Diosgenin attenuates cardiac oxidative stress in streptozotocin-induced diabetic rat

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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.

Key Words: Diosgenin, Diabetes mellitus, Streptozotocin, Heart, Oxidative stress, Antioxidant

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).

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Diosgenin is a naturally occurring aglycone that is abundantly present in fenugreek. This steroideal 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 4°C 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 1000xg 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 °C) 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 hypoglycemic 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


