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Combination of Grape Seed Extract and Exercise Training Improves Left Ventricular Dysfunction in STZ-Induced Diabetic Rats

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ABSTRACT

Background: Cardiomyopathy is one of the major complications of diabetes. It has been indicated that regular Exercise Training (ET) and antioxidants could reduce cardiomyopathy and cardiovascular morbidity and mortality of diabetes.

Objectives: The present study aimed to determine the effects of Grape Seed Extract (GSE) with and without ET on left ventricular function in Streptozotocin (STZ)-induced diabetic rats.

Materials and Methods: In this study, 45 male Wistar rats weighing 200 - 232 grams were randomly assigned to five groups: sedentary control, sedentary diabetic, trained diabetic, GSE treated sedentary diabetic, and GSE treated trained diabetic. ET was conducted on a treadmill daily. After eight weeks, left ventricular function was determined by Langendorff system. Then, the data were statistically analyzed using one-way and repeated measures ANOVA followed by LSD test.

Results: Systolic pressure gradient associated with diastolic pressure and maximum ± dP/dT that was reduced by STZ was partially improved with GSE or ET, but was completely corrected by their combination. Moreover, heart rate and left ventricular developed pressure that were reduced by STZ did not change by GSE or ET alone, but improved by their combination.

Conclusions: The results indicated that a combination of ET and GSE had more significant improving effects on left ventricular dysfunction compared to ET or GSE alone. Thus, it may constitute a convenient and inexpensive therapeutic approach to diabetic cardiomyopathy.

►*Implication for health policy/practice/research/medical education:*
Our data showed that combination of exercise training and grape seed extract had more significant improving effects on left ventricular dysfunction induced by STZ compared to exercise training or grape seed extract alone. This indicates that administration of an antioxidant with exercise increases their beneficial effects.

1. Background

Cardiomyopathy is one of the major complications of diabetes mellitus. Cardiomyopathy is a cardiac contractile dysfunction in the absence of any known risk factors for heart disease, such as coronary artery disease or hypertension (1). Early symptoms of cardiomyopathy were seen in young diabetics treated with insulin (2). These findings show that although control of blood glucose, blood lipid, and blood pressure are effective in slowing the progression of cardiac complications of diabetes, they do not completely prevent them (3, 4).

Based on clinical and experimental studies, Exercise Training (ET) is one of the most effective treatments in reduction of cardiomyopathy and cardiovascular morbidity and mortality of diabetes (5-7). Some of the beneficial effects of ET on diabetes include improvement of cardiac function (8), increase of cardiac output under high preload condition (9), and prevention of cardiac autonomic nervous system dysfunction (10).

The common consensus is that diabetes causes excessive

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production of reactive oxygen species, which leads to oxidative myocardial damage and heart dysfunction (11). It has been shown that antioxidant properties of luteolin (12) and coenzyme Q10 (13) can ameliorate diabetic cardiomyopathy.

Up to now, extensive studies have investigated the composition and properties of Grape Seed Extract (GSE) and reported its many beneficial effects on human health, such as reduction of cardiovascular disease (14, 15) and lowering lead-induced hypertension in rats (16). Furthermore, other studies have shown that the compound of GSE possesses a broad-spectrum of antioxidant properties with greater potency compared to vitamin E and C (17), protecting body organs against free radicals and oxidative stress. However, to our knowledge, no researches have been conducted on the effects of GSE alone or combined with exercise on myocardial function in Streptozotocin (STZ)-induced diabetic rats.

2. Objectives

We hypothesize that GSE could play a favorable role in scavenging free radicals and could thereby ameliorate myocardial dysfunction and potentiate the beneficial effects of ET on the myocardium. Therefore, this study aims to determine the effects of GSE and ET alone or in combination on left ventricular function in STZ-induced diabetic rats.

3. Materials and Methods

3.1. Animals and Treatment

In this experimental study, 45 male Wistar strain rats weighing 200 - 232 grams from the Animal House of Physiology Research Center at Ahvaz Jundishapoor University of Medical Sciences, Iran were randomly assigned to five groups each containing 9 rats: Sedentary Control (SC), Sedentary Diabetic (SD), Trained Diabetic (TrD), GSE treated sedentary diabetic (ExD), and GSE treated trained diabetic (TrExD). STZ (60 mg/kg body weight dissolved in 0.3 mL normal saline) was used to induce diabetes. An equivalent volume of the vehicle was injected to the control group. GSE (200 mg/kg) was administered orally via gavage once a day. Besides, ET was conducted on a treadmill. The study lasted for eight weeks. During this period, the animals were supplied with food and water ad-libitum and were maintained in a temperature-controlled room at 22 °C with a 12-h dark-light cycle. After 5 days, blood glucose levels above 300 mg/dL were considered as diabetic state (18). The experimental protocol and procedures were submitted to and approved by the Institutional Animal Care and Use Committee of the University based on the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication, revised 1996).

3.2. Drugs

STZ and heparin sodium were obtained from Sigma (St. Louis, Mo). Sodium chloride, potassium chloride, magnesium sulfate, sodium hydrogen carbonate, potassium hydrogen orthophosphate, D-glucose, and calcium chloride were obtained from Merck laboratories and sodium pentothal was purchased from Rotex Medica, Germany.

3.3. Preparation of GSE

Vitis Vinifera (grape seeds) was confirmed by Qazvin Agricultural Research Center, Qazvin, Iran. Voucher specimen was available in the herbarium at the Department of Phamacognosy, Faculty of Pharmacy, Joundishapour University of Medical Sciences, Ahwaz, Iran. After manually separating the grape seeds (Vitis Vinifera) from the grapes, they were dried at room temperature for 7 days and milled to fine powder. The powders were macerated in 70 % ethanol (25% w/v) for 3 days in shade (25 - 30 °C) and were stirred 3 times a day. After filtration of the mixture with cheese cloth, the filtrate was dried at room temperature to remove ethanol. Finally, GSE was obtained in form of powder (19).

3.4. ET Protocol

As shown in Table 1, ET was conducted on a treadmill daily for 8 weeks 1 day after diabetic verification after gavage of GSE.

3.5. Isolation of the Heart

The animals were anesthetized with sodium pentothal (80 mg/kg, i.p) containing 1000 U/kg heparin. After opening the chest, the heart was rapidly excised and placed in a Petri dish containing ice-cold oxygenated modified Krebs-Henseleit Solution (KHS). After washing with ice-cold KHS and arresting, the hearts were cannulated via the ascending aorta and were immediately transferred to Langendorff system. A modified KHS of the following composition (mM): NaCl 118.4, KCl 4.7, MgSO4 H2O 1.2, KH2PO4 2H2O 1.2, NaHCO3 25, CaCl2 2.5, and glucose 11.1 in distilled water retroperfused the aorta. This solution was maintained at 37 °C, bubbled with 5% CO2 and 95% O2, and perfused the isolated hearts at a constant flow (4 mL/min) via peristaltic pumps (Gilson-France). The maximum interval between the excision of the heart and

Table 1. Exercise Training Protocol for the Rats on the Treadmill

Week	Belt Speed (m/min)	Inclination (°)	Total Time (min)
1	16	0	30
2	16	5	30
3	16	10	45
4	16	12	45
5	16	12	60
6	16	12	60
7	16	12	60
8	16	12	60

beginning of perfusion was 2 minutes. In order to record the left ventricular pressure, a latex balloon filled with water was placed in the left ventricle and was connected to a pressure transducer (Powerlab 8/30 AD Instrument, Australia). After the stabilization period (30 minutes with a diastolic pressure between 1 - 6 mm Hg), left ventricular diastolic pressure was increased by 10 mm Hg every 5 minutes, achieving 10, 20, 30, and 40 mmHg (10). For each diastolic pressure, the following variables were calculated: left ventricular systolic pressure and maximum rate of pressure rise (+dP/dT max) and fall (-dP/dT max). In the basal state, the difference between left ventricular systolic and diastolic pressures was considered as the Left Ventricular Developed Pressure (LVDP).

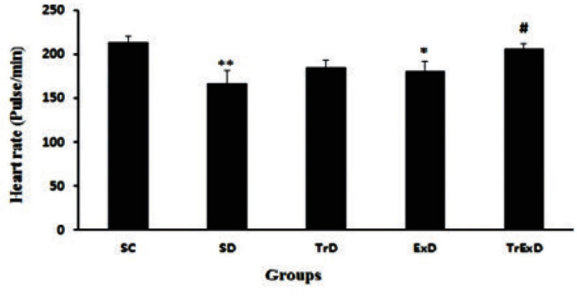
3.6. Statistical Analysis

The results were expressed as mean ± Standard Error of Mean (SEM). The comparisons between the study groups in each protocol were performed using one-way and repeated measures ANOVA followed by LSD multiple comparison test. All the analyses were performed using the SPSS statistical software, version 16.0 (Chicago, IL, USA) and P value < 0.05 was considered as statistically significant.

4. Results

STZ-induced diabetes (167 ± 14) could significantly (P = 0.003) decrease isolated heart rate compared to the control group (214 ± 7). The isolated heart rate was not corrected by ET (185 ± 8) or GSE (181 ± 12) alone, but was significantly

Figure 1. Heart Rate in Different Groups (Mean ± SEM, n = 7 - 8)



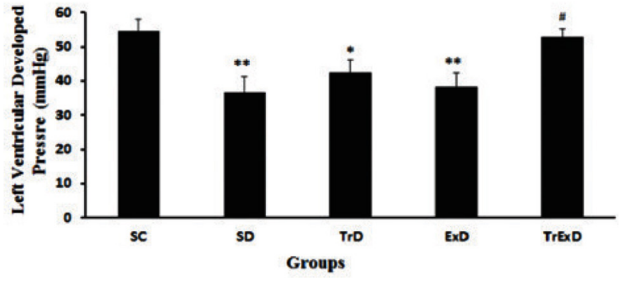
Abbreviations: SC, sedentary control; SD, sedentary diabetic; TrD, trained diabetic; ExD, grape seed extract treated sedentary diabetic; TrExD, grape seed extract treated trained.* and #, P < 0.05 significantly different from SC and SD, respectively; **, P<0.01 significantly different from SC

improved by the combination of these two factors (206 ± 7, P = 0.01) (Figure 1).

In comparison to the control group (55 ± 4), STZ-induced diabetes (37 ± 5) significantly decreased LVDP (P = 0.002). This could not be modified by ET (43 ± 4) or GSE (38 ± 4) alone, but was significantly improved by their combination (53 ± 3, P = 0.004) (Figure 2).

As Table 2 depicts, by increasing the diastolic pressure of the left ventricle from base (1 - 6 mm Hg) to 40 mmHg, systolic pressure also increased in all the study groups. Nevertheless, systolic pressure in the diabetic group (71 ± 5) was significantly (P = 0.001) lower compared to the control group (105 ± 4) in all diastolic pressures. Systolic pressure was improved partially by GSE (85 ± 5, P = 0.045) or ET (89 ± 6, P = 0.019) alone and perfectly by their combination (101

Figure 2. Left Ventricular Developed Pressure of the Hearts Isolated from Different Groups (Mean ± SEM, n = 7 - 8)



Abbreviations: SC, sedentary control; SD, sedentary diabetic; TrD, trained diabetic; ExD, grape seed extract treated sedentary diabetic; TrExD, grape seed extract treated trained, * and #, P < 0.05 significantly different from SC and SD, respectively; **, P<0.01 significantly different from SC

± 3, P = 0.001). STZ-induced diabetes significantly reduced the maximum rate of rise and fall (-dP/dT) compared to the control group (P < 0.001 for both). This was corrected partially by GSE or ET (both P < 0.001) and completely by their combination (P = 0.001) (Tables 3 and 4).

5. Discussion

The present study showed that systolic pressure gradient associated with diastolic pressure and maximum ± dP/dT that was reduced by diabetes were partially improved with GSE or ET, but were completely corrected by their combination. Moreover, heart rate, LVDP, and rate pressure product did not change by GSE or ET alone, but were improved with the combination of these two factors.

Diabetic cardiomyopathy includes heart dysfunction caused by diabetes in the absence of any known cardiovascular diseases, such as coronary artery disease, congenital or valvular heart disease, and hypertension. Left ventricular dysfunction is generally presented as cardiomyopathy (20).

In this study, heart rate, LVDP, increment in left ventricular systolic pressure proportional to the increment in diastolic pressure, and ± dP/dT were measured to assess the left ventricular function. Reduction of all the indices of left ventricle affected by diabetes represented diabetic cardiomyopathy.

Evidence has indicated that although hyperglycemia, hyperlipidemia, inflammatory cytokines, and peptides by different mechanisms are involved in diabetic cardiomyopathy, they all are related to oxidative stress. Heart is one of the organs in the body that has high oxygen consumption rate and slow turnover of antioxidant enzymes (21); thus, heart has a high probability of oxidative damage (22). On the other hand, diabetes not only increases the production of free radicals, but it also impairs the antioxidant defense system (23). Reduction of expression of heat shock protein 60 and heme oxygenase-1 in the diabetic heart also increases the risk of oxidative damage (24). It seems that adding external antioxidants or boosting internal antioxidants might be useful in controlling oxidative stress. In the present study, GSE as an antioxidant (19) could partially improve diabetic cardiomyopathy. Based on the available information, there are no studies on the

Table 2. Systolic Pressure Gradient Associated with Diastolic Pressure of Left Ventricles Isolated from Different Groups (Mean ± SEM, n = 7 - 8)

Groups\Diastolic P.	Basal (1 - 6 mmHg)	10 (mmHg)	20 (mmHg)	30 (mmHg)	40 (mmHg)
SC	58 ± 4	77 ± 4	88 ± 4	101 ± 4	105 ± 4
SD	40 ± 5 [a]	44 ± 5 [a]	55 ± 5 [a]	68 ± 5 [a]	71 ± 5 [a]
TrD	46 ± 4 [a]	61 ± 6 [a, b]	73 ± 7 [b]	85 ± 6 [a, b]	89 ± 6 [a, b]
ExD	42 ± 4 [a]	56 ± 5 [a]	69 ± 5 [a]	82 ± 5 [a, b]	85 ± 5 [a, b]
TrExD	57 ± 2 [b]	71 ± 5 [b]	85 ± 5 [b]	98 ± 4 [b]	101 ± 3 [b]

Abbreviations: SC, sedentary control; SD, sedentary diabetic; TrD, trained diabetic; ExD, grape seed extract treated sedentary diabetic; TrExD, grape seed extract treated trained diabetic

[a] $P < 0.05$ significantly different from SC, [b] $P < 0.05$ significantly different from SD (repeated measures ANOVA followed by LSD).

Table 3. Maximum + dP/dT of the Left Ventricles Isolated from Different Groups in Response to Different Diastolic Pressures (Mean ± SEM, n = 7 - 8)

Groups\Diastolic P.	Basal (1 - 6 mmHg)	10 (mmHg)	20 (mmHg)	30 (mmHg)	40 (mmHg)
SC	1442 ± 80	1667 ± 89	1933 ± 71	2020 ± 74	2079 ± 77
TrD	932 ± 24 [a, b]	1116 ± 30 [a, b]	1316 ± 52 [a, b]	1410 ± 51 [a, b]	1507 ± 65 [a, b]
ExD	875 ± 21 [a, b]	1046 ± 33 [a, b]	1221 ± 70 [a, b]	1325 ± 69 [a, b]	1386 ± 58 [a, b]
TrExD	1419 ± 76 [b]	1587 ± 86 [b]	1801 ± 88 [b]	1925 ± 84 [b]	1997 ± 75 [b]

Abbreviations: SC, sedentary control; SD, sedentary diabetic; TrD, trained diabetic; ExD, grape seed extract treated sedentary diabetic; TrExD, grape seed extract treated trained diabetic

[a] $P < 0.05$ significantly different from SC, [b] $P < 0.05$ significantly different from SD (repeated measures ANOVA followed by LSD)

Table 4. Maximum -dP/dT of the Left Ventricles Isolated from Different Groups in Response to Different Diastolic Pressures (Mean ± SEM, n = 7 - 8)

Groups\Diastolic P.	Basal (1 - 6 mmHg)	10 (mmHg)	20 (mmHg)	30 (mmHg)	40 (mmHg)
SC	-812 ± 15	-942 ± 22	-1290 ± 32	-1372 ± 43	-1400 ± 47
SD	-516 ± 23 [a]	-588 ± 29 [a]	-739 ± 41 [a]	-792 ± 45 [a]	-842 ± 59 [a]
TrD	-642 ± 20 [a, b]	-774 ± 37 [a, b]	-1070 ± 40 [a, b]	-1138 ± 45 [a, b]	-1188 ± 53 [a, b]
ExD	-610 ± 21 [a, b]	-732 ± 33 [a, b]	-986 ± 46 [a, b]	-1070 ± 32 [a, b]	-1115 ± 30 [a, b]
TrExD	-789 ± 20 [b]	-939 ± 13 [b]	-1228 ± 30 [b]	-1294 ± 35 [b]	-1353 ± 34 [b]

Abbreviations: SC, sedentary control; SD, sedentary diabetic; TrD, trained diabetic; ExD, grape seed extract treated sedentary diabetic; TrExD, grape seed extract treated trained diabetic

[a] $P < 0.05$ significantly different from SC, [b] $P < 0.05$ significantly different from SD (repeated measures ANOVA followed by LSD)

effect of GSE on the diabetic heart. Thus, further studies are required to determine the mechanisms of this effect. According to the studies that have examined the effect of other antioxidants on diabetic heart, several mechanisms can be proposed, including prevention of myocardial apoptosis, angiogenesis, and improvement of endothelial function. Some studies have shown that antioxidants, such as resveratrol (25) and alpha lipoic acid (26), could prevent progression of diabetic cardiomyopathy. Hyperglycemia can also cause cardiomyocyte apoptosis which can have an important role in progression of heart failure and diabetic cardiomyopathy (26). It has been shown that antioxidants can prevent myocardial apoptosis (25, 26). Angiogenic properties of resveratrol derived from grape seeds may also play a protective role in the heart (25). Furthermore, diabetes-induced endothelial dysfunction can influence the pathogenesis of diabetic cardiomyopathy (27). It has been shown that GSE can improve endothelial dysfunction induced by diabetes (19, 28).

The present study demonstrated that ET could partially improve diabetic heart function, which is consistent with other studies conducted on the issue (8, 10, 29). Upregulation of sarcolemmal GLUT-4 protein and mRNA has been seen by ET (30) that may increase myocardial glucose oxidation (31) and improve cardiac pump performance (32). Improvement of left ventricular geometry features can also be involved in this beneficial effect of exercise on the heart (33). Exercise reverses bradycardia (34) and myocardial depression induced by diabetes, thus maintaining the cardiac output (6, 10). Blood levels of catecholamines, angiotensin II, vasopressin, neuropeptide Y, atrial natriuretic peptide, and pre-inflammatory mediators can also be normalized by exercise (7).

It seems that the combination of GSE and ET has been more effective than any of them alone in control of oxidative stress by adding an external antioxidant (GSE) and strengthening of internal antioxidants with ET (35).

The above-mentioned mechanisms or other unknown mechanisms might have contributed to the beneficial effects of the combination of exercise and GSE on left ventricular function of diabetic hearts.

The results of the present study showed that the combination of ET and GSE was more effective than either of them alone in diabetic cardiomyopathy.

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Authors' Contribution

Study concept and design: Badavi, Abedi, Dianat, and Sarkaki; Analysis and interpretation of the data: Badavi and Abedi; Drafting of the manuscript: Abedi; Critical revision of the manuscript for important intellectual content: Badavi, Abedi, Dianat, and Sarkaki; Statistical analysis: Badavi and Abedi.

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The Physiology Research Center had no role in the design and conduct of the study, collection, management, and analysis of the data, and preparation, review, and approval of the manuscript, but prepared the needed equipment and a location for performing the research.

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References

1. Scognamiglio R, Avogaro A, Negut C, Piccolotto R, Vigili de Kreutzenberg S, Tiengo A. Early myocardial dysfunction in the diabetic heart: current research and clinical applications. Am J Cardiol. 2004;93(8A):17A–20A.
2. Mildenberger RR, Bar-Shlomo B, Druck MN, Jablonsky G, Morch JE, Hilton JD, et al. Clinically unrecognized ventricular dysfunction in young diabetic patients. J Am Coll Cardiol. 1984;4(2):234–8.
3. Li B, Liu S, Miao L, Cai L. Prevention of diabetic complications by activation of Nrf2: diabetic cardiomyopathy and nephropathy. Exp Diabetes Res. 2012;2012:216512.
4. Miki T, Yuda S, Kouzu H, Miura T. Diabetic cardiomyopathy: pathophysiology and clinical features. Heart Fail Rev. 2013;18(2):149–66.
5. Riddell MC, Iscoe KE. Physical activity, sport, and pediatric diabetes. Pediatr Diabetes. 2006;7(1):60–70.
6. Saraceni C, Broderick TL. Cardiac and metabolic consequences of aerobic exercise training in experimental diabetes. Curr Diabetes Rev. 2007;3(1):75–84.
7. Shao CH, Wehrens XH, Wyatt TA, Parbhu S, Rozanski GJ, Patel KP, et al. Exercise training during diabetes attenuates cardiac ryanodine receptor dysregulation. J Appl Physiol (1985). 2009;106(4):1280–92.
8. Korte FS, Mokelke EA, Sturek M, McDonald KS. Exercise improves impaired ventricular function and alterations of cardiac myofibrillar proteins in diabetic dyslipidemic pigs. J Appl Physiol (1985). 2005;98(2):461–7.
9. DeBlieux PM, Barbee RW, McDonough KH, Shepherd RE. Exercise training improves cardiac performance in diabetic rats. Proc Soc Exp Biol Med. 1993;203(2):209–13.
10. De Angelis KL, Oliveira AR, Dall'Ago P, Peixoto LR, Gadonski G, Lacchini S, et al. Effects of exercise training on autonomic and myocardial dysfunction in streptozotocin-diabetic rats. Braz J Med Biol Res. 2000;33(6):635–41.
11. Stevens MJ. Oxidative-nitrosative stress as a contributing factor to cardiovascular disease in subjects with diabetes. Curr Vasc Pharmacol. 2005;3(3):253–66.
12. Wang G, Li W, Lu X, Bao P, Zhao X. Luteolin ameliorates cardiac failure in type I diabetic cardiomyopathy. J Diabetes Complications. 2012;26(4):259–65.
13. Huynh K, Kiriazis H, Du XJ, Love JE, Jandeleit-Dahm KA, Forbes JM, et al. Coenzyme Q10 attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the db/db mouse model of type 2 diabetes. Diabetologia. 2012;55(5):1544–53.
14. Charradi K, Sebai H, Elkahoui S, Ben Hassine F, Limam F, Aouani E. Grape seed extract alleviates high-fat diet-induced obesity and heart dysfunction by preventing cardiac siderosis. Cardiovasc Toxicol. 2011;11(1):28–37.
15. Leifert WR, Abeywardena MY. Cardioprotective actions of grape polyphenols. Nutr Res. 2008;28(11):729–37.
16. Badavi M, Mehrgerdi FZ, Sarkaki A, Naseri MK, Dianat M. Effect of grape seed extract on lead induced hypertension and heart rate in rat. Pak J Biol Sci. 2008;11(6):882–7.
17. Shi J, Yu J, Pohorly JE, Kakuda Y. Polyphenolics in grape seeds-biochemistry and functionality. J Med Food. 2003;6(4):291–9.
18. Li BY, Cheng M, Gao HQ, Ma YB, Xu L, Li XH, et al. Back-regulation of six oxidative stress proteins with grape seed proanthocyanidin extracts in rat diabetic nephropathy. J Cell Biochem. 2008;104(2):668–79.
19. Badavi M, Abedi H, Dianat M, Sarkaki A. Dysfunction of Mesenteric Vascular Bed in STZ-induced Diabetic Rats. International Journal of Pharmacology. 2011;7(8):813–20.
20. Wold LE, Ceylan-Isik AF, Ren J. Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. Acta Pharmacol Sin. 2005;26(8):908–17.
21. Asha Devi S, Prathima S, Subramanyam MV. Dietary vitamin E and physical exercise: II. Antioxidant status and lipofuscin-like substances in aging rat heart. Exp Gerontol. 2003;38(3):291–7.
22. Hamblin M, Friedman DB, Hill S, Caprioli RM, Smith HM, Hill MF. Alterations in the diabetic myocardial proteome coupled with increased myocardial oxidative stress underlies diabetic cardiomyopathy. J Mol Cell Cardiol. 2007;42(4):884–95.
23. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med. 2006;40(2):183–92.
24. Cai L. Diabetic cardiomyopathy and its prevention by metallothionein: experimental evidence, possible mechanisms and clinical implications. Curr Med Chem. 2007;14(20):2193–203.
25. Thirunavukkarasu M, Penumathsa SV, Koneru S, Juhasz B, Zhan L, Otani H, et al. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. Free Radic Biol Med. 2007;43(5):720–9.
26. Li CJ, Zhang QM, Li MZ, Zhang JY, Yu P, Yu DM. Attenuation of myocardial apoptosis by alpha-lipoic acid through suppression of mitochondrial oxidative stress to reduce diabetic cardiomyopathy. Chin Med J (Engl). 2009;122(21):2580–6.
27. Malhotra A, Sanghi V. Regulation of contractile proteins in diabetic heart. Cardiovasc Res. 1997;34(1):34–40.
28. Badavi M, Abedi HA, Sarkaki AR, Dianat M. Co-administration of Grape Seed Extract and Exercise Training Improves Endothelial Dysfunction of Coronary Vascular Bed of STZ-Induced Diabetic Rats. Iran Red Crescent Med J. 2013;15(10)
29. Le Douairon Lahaye S, Gratas-Delamarche A, Malarde L, Zguira S, Vincent S, Lemoine Morel S, et al. Combined insulin treatment and intense exercise training improved basal cardiac function and Ca(2+)-cycling proteins expression in type 1 diabetic rats. Appl Physiol Nutr Metab. 2012;37(1):53–62.
30. Osborn BA, Daar JT, Laddaga RA, Romano FD, Paulson DJ. Exercise training increases sarcolemmal GLUT-4 protein and mRNA content in diabetic heart. J Appl Physiol (1985). 1997;82(3):828–34.
31. Paulson DJ, Mathews R, Bowman J, Zhao J. Metabolic effects of treadmill exercise training on the diabetic heart. J Appl Physiol (1985). 1992;73(1):265–71.
32. Paulson DJ, Kopp SJ, Peace DG, Tow JP. Myocardial adaptation to endurance exercise training in diabetic rats. Am J Physiol. 1987;252(6 Pt 2):R1073–81.
33. Bakth S, Arena J, Lee W, Torres R, Haider B, Patel BC, et al. Arrhythmia susceptibility and myocardial composition in diabetes. Influence of physical conditioning. J Clin Invest. 1986;77(2):382–95.
34. Badavi M, Abedi HA, Dianat M, Sarkaki AR. Exercise Training

and Grape Seed Extract Co-Administration Improves Lipid Profile, Weight Loss, Bradycardia, and Hypotension of STZ-Induced Diabetic Rats. Int Cardiovasc Res J. 2013;7(4):111–7.
35. Husain K. Interaction of physical training and chronic nitroglycerin treatment on blood pressure, nitric oxide, and oxidants/antioxidants in the rat heart. Pharmacol Res. 2003;48(3):253–61.