Effect of Grape Seed Extract Consumption along with Moderate Intensity Aerobic Training on Some Biomarkers of Apoptosis of Cardiomyocytes in Wistar Rats with Type 1 Diabetes

Azam Ghauor Najaf Abadi[1], Saeed Keshavarz[1*], Mehdi Kargarfard[2], Jamshid Banaii Boroujeni[1]

1. Department of Physical Education and Sport Sciences, Najafabad Branch, Islamic Azad University, Najafabad, Iran
2. Faculty of Physical Education and Sport Sciences, University of Isfahan, Isfahan, Iran

*** Corresponding author:**Tel: +98 913 270 46 83 ; Fax: +98 +98 3142292929
Address: Department of Exercise Physiology, Najafabad branch, Islamic Azad University, Najafabad, Iran
E-mail: keshavarz1357@gmail.com
Received; 29/05/2021 revised; 21/07/2021 accepted; 29/08/2021

Abstract

Introduction: Today, diabetes is one of the leading causes of death in worldwide. Nutritional intervention and exercise training known as main strategies for control and management diabetes. The aim of the present study was to investigate the effect of grape seed extract consumption with moderate intensity aerobic exercise on some markers of cardiac myocyte apoptosis in male Wistar rats with type 1 diabetic.

Materials and Methods: Forty adult male Wistar rats with an initial weight range of 160-220 g were divided into 5 groups of including diabetic / training + extract, diabetic / training, diabetic / extract, diabetic / control and healthy / control (8 heads per group). The rats of training groups performed aerobic exercise for 60 minutes a day at an average speed of 28 meters per minute (intensity equivalent to 70 to 75% of maximum oxygen consumption and training volume of 8.4 km per week). Grape seed extract was consumed by gavage with dosage of 40 mg / kg per day. All groups 48 hours after the last training session, was dissected and heart tissue was collected and Bcl-2, sFas and FasL analysis was performed.

Results: Aerobic training and consumption of grape seed extract (both and each alone) significantly increased FasL and BcL-2 and also significantly decreased sFas and sFas/FasL in type 1 diabetic rats ($P < 0.05$); however, the combination of aerobic training and consumption of grape seed extract increased FasL and BcL-2 further and also decreased sFas and sFas/FasL in these rats compared to the training and consumption of the extract alone, which was a statistically significant difference ($P < 0.05$).

Conclusion: By combining the antioxidant and anti-inflammatory effects of moderate-intensity aerobic exercise along with the consumption of grape seed extract, more anti-apoptotic effects may be induced in the myocytes of type 1 diabetic rats.

Keywords: Diabetes, Apoptosis, Grape seed, Aerobic training, Cardiomyocytes

Introduction

Diabetes mellitus is one of the leading causes of death in developing countries (1). Cardiovascular diseases are the leading cause of death in patients with diabetes. Apoptosis has been shown to play a major role in the process of heart disease. In fact, various studies have shown that diabetes significantly increases the rate of apoptosis in cardiac cells (2). evidences suggests that increased signaling through positive regulation of tumor necrosis factor (TNF) is common in heart failure, and that degradation of FAS receptor inhibitors such as cFLIP is another mechanism for apoptosis to occur (3). The TNF and FAS

pathways in apoptosis have been shown to be involved in the pathogenesis of myocardial infarction, ischemia-reperfusion injury, myocardial injury, and congestive heart failure (4). The interaction between the FAS ligand (FasL) and Fas is also likely to mediate apoptotic cardiac cell death under certain pathophysiological conditions, while normal heart and myocardial cells offer some resistance to Fas-induced cytotoxicity (5). Fas is a receptor molecule characteristic of apoptosis that is present in a number of cell types, including cardiac cells (6).

FAS antigen is expressed in various tissues and its soluble form sFas lacks a transfer membrane. Studies have shown that plasma levels of sFasL are significantly lower in people with heart damage compared to healthy people (7). However, sFas is significantly higher in people with heart damage compared to healthy people (7). Therefore, the researchers concluded that plasma and tissue levels of sFas could potentially be a strong predictor of the diagnosis of myocardial infarction.

The process of cell apoptosis by some mitochondrial proteins including B-cell lymphoma-2 (Bcl-2) family proteins which are divided into two parts: anti-apoptotic proteins (Bcl-2, Bcl-W, Bcl-XL, Bfl-1 and Mcl-1) and pre-apoptotic proteins (Bim, Bik, Bid, BadBcl-Xs, Bak, Bax and Hrk) regulated that have a major role in accelerating the onset or preventing its occurrence (8). It has been shown that exercise training is one of the effective strategies to reduce the development of cardiac injury and the incidence of cardiovascular complications and mortality in the length of diabetes (9). Exercise training plays a protective role against the complications of diabetes by reducing oxidative stress and apoptosis in heart cells (10-12). On the other hand, black grape seed extract is one of the supplements that has flavonoid compounds and has very high antioxidant effects (13). Studies show that grape seed extract has a very high potential in eliminating free radicals and inhibiting oxidative stress (14, 15). One possible mechanism for this effect is reduced apoptosis, reduced endoplasmic reticulum stress, and equally negative regulation of PERK eIF2α CHOP signaling pathway activity (16).

However, the effect of grape seed extract consumption with exercise on apoptotic indices, has not been studied yet, therefore, the aim of the present study was to evaluate the effect of grape seed extract consumption with moderate intensity aerobic exercise on some markers of cardiac myocyte apoptosis in male Wistar type 1 diabetic rats.

Materials and Methods

This research is an experimental study that was performed with a post-test design with a control group. The statistical population of this study was adult male Wistar rats from the laboratory animal center of Shiraz University of Medical Sciences with an initial weight range of 160-220 g. The sample size was 40 rats, which was selected based on related articles. After selection, 1- Diabetic / training + Extract (DTE), 2- Diabetic / training (DT), 3- Diabetic / Extract (DE), 4- Diabetic / Control (DC) and 5- Healthy / Control (HC) (8 heads per group). The study procedures were reviewed and approved by the Research and Ethics Committee of the Islamic Azad University (Ethics code: IR-IAU1388-22). All procedures were performed according to the latest revision of the Declaration of Helsinki.

Induction of type 1 diabetes with a single intraperitoneal injection of streptozotocin (STZ), solution prepared from Sigma-Aldrich, Germany, dissolved in citrate buffer (pH = 4.5 and concentration of 0.1 M) at a rate of 55 mg performed per kilogram of animal body weight.

14 days after STZ injection, blood glucose concentrations were measured in blood samples collected from animal tails using a glucometer (manufactured by Medisign, South Korea). The criterion for being

diabetic was fasting blood glucose concentration higher than 250 mg / dl (17). Glucose oxidase method was used to measure glucose. For the control group, in order to equalize the effect of injection, 0.1 M citrate buffer with the same volume was injected intraperitoneally (17). All mice were kept in controlled environmental conditions with an average temperature of 22 ± 3°C, light cycle to dark 12:12 hours and with free access to water and food for mice (prepared in the form of pellets from Behparvar Iran Company).

The stage of familiarity and aerobic training was such that in the stage of familiarity with the treadmill (the first week) the rats walked for 15 minutes on a 10-channel rodent's treadmill for 15 consecutive days at a speed of 10 meters per minute with a zero-degree slope. Training started between 7 to 11 in the morning every day. During the second and third weeks, the treadmill speed and duration of exercise increased gradually.

The rats performed aerobic exercise for 60 minutes a day at an average speed of 28 meters per minute (intensity equivalent to 70 to 75% of maximum oxygen consumption and training volume of 8.4 km per week). At the end of the exercise program, in order to perform cooling, the speed of the device was reduced inversely until the speed of the device reached zero. This procedure continued until the end of the eighth week of training (18). Grapes used by the family of vitis vinifera, genus amplidase, Genus vitis, subgenus uveitis and Iranian grape species were selected and approved by the cultivation and development group of the Tehran institute of medicinal Plants. After purchasing red grapes, the seeds were separated from red grape pulp manually. The seeds were then washed in the open air and dried in direct sunlight at 50°C for 30 minutes. The dried grains were crushed and grounded to the stage of powder formation. Separation of grain fat was done by soxhlet method using hexane solvent. In this method, first 500 mg of grape seed powder was poured into filter paper and placed in a Soxhlet apparatus and the suction operation was performed on 500 ml of hexane solvent for one hour.

The hexane extract prepared with a rotary apparatus was concentrated at 50 ° C and then dried in an arc at the same temperature. The resulting powder was processed by Soxhlet method using methanol solvent twice and filtered through filter paper. Finally, the methanol solution containing the extract was dried in a vacuum at 40 ° C by evaporator to separate the extract from the methanol solvent. After evaporation of methanol, the pure extract remained in a container, which after collection, was kept away from light and moisture (19). Grape seed extract was consumed by gavage at a dosage of 40 mg / kg per day.

All groups 48 hours after the last training session, in exactly the same condition and in the fasting state of animals by intraperitoneal injection of ketamine (30-50 mg / kg body weight) and xylazine (3-5 mg / kg body weight) fainted and the chest was dissected and heart tissue was collected.

The heart tissue was separated and transferred to microtubes that were kept in liquid nitrogen and then stored at -80°C for later assessment. All the experimental procedures including laboratory measurements and training and sacrificing the animals were performed in the morning time between 8 - 11 a.m. The tissues were transported to a Chinese oven and homogenized with powder. Commercial immunoassay kits for measuring Bcl-2 (Mybiosource, USA), sFas (Abcam, USA) and FasL (Abcam, USA) were used. Also, the sFas/FasL ratio was calculated.

Statistical Analysis

One-way analysis of variance was used to compare the groups for the studied variables and Bonferroni post hoc test was used to perform additional tests. Significance level was considered $P < 0.05$ and SPSS software version 19 was used for statistical analysis.

Results

Examination of intergroup changes for Bcl-2, sFas and FasL indices showed that there was a significant difference between groups ($P < 0.05$) (Table 1). Induction of diabetes significantly increased sFas and sFas/FasL ($P = 0.001$), but the three interventions extract consumption, training and training + extract consumption significantly decreased sFas and sFas/FasL ($P < 0.05$) (Table 2). However, the training + extract group showed a greater decrease in sFas and sFas/FasL compared to the two training and extract groups alone, which was statistically significant ($P < 0.05$). Furthermore, induction of diabetes significantly decreased FasL and Bcl-2 ($P = 0.001$), but all three interventions of extract consumption, training and training + extract consumption significantly increased FasL and Bcl-2 ($P < 0.05$) (Table 2). On the other hand, the training + extract group showed a greater increase in FasL and Bcl-2 compared to the two training and extract groups, which was statistically significant ($P < 0.05$) (Table 2).

Table1. The levels of Bcl-2, sFas and FasL in heart tissue of different rat groups after exerting the allocated treatments.

Variables	Group	Mean ± SD	F	P value
sFas (ng/100mg tissue)	DTE	275.91 ± 48.05	40.538	0.001
	DT	494.15 ± 100.91		
	DE	546.22 ± 121.66		
	DC	775.74 ± 128.05		
	HC	254.71 ± 40.83		
FasL (ng/100mg tissue)	DTE	1055.87 ± 159.92	39.056	0.001
	DT	871.76 ± 97.88		
	DE	857.14 ± 102.12		
	DC	409.62 ± 107.005		
	HC	1060.87 ± 122.42		
sFas/FasL (ng/100mg tissue)	DTE	0.26 ± 0.012	230.492	0.001
	DT	0.56 ± 0.562		
	DE	0.63 ± 0.631		
	DC	1.94 ± 0.949		
	HC	0.23 ± 0.239		
Bcl-2 (ng/100mg tissue)	DTE	969.37 ± 77.85	25.845	0.001
	DT	707.44 ± 161.02		
	DE	666.06 ± 158.36		
	DC	417.17 ± 95.17		
	HC	994.12 ± 147.77		

DTE: Diabetic / Training + Extract, DT: Diabetic / Training, DE: Diabetic / Extract, DC: Diabetic / Control, HC: Healthy / Control.

Discussion

Based on the present study findings, moderate intensity aerobic training and consumption of grape seed extract (alone or together) result in significant increase in FasL and BcL-2 and also significant decrease in sFas and sFas / FasL in type 1 diabetic rats' cardiomyocytes. However, aerobic training combined with grape seed extract supplementation cause to further increase in FasL and BcL-2 levels, and further decrease in sFas level and sFas/FasL, which was statistically significant compared to aerobic training and consumption of grape seed extract alone.

It has been shown that induction of diabetes caused a significant increase in sFas level and sFas/FasL, and no change in and Bcl-2 levels in rats, but 4 weeks of training significantly reduced FasL, no change in sFas and sFas/ FasL and Bcl-2 were detected in diabetic rats. The lack of significant change in sFas, sFas/ FasL and Bcl-2 in this study is probably due to the

short duration (4 weeks) of training. Above mentioned findings confirmed the progressive effect of diabetes-induced apoptosis on heart tissue and suggested that regular aerobic training can be considered as an effective non-pharmacological method to reduce the effects of diabetes-induced apoptosis on the cardiac tissue of diabetics (20).

Table 2. The results of Bonferroni post hoc test to demonstrate the differences between groups for Bcl-2, sFas and FasL levels in heart tissue of the rats after exerting the allocated treatments.

Variables	Paired comparison		P value
sFas (ng/100mg tissue)	DTE	DT	0.001
		DE	0.001
		DC	0.001
		HC	1
	DT	DE	1
		DC	0.001
		HC	0.001
	DE	DC	0.001
		HC	0.001
	DC	HC	0.001
FasL (ng/100mg tissue)	DTE	DT	0.041
		DE	0.022
		DC	0.001
		HC	1
	DT	DE	1
		DC	0.001
		HC	0.033
	DE	DC	0.001
		HC	0.017
	DC	HC	0.001
sFas/FasL (ng/100mg tissue)		DT	0.001
	DTE	DE	0.001
		DC	0.001
		HC	1
		DE	1
	DT	DC	0.001
		HC	0.001
	DE	DC	0.001
		HC	0.001
	DC	HC	0.001
Bcl-2 (ng/100mg tissue)	DTE	DT	0.004
		DE	0.001
		DC	0.001
		HC	1
	DT	DE	1
		DC	0.001
		HC	0.001
	DE	DC	0.006
		HC	0.001
	DC	HC	0.001

DTE: Diabetic / Training + Extract, DT: Diabetic / Training, DE: Diabetic / Extract, DC: Diabetic / Control, HC: Healthy / Control.

In another study, researchers investigated the effect of aerobic training on treadmill (60 minutes a day, 5 days a week for 10 weeks) on the Fas receptor-dependent and mitochondrial apoptosis pathways in the rat ovaries, and indicated the abnormal structure of the heart. On the other hand, cardiac fibrosis and apoptotic cells improved following aerobic training. Moreover, FAS ligand level, FAS receptor,

FAS death domain, activated caspase 8, activated caspase 3 (Fas receptor-dependent apoptotic pathways) as well as t-Bid, Bad, Bak, Bax, cytochrome C of cytosol, activated caspase 9 and caspase 3 active (mitochondrial-dependent pathways) were significantly reduced with aerobic training. The evidences of this study also confirm the present findings (21).

Increased oxidative stress following diabetes has been shown to increase the amount of reactive oxygen species and decrease antioxidant defense capacity, resulting in programmed death of heart cells or an apoptotic pattern (22, 23).

Studies have reported that the cause of cardiac apoptosis due to diabetes, in addition to increased oxidative stress, is the inflammatory process and the presence of cytokines such as TNF-α, IL-1β and IFN-γ. Their effect on nitric oxide production also increases FAS ligand by inflammatory and cardiac cells and eventually apoptotic cell death occurs in cardiac cells (24). However, the exact molecular mechanisms of apoptosis due to high glucose concentrations have not been identified. The researchers reported that these mechanisms varied, depending on the cell and tissue studied (25). Previous research has shown that the increasing in the activity of antioxidant enzymes and decreasing in the level of lipid peroxidation, that results from exercise, have significant effects in preventing the complications of diabetes-induced apoptosis and tissue damage caused by stress (26).

Regular exercise has been shown to increase the activity of antioxidant enzymes, increase resistance to oxidative stress, and thus reduce oxidative damage (27). In addition, regular exercise has been shown to be effective in preventing and delaying diabetes, increasing insulin sensitivity, and improving glucose metabolism (28). Exercise training also has been shown to reduce the ratio between pre-apoptotic proteins and anti-apoptotic proteins such as, Bcl-2 and to reduce caspase-3 activation signaling (the final caspase of the apoptotic pathway) (29).

One of the most important possible mechanisms in the field of cellular protection due to exercise can be the ability to block the formation of free radicals. Oxygen-activated species are produced in the mitochondrial electron transfer chain as a natural product, but can lead to cell death when their levels exceed the antioxidant capacity of the cell. Oxidative stress from reactive oxygen species is strongly associated with diabetes and its complications can trigger cell death through a variety of pathways (30). Cellular and molecular factors communicate with each other through cascading signaling. Intercellular cascade signaling occurs in response to external stimuli and stress.

One of the factors involved in signaling stress and stimuli is protein B kinase. Protein kinase B is the major operator in the phosphatidylinositol-3 kinase signaling pathway, which is involved in many cellular processes, including cell survival, metabolism, cell growth and proliferation. Increased expression and increased protein kinase B activity by phosphorylation of anti-apoptotic proteins of the Bcl-2 family and inactivation of apoptosis-promoting proteins such as Bax or by direct inhibition of caspase, activity block apoptotic pathways (31). Studies have reported that diabetes lower protein kinase B levels in animal specimens (31). Probably another mechanism of cellular protection against apoptosis is the increased expression of protein kinase B (32). It has also been shown that grape seed extract has a protective role in pancreatic tissue due to its antioxidant properties. In addition, increasing the enzyme glutathione peroxidase and decreasing fat peroxidation due to the presence of grape seed extract was able to significantly reduce the damage caused by streptozotocin in pancreatic tissue (33). Grape seed extract as an antioxidant can significantly prevent heart cell damage in diabetic cardiomyopathy by reducing oxidative stress and increasing

antioxidant defense (34). Any intervention that increases antioxidant defense and reduces the production of free radicals can reduce the damage caused by oxidative stress that threatens heart cells in diabetes (35). However, this was the first time that a study examined the simultaneous effect of training and consumption of grape seed extract on cardiomyocyte apoptosis. The results showed that by adding the anti-apoptotic effect of aerobic training and consumption of grape seed extract, we see more effects, but to achieve more reliable results, more studies are needed in this area.

Conclusion

It is concluded that both moderate-intensity aerobic exercise and grape seed extract consumption can reduce the cardiac complications of type 1 diabetes by reducing the apoptosis factors of cardiac myocytes in male Wistar type 1 diabetic rats. It seems that if moderate intensity aerobic training and consumption of grape seed extract are used simultaneously, which can lead to more beneficial effects. Reducing oxidative stress and inflammation may play an important role. By combining the antioxidant and anti-inflammatory effects of moderate-intensity aerobic exercise with the consumption of grape seed extract, more anti-apoptotic effects may be induced in the myocytes of type 1 diabetic rats.

Acknowledgment

This study was supported by Islamic Azad University of Najafabad.

References

1. Saeidi A, Soltani M, Daraei A, Nohbaradar H, Haghighi MM, Khosravi N, et al. The Effects of Aerobic-Resistance Training and Broccoli Supplementation on Plasma Dectin-1 and Insulin Resistance in Males with Type 2 Diabetes. Nutrients. 2021;13(9):3144. doi: 10.3390/nu13093144.
2. Mattisson IY, Björkbacka H, Wigren M, Edsfeldt A, Melander O, Fredrikson GN, et al. Elevated markers of death receptor-activated apoptosis are associated with increased risk for development of diabetes and cardiovascular disease. EBioMedicine. 2017;26:187-97. doi: 10.1016/j.ebiom.2017.11.023.
3. Chen Z, Jiang H, Wan Y, Bi C, Yuan Y. H2O2-induced secretion of tumor necrosis factor-α evokes apoptosis of cardiac myocytes through reactive oxygen species-dependent activation of p38 MAPK. Cytotechnology. 2012;64(1):65-73. doi: 10.1007/s10616-011-9392-3.
4. Kinugawa T, Kato M, Yamamoto K, Hisatome I, Nohara R. Proinflammatory cytokine activation is linked to apoptotic mediator, soluble Fas level in patients with chronic heart failure. Int Heart J.. 2012;53(3):182-6. doi: 10.1536/ihj.53.182.
5. Li S, Li X, Rozanski GJ. Regulation of glutathione in cardiac myocytes. JMCC. 2003;35(9):1145-52. doi: 10.1016/S0022-2828(03)00230-X.
6. Ramaswamy M, Siegel RM. Autoimmunity: twenty years in the Fas lane. J Immunol. 2012;189(11):5097-100. doi: 10.4049/jimmunol.1202833.
7. Watanabe-Fukunaga R, Brannan CI, Itoh N, Yonehara S, Copeland NG, Jenkins NA, et al. The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J Immunol. 1992;148(4):1274-9.
8. Marzetti E, Privitera G, Simili V, Wohlgemuth SE, Aulisa L, Pahor M, et al. Multiple pathways to the same end: mechanisms of myonuclear apoptosis in sarcopenia of aging. Sci World J. 2010;10:340-9. doi: 10.1100/tsw.2010.27.
9. Riddell M, Iscoe K. Physical activity, sport, and pediatric diabetes. Pediatr

Diabet. 2006;7(1):60-70. doi: 10.1111/j.1399-543X.2006.00146.x.
10. Saeidi A, Haghighi MM, Kolahdouzi S, Daraei A, Abderrahmane AB, Essop MF, et al. The effects of physical activity on adipokines in individuals with overweight/obesity across the lifespan: A narrative review. Obes Rev. 2021;22(1):e13090. doi: 10.1111/obr.13090.
11. Tayebi SM, Saeidi A, Khosravi M. Single and concurrent effects of endurance and resistance training on plasma visfatin, insulin, glucose and insulin resistance of non-athlete men with obesity. AASS. 2016;4(4):21-31. doi: 10.18869/acadpub.aassjournal.4.4.21.
12. Hafstad AD, Boardman N, Aasum E. How exercise may amend metabolic disturbances in diabetic cardiomyopathy. ARS. 2015;22(17):1587-605. doi: 10.1089/ars.2015.6304.
13. Terauchi M, Horiguchi N, Kajiyama A, Akiyoshi M, Owa Y, Kato K, et al. Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: a randomized, double-blind, placebo-controlled pilot study. Menopause. 2014;21(9):990-6. doi: 10.1097/GME.0000000000000200.
14. Ghanbari Niaki A, Ardeshiri S, AliakbariBaydokhty M, Saeidi A. Effects of circuit resistance training with crocus sativus supplementation on insulin and estradiol hormones response. HMS. 2016;22(2):125-30. doi: 10.18869/acadpub.hms.22.2.125.
15. Zhang F-l, Gao H-q, Wu J-m, Ma Y-b, You B-a, Li B-y, et al. Selective inhibition by grape seed proanthocyanidin extracts of cell adhesion molecule expression induced by advanced glycation end products in endothelial cells. J Cardiovasc. Pharmacol. 2006;48(2):47-53. doi: 10.1097/01.fjc.0000242058.72471.0c.
16. Wang X, Jia D, Zhang J, Wang W. Grape seed proanthocyanidins protect cardiomyocytes against hypoxia/reoxygenation injury by attenuating endoplasmic reticulum stress through PERK/eIF2α pathway. Mol Med Rep. 2017;16(6):9189-96. doi: 10.3892/mmr.2017.7756.
17. Bugger H, Abel ED. Rodent models of diabetic cardiomyopathy. DMM. 2009;2(9-10):454-66. doi: 10.1242/dmm.001941.
18. Garekani ET, Mohebbi H, Kraemer RR, Fathi R. Exercise training intensity/volume affects plasma and tissue adiponectin concentrations in the male rat. Peptides. 2011;32(5):1008-12. doi: 10.1016/j.peptides.2011.01.027.
19. Krueger RJ. Drugs of Natural Origin. A Textbook of Pharmacognosy. By Gunnar Samuelson. Swedish Pharmaceutical Press, Stockholm. ACS Publications; 2005. doi: 10.1021/np058229l.
20. Tanoorsaz S, Behpour N, Tadibi V. Investigating the Effect of mid-term of aerobic exercise on apoptosis biomarkers in the cardiomyocytes of streptozotocin-induced diabetic rats. J Fasa Uni Med Sci. 2018;7(4):488-97. doi: 20.1001.1.22285105.2018.7.4.15.4.
21. Huang CY, Lin Y, Hsu CC, Cheng SM, Shyu WC, Ting H, et al. Antiapoptotic effect of exercise training on ovariectomized rat hearts. J Appl Physiol. 2016;121(2):457-65. doi: 10.1152/japplphysiol.01042.2015.
22. Wang J, Song Y, Wang Q, Kralik PM, Epstein PN. Causes and characteristics of diabetic cardiomyopathy. Rev Diabet Stud. 2006;3(3):108. doi: 10.1900/RDS.2006.3.108.
23. Podestà F, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C, et al. Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis in vivo and in vitro. Am. J. Clin. Pathol. 2000;156(3):1025-

32. doi: 10.1016/S0002-9440(10)64970-X.
24. Mauricio D, Orlinick J, Vaishnam A. Role of Fas-FasL in insulitis in nonobes diabetic mouse. Chin Med J. 2018;43:1537-47.
25. Allen DA, Yaqoob MM, Harwood SM. Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications. J Nutr Biochem. 2005;16(12):705-13. doi: 10.1016/j.jnutbio.2005.06.007.
26. SA F. Effect of swimming exercise on oxidative stress in hippocampus of diabetic male Rats. Med J Tabriz Univ Med Sci. 2009;30(2):111-8.
27. Mazzola PN, Terra M, Rosa AP, Mescka CP, Moraes TB, Piccoli B, et al. Regular exercise prevents oxidative stress in the brain of hyperphenylalaninemic rats. Metab Brain Dis. 2011;26(4):291-7. doi: 10.1007/s11011-011-9264-8.
28. Derouich M, Boutayeb A. The effect of physical exercise on the dynamics of glucose and insulin. J Biomech. 2002;35(7):911-7. doi: 10.1016/S0021-9290(02)00055-6.
29. Aboutaleb N, Shamsaei N, Khaksari M, Erfani S, Rajabi H, Nikbakht F. Pre-ischemic exercise reduces apoptosis in hippocampal CA3 cells after cerebral ischemia by modulation of the Bax/Bcl-2 proteins ratio and prevention of caspase-3 activation. Physiol Sci. 2015;65(5):435-43. doi: 10.1007/s12576-015-0382-7.
30. Hong JH, Kim MJ, Park MR, Kwag OG, Lee IS, Byun BH, et al. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. Clin Chim. 2004;340(1-2):107-15. doi: 10.1016/j.cccn.2003.10.003.
31. Kim DY, Jung SY, Kim CJ, Sung YH, Kim JD. Treadmill exercise ameliorates apoptotic cell death in the retinas of diabetic rats. Mol Med Rep. 2013;7(6):1745-50. doi: 10.3892/mmr.2013.1439.
32. Libonati JR. Cardiac effects of exercise training in hypertension. Int Sch Res Notices. 2013;2013. doi: 10.5402/2013/980824.
33. Du Y, Guo H, Lou H. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. J Agric Food Chem. 2007;55(5):1695-701. doi: 10.1021/jf063071b.
34. Saad AA, Youssef MI, El-Shennawy LK. Cisplatin induced damage in kidney genomic DNA and nephrotoxicity in male rats: the protective effect of grape seed proanthocyanidin extract. Food Chem Toxicol. 2009;47(7):1499-506. doi: 10.1016/j.fct.2009.03.043.
35. Ghosh S, Pulinilkunnil T, Yuen G, Kewalramani G, An D, Qi D, et al. Cardiomyocyte apoptosis induced by short-term diabetes requires mitochondrial GSH depletion. Am J Physiol Heart Circ Physiol. 2005;289(2):H768-H76. doi: 10.1152/ajpheart.00038.2005.