03 Nov 2016
Revised: 10 Feb 2016
Accepted: 17 Feb 2016

p-ISSN:2322-1895
e-ISSN: 2345-4334

Key Words:
Diosgenin
Diabetes mellitus
Streptozotocin
Heart
Oxidative stress
Antioxidant

ABSTRACT

Background and Objective: Chronic diabetes mellitus (DM) leads to cardiovascular dysfunction. Diosgenin is a natural steroidal saponin with cardiovascular protective potential. In this research study, the beneficial effect of diosgenin was evaluated on some markers of oxidative stress in cardia tissue of streptozotocin (STZ)-diabetic rats.

Materials and Methods: Male Wistar rats (n = 28) were divided into equal-sized control, diosgenin-treated control, diabetic, and diosgenin-treated diabetic groups. Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg). Diosgenin was administered *p.o.* at a dose of 40 mg/kg for 7 weeks. Some markers of oxidative stress including malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) were measured in cardiac tissue homogenate.

Results: It was found out that diabetic group had an elevated MDA content ($p<0.01$), reduced activity of SOD ($p<0.05$) and catalase ($p<0.05$) and a lower content of GSH ($p<0.01$) versus control group and chronic diosgenin treatment significantly reversed only MDA ($p<0.05$) and GSH ($p<0.05$) with no significant effect on SOD activity and catalase activity.

Conclusion: Diosgenin could attenuate cardiac lipid peroxidation and improves non-enzymatic antioxidant defensive system in diabetic condition and it may be considered as a potential therapeutic agent to mitigate cardiac dysfunction in DM.

1. Introduction

Diabetes mellitus (DM) is accompanied with significant rate of morbidity and mortality and markedly contributes to pathogenesis of cardiovascular diseases (1). Chronic vascular abnormalities are usually the most complex and consequence of DM in the society (2). Hyperglycemia and enhanced oxidative stress are mainly responsible for endothelium dysfunction and cardiac dysfunction in DM (1, 3) (4-6). Reduced generation and release of endothelium-derived relaxing factors like nitric oxide (NO) in combination with enhanced production of reactive oxygen species (ROS) is an important determinant of cardiovascular dysfunction in DM (7).

***Corresponding Author:** *Farshad Roghani-Dehkordi*
Department of Cardiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: frdehkordi@gmail.com

Diosgenin is a naturally occurring aglycone that is abundantly present in fenugreek. This steroidal saponin is being used as a traditional medicine for DM (1, 8, 9). This substance has shown cardiovascular protective effect (10, 11), hypoglycemic and anti-hyperglycemic effect (12, 13) and attenuation of hyperlipidemia (14) and reduction of oxidative stress and inflammation (15). In addition, diosgenin could prevent aortic damage due to enhanced oxidative stress in DM by potentiation of antioxidant defensive system and mitigation of lipid peroxidation (16). It has been suggested that diosgenin could possess therapeutic efficacy for cardiovascular disorders of DM. Meanwhile, diosgenin could improve insulin resistance by alleviating metabolic dysregulation of lipid profile in both plasma and affected tissues (17). Therefore, this study was designed to evaluate the effect of diosgenin treatment on mitigation of oxidative stress in cardiac tissue from STZ-diabetic rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats at an initial body weight of 210-280 g (n = 28) were procured from Pasteur's Institute (Tehran, Iran) and kept at 21 ± 2°C with free access to diet and water. Procedures for animal use and care were according to NIH guidelines and those of Ethics Committee of Shahed University (Tehran, Iran).

2.2. Experimental procedure

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg; Sigma-Aldrich, USA) freshly dissolved in cold normal saline. Control group received normal saline. After one week, overnight fasting serum glucose was determined using glucose oxidation method and animals with serum glucose higher than 250 mg/dl were considered diabetic for further research. Control and hyperglycemic rats were randomly divided into four equal-sized groups, i.e. control, diosgenin-treated control, diabetic, and diosgenin-treated diabetic. Diosgenin (Sigma-Aldrich, USA) was dissolved in carboxymethylcellulose and administered p.o. at a dose of 40 mg/kg/day for 7 weeks. Dose of diosgenin was according to our earlier report on its beneficial effect in STZ-diabetic rats (18).

2.3. Measurement of cardiac oxidative stress

Isolated hearts form rats were immediately made into 10% homogenate in ice-cold normal saline in the presence of protease inhibitor cocktail, centrifuged at 4ºC and the obtained supernatant was aliquotted and stored at -70°C until being used for further experiments.

2.3.1. Determination of cardiac MDA content

The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the supernatant was measured as described before (19). Briefly, trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 1000×g for 10 min and the absorbance of the supernatant was read at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard.

2.3.2. Measurement of cardiac SOD activity

SOD activity measurement was according to our previous work (20). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 ºC) for 40 min and NBT was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity.

2.3.3. Measurement of cardiac reduced glutathione (GSH)

GSH was measured as described before (21, 22). Briefly, the supernatant was centrifuged with trichloroacetic acid. Then, phosphate buffer and 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) was added. The mixture was vortexed and the absorbance was read at 412 nm.

2.3.4. Assay of cardiac catalase activity

For this purpose, the Claiborne's method was used (23). Briefly, H2O2 was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and the rate of H2O2 decomposition was assessed by measuring the absorbance changes at 240 nm.

2.3.5. Protein assay

The protein content of the supernatant was measured with Bradford method using bovine serum albumin as the standard (24).

2.4. Data and statistical analysis

Data were expressed as means ± SEM. Statistical analysis was done by one-way ANOVA followed by Tukey *post-hoc* test. A p value less than 0.05 considered significant.

3. Results

After 8 weeks, diabetes caused a significant reduction of body weight relative to control ($p<0.05$) and diosgenin treatment non-significantly attenuated this. Serum glucose level was also significantly higher in diabetic group as compared to control ($p<0.001$) and diosgenin treatment significantly reduced this ($p<0.05$).

Regarding cardiac markers of oxidative stress, diabetic group had an elevated MDA content ($p<0.01$), reduced activity of SOD ($p<0.05$) and catalase ($p<0.05$) and a lower content of GSH ($p<0.01$) versus control group and diosgenin significantly reversed only MDA ($p<0.05$) and GSH ($p<0.05$) with no significant effect on SOD and catalase activity (Figures 1-2).

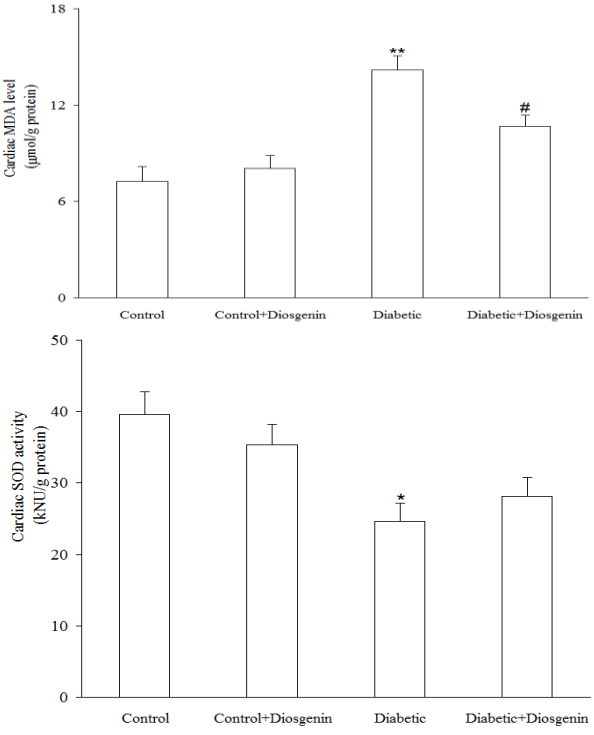

Fig. 1. Malondialdehyde (MDA) content (upper panel) and superoxide dismutase (SOD) activity (lower panel) in cardiac tissue homogenate. Data are shown as Mean ± S.E.M. n=6-7 for each group. * $p<0.05$, ** $p<0.01$ (vs. control); # $p<0.05$ (vs. diabetic). Statistical test included one-way ANOVA followed by Tukey *post-hoc* test.

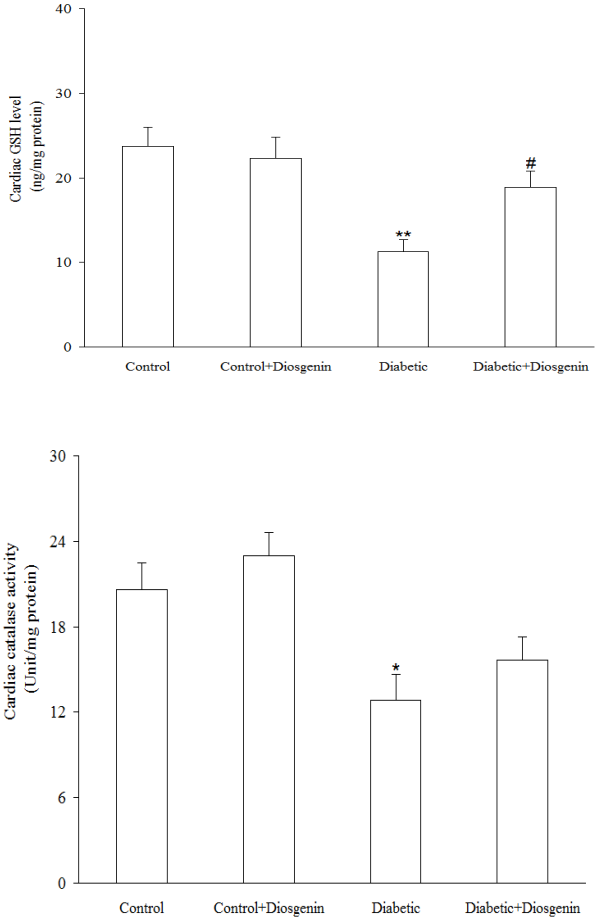

Fig. 2. Reduced glutathione (GSH) content (upper panel) and catalase activity (lower panel) in cardiac tissue homogenate. Data are shown as Mean ± S.E.M. n=6 for each group. * $p<0.05$, ** $p<0.01$ (vs. control); # $p<0.05$ (vs. diabetic). Statistical test included one-way ANOVA followed by Tukey *post-hoc* test.

4. Discussion

This study showed that chronic diosgenin treatment of diabetic rats has hypoglycemic effect and could attenuate cardiac lipid peroxidation and improves non-enzymatic antioxidant defensive system in diabetic condition.

According to previous studies, it has been shown that diosgenin is capable to exert hyperglycemic effect in STZ-induced DM via modulation of the activity of carbohydrate metabolic key enzymes in muscle and kidney tissues and through increasing plasma insulin level (25).

Damaging effect of DM is somewhat attributed to enhanced oxidative stress, as shown by a higher level of MDA and lower activity of the defensive enzymes like SOD (19). In this study, chronic diabetes was followed by a significant increase of malondialdehyde as a reliable marker of lipid peroxidation and a significant decrease of the defensive enzyme SOD and catalase and a lower content of GSH in cardiac tissue homogenate that was consistent with previous reports (19). The results of the present study showed that diosgenin could mitigate MDA content and enhance GSH quantity in cardiac tissue that may have beneficial effect on cardiac performance in diabetic state. Previous studies have indicated that diosgenin is able to protect vascular system in diabetic state through modulation of the antioxidant defense and mitigation of the lipid peroxidation in aorta (16). Part of beneficial effects of diosgenin in our study may be related to its anti-inflammatory and anti-oxidative potential. This agent is capable to protect against inflammatory processes and insulin resistance in the endothelium (26) and in this way could attenuate cardiovascular dysfunction.

In conclusion, diosgenin could attenuate cardiac lipid peroxidation and improves non-enzymatic antioxidant defensive system in diabetic condition and it may be considered as a potential therapeutic agent to mitigate cardiac dysfunction in DM.

Acknowledgment

The authors thanks Dr. Mehrdad Roghani for his technical assistance.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

References

1. Hao S, Xu R, Li D, Zhu Z, Wang T, Liu K. Attenuation of Streptozotocin-Induced Lipid Profile Anomalies in the Heart, Brain, and mRNA Expression of HMG-CoA Reductase by Diosgenin in Rats. Cell Biochemistry and Biophysics 2015.

2. Delbin MA, Trask AJ. The diabetic vasculature: physiological mechanisms of dysfunction and influence of aerobic exercise training in animal models. Life Sciences 2014;102(1):1-9.

3. Kizub IV, Klymenko KI, Soloviev AI. Protein kinase C in enhanced vascular tone in diabetes mellitus. International Journal of Cardiology 2014;174(2):230-42.

4. Yin Y, Qi F, Song Z, Zhang B, Teng J. Ferulic acid combined with astragaloside IV protects against vascular endothelial dysfunction in diabetic rats. Bioscience Trends 2014;8(4):217-26.

5. Tousoulis D, Papageorgiou N, Androulakis E, Siasos G, Latsios G, Tentolouris K, et al. Diabetes mellitus-associated vascular impairment: novel circulating biomarkers and therapeutic approaches. Journal of the American College of Cardiology 2013;62(8):667-76.

6. Polovina MM, Potpara TS. Endothelial dysfunction in metabolic and vascular disorders. Postgraduate Medicine 2014;126(2):38-53.

7. Fraga-Silva RA, Costa-Fraga FP, Murca TM, Moraes PL, Martins Lima A, Lautner RQ, et al. Angiotensin-converting enzyme 2 activation improves endothelial function. Hypertension 2013;61(6):1233-8.

8. Au AL, Kwok CC, Lee AT, Kwan YW, Lee MM, Zhang RZ, et al. Activation of iberiotoxin-sensitive, Ca2+-activated K+ channels of porcine isolated left anterior descending coronary artery by diosgenin. European Journal of Pharmacology 2004;502(1-2):123-33.

9. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. Proceedings of the Society for Experimental Biology and Medicine 1998;217(3):369-78.

10. Kalailingam P, Kannaian B, Tamilmani E, Kaliaperumal R. Efficacy of natural diosgenin on cardiovascular risk, insulin secretion, and beta cells in streptozotocin (STZ)-induced diabetic rats. Phytomedicine 2014;21(10):1154-61.

11. Manivannan J, Balamurugan E, Silambarasan T, Raja B. Diosgenin improves vascular function by increasing aortic eNOS expression, normalize dyslipidemia and ACE activity in chronic renal failure rats. Molecular and Cellular Biochemistry 2013;384(1-2):113-20.

12. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, et al. Diosgenin from Dioscorea bulbifera: novel hit for treatment of type II diabetes mellitus with inhibitory activity against alpha-amylase and alpha-glucosidase. PloS One 2014;9(9):e106039.

13. Sato K, Fujita S ,Iemitsu M. Acute administration of diosgenin or dioscorea improves hyperglycemia with increases muscular steroidogenesis in STZ-induced type 1 diabetic rats. Journal of Steroid Biochemistry and Molecular Biology 2014;143:152-9.

14. Uemura T, Goto T, Kang MS, Mizoguchi N, Hirai S, Lee JY, et al. Diosgenin, the main aglycon of fenugreek, inhibits LXRalpha activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. Journal of Nutrition 2011;141(1):17-23.

15. Ahmed LA, Obaid AA, Zaki HF, Agha AM. Role of oxidative stress, inflammation, nitric oxide and transforming growth factor-beta in the protective effect of diosgenin in monocrotaline-induced pulmonary hypertension in rats. European Journal of Pharmacology 2014;740:379-87.

16. Pari L, Monisha P, Mohamed Jalaludeen A. Beneficial role of diosgenin on oxidative stress in aorta of streptozotocin induced diabetic rats. European Journal of Pharmacology 2012;691(1-3):143-50.

17. Naidu PB, Ponmurugan P, Begum MS, Mohan K, Meriga B, RavindarNaik R, et al. Diosgenin reorganises hyperglycaemia and distorted tissue lipid profile in high-fat diet-streptozotocin-induced diabetic rats. Journal of the Science of Food and Agriculture 2015;95(15):3177-82.

18. Roghani-Dehkordi F, Roghani M , Baluchnejadmojarad T. Diosgenin Mitigates Streptozotocin Diabetes-Induced Vascular Dysfunction of the Rat Aorta: The Involved Mechanisms. Journal of Cardiovascular Pharmacology 2015;66(6):584-92

19. Baluchnejadmojarad T, Roghani M, Jalali Nadoushan MR, Vaez Mahdavi MR, Kalalian-Moghaddam H, Roghani-Dehkordi F, et al. The sesame lignan sesamin attenuates vascular dysfunction in streptozotocin diabetic rats: involvement of nitric oxide and oxidative stress. European Journal of Pharmacology 2013;698(1-3):316-21.

20. Roghani M, Niknam A, Jalali-Nadoushan MR, Kiasalari Z, Khalili M, Baluchnejadmojarad T. Oral pelargonidin exerts dose-dependent neuroprotection in 6-hydroxydopamine rat model of hemi-parkinsonism. Brain Research Bulletin 2010;82(5-6):279-83.

21. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry 1968;25(1):192-205.

22. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959;82(1):70-7.

23. Claiborne A. Catalase activity. In: CRC Handbook of Methods for Oxygen Radical Research, edited by Greenwald RA. Boca Raton, FL: CRC. 1985:283–4.

24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976;248:54-72.

25. Saravanan G, Ponmurugan P, Deepa MA, Senthilkumar B. Modulatory Effects of Diosgenin on Attenuating the Key Enzymes Activities of Carbohydrate Metabolism and Glycogen Content in Streptozotocin-Induced Diabetic Rats. Canadian Journal of Diabetes 2014; 38(6):409-14.

26. Liu K, Zhao W, Gao X, Huang F, Kou J, Liu B. Diosgenin ameliorates palmitate-induced endothelial dysfunction and insulin resistance via blocking IKKbeta and IRS-1 pathways. Atherosclerosis 2012;223(2):350-8.