

## The Effect of Interval and Continued Trainings with Crocin on Apoptotic Markers in the Heart Tissue of High-Fat Diet and Streptozotocin Induced Type 2 Diabetic Rats

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### Abstract

**Introduction:** Diabetes is a metabolic disease that has a close relationship with increased physical disabilities and muscle tissue damage. The aim of this study was to investigate the effect of interval and continued trainings with crocin consumption on Bcl-2, Bax and P53 gene expression of heart tissue in high-fat diet- and streptozotocin induced type 2 diabetic rats.

**Methods:** In this experimental study, 49 adult high-fat diet- and streptozotocin induced diabetic rats were selected and randomly assigned to 7 groups (1) high intensity interval training (HIIT) (2) low intensity continued training (LICT) (3) HIIT with crocin consumption, (4) LICT with crocin consumption, (5) crocin consumption, (6) sham and (7) control. HIIT and LICT groups ran the treadmill for eight weeks, three sessions per week with intensity 80- 85 and 50- 55 percent of maximum running speed, respectively, and crocin consumption groups received crocin 25 mg/kg per week for eight weeks. To analyze the research findings, paired sample t-test, two- way ANOVA with Bonferroni post hoc test were used ( $p \leq 0.05$ ).

**Results:** Eight weeks of HIIT and LICT had significant effect on reduction of Bax gene expression in heart tissue of diabetic rats ( $p \leq 0.05$ ), and HIIT had significant effect on reduction of P53 gene expression in heart tissue of diabetic rats ( $p \leq 0.05$ ), crocin consumption had significant effect on reduction of P53 gene expression in heart tissue of diabetic rats ( $p \leq 0.05$ ), endurance training with crocin consumption had interaction effect on increase of Bcl-2 gene expression and reduction of P53 gene expression in heart tissue of diabetic rats ( $p \leq 0.05$ ).

**Conclusion:** It seems that interval and continued with crocin consumption in high- fat diet- and streptozotocin induced diabetic rats have interaction anti-apoptotic effects.

**Keywords:** Training, Crocin, Apoptosis, Diabetes

### Introduction

Type 2 diabetes mellitus (DM) is a complex metabolic disease in which concomitant insulin resistance and beta-cell impairment lead to hyperglycemia, which is the hallmark of the disease (1). Researches show, most patients with type 2 diabetes are obese, and epidemic of obesity largely explains the dramatic increase in the incidences and prevalence of type 2 diabetes (2). Obesity and diabetes are well-established risk factors for several types of cardiovascular disease (CVD), but the pathways under-lying these associations are incompletely understood (2, 3). This association is probably due to genetic, environmental and immunological factors,

such as T helper 1 cells (Th1) and T helper 17 cells (Th17) pathway activation, proinflammatory cytokines and increased oxidative stress (4). Researchers believe that oxidative stress has been shown to be involved in triggering cardiomyocyte apoptosis associated with diabetic cardiomyopathy (5). In the diabetic state reactive oxygen species (ROS) activates various redox-sensitive signaling molecules, leading to cellular dysfunction and injury and ultimately micro and macrovascular complications (6, 7). These free radicals all might play a role in DNA damage, glycation, protein modification reactions, and in lipid oxidative modification and heart tissue apoptosis in diabetes (5-7). By

increasing oxidative stress, the tumor suppressor protein (P53), by activating the apoptosis process, prevents the proliferation and repair of cells and accelerates cell death. During the mechanism, P53 activates caspase 9, which is one of the major enzymes for the onset of apoptosis (8, 9). The activation of caspase 9 from the intrinsic pathway increases the Bcl-2-associated X protein (Bax) gene as the precipitant of apoptosis and decreases the expression of B-cell lymphoma 2 (Bcl-2) as an anti-apoptotic agent (9, 10). Regular physical activity moderator effects have been reported on the inherent immune system, reduction of cardiovascular complications, improvements in strength and functional ability in diabetic patients (11, 12). Some researchers believe that exercise reduces apoptosis by reducing the oxidative stress (5). However, there is uncertainty regarding the effect of exercise on apoptotic markers. For example, four weeks of endurance training resulted in a significant increase in the first apoptosis signal receptor (Fas) and Fas ligand (FasL) levels, and no change in Bcl-2 levels in the heart tissue of diabetic and non-diabetic rats (13); But high intensity interval trainings did not significantly effect on increase Bcl-2 levels, but increased Bax in rats with myocardial infarction. However, endurance trainings with an intensity of 80- 75% maximum run speed had no significant effect on P53 changes, but significantly decreased cytochrome C in soleus muscle tissue in male rats (8). Also, for treating or controlling diabetes, several therapeutic approaches, such as the use of medicinal plants, are recommended for diabetic patients (14, 15). Research suggests that the compounds contained in food supplements and medicinal plants used in traditional medicine, including flavonoids, food fibers and other antioxidant compounds, in addition to reducing blood lipids, can contribute to the inhibition of oxidation and ROS, and have an effect on the immune system and the improvement of metabolic abnormalities in the body (16). Among the

medicinal plants, saffron belonging to iridaceae family, contains a substance called crocin, which researchers have studied the beneficial effects of this substance on glycemic indexes and fat profiles (14). In this regard, six weeks of 25 mg/kg daily aqueous extract of saffron reduced hemoglobin glycolysis and insulin resistance in diabetic rats (14); Four weeks of 25 mg/kg daily aqueous extract of saffron reduced Low-density lipoprotein (LDL), Very-low-density lipoprotein (VLDL), Triglycerides (TG) and Cholesterol (Cho) of diabetic rats, nevertheless it has no significant effect on High-density lipoprotein (HDL) (15); nine weeks intake of 50 mg/kg of aqueous extract of saffron stigma had a significant effect on oxidative stress reduction due to intensive training in the heart and brain tissues of young male rats (17); receiving 0.5 ml of crocin reduced Bax and increased Bcl-2 and antioxidant enzymes, or decreased apoptosis by decreasing lipid peroxidation (18), treated with crocin (10, 20, 40 mg/kg, i.p.), reduced lipid peroxidation in the liver and brain tissues while elevated Glutathione Peroxidase (GSH) content. Also, a decline in serum levels of Tumor necrosis factor alpha (TNF- $\alpha$ ) and Interleukin 6 (IL-6) (19). Regarding the relationship of diabetes and obesity with cardiovascular disease, the role of sports activities in reducing the complications of diabetes and the use of medicinal plants with antioxidant properties is significant. Also, despite the conducted research, this study has been found to examine the simultaneous effects of crocin and endurance trainings on apoptotic indices in diabetic rat heart tissue, therefore, the aim of this study was to investigate the interactive effect of endurance training and crocin on apoptosis indices in heart tissue of high-fat diet-induced type 2 diabetic rats.

## Methods

In this experimental study, 49 male Sprague-Dawley rats, eight weeks old and a weighing range of  $150 \pm 30$  g, were purchased from the

Animals Reproduction and Breeding Center of Islamic Azad University, Marvdasht Branch. After moving to the animal physiological lab, they were transferred under standard conditions with an ambient temperature of 22 to 27 ° C, a relative humidity of 50%, and a controlled light (12-hour cycle of light and dark) and passed the seven-day adaptation period. Animals access to water and food was free during the period. In the present study, for the induction of type 2 diabetes, a combination of high-fat diet and Streptozotocin (STZ) was used. For this purpose, all rats were subject to a period of eight weeks with a fatty diet of 45% total fat (derived from animal fat), containing 24 grams of fat, 24 grams of protein and 41 grams of carbohydrate per 100 grams (20). After eight weeks, diabetes was induced with a single dose STZ dissolved in sodium citrate buffer with PH = 4.5 in 30 mg/kg intraperitoneally (20). For confirmation of diabetes, 96 h after injection, rats with glucose levels higher than 300 mg / dL were selected as samples (20). Based on the blood glucose, rats were divided into seven groups of 7 including: (1) high intensity interval training (2) low intensity continued training (3) high intensity interval training with crocin consumption, (4) low intensity continued training with crocin consumption, (5) crocin consumption, (6) sham and (7) control. To evaluate the maximum running speed, the sport performance test graded with a zero gradient was performed. To perform this test, the rats started at a speed of 10 m / min, and then the speed of the treadmill was increased to 1 m / min for each 1 minute. This process continued until the rats were no more able to run (exhaustion) (20). After speed estimation, groups 1 and 3 trained for eight weeks, three sessions per week with an intensity of 80 to 85% of maximum speed for 2 minutes and active rest periods of one minute. High intensity interval trainings from six exercises in the first week of training reached 12 exercises in the last week. Groups 2 and 4 were also trained for eight weeks, three

sessions per week with an intensity ranging from 50 to 55% of maximum running. Low-intensity continued trainings began in the first week of 25 minutes and reached 50 minutes in the last week. It should be noted that the total volume of exercise activity (intensity, duration and repetition) was matched between the two groups of low intensity training and high intensity interval trainings (20). Also, groups 3, 4 and 5 received 25 mg / kg of crocin (dissolved in normal saline) intraperitoneally (21). In order to control the effects of injection on the variables of the study, the sham group received crocin (Sigma, Cat No: 17304) soluble intraperitoneally daily. 24 hours after the last training session at the end of the eighth week, the rats were surgically treated to measure the parameters. Rats were anesthetized after about 5 minutes with ketamine 10% (50 mg/kg) and xylosin 2% (10 mg / kg), the heart tissue was then extracted by experts and placed in liquid nitrogen after being placed in the micro tube and stored for further investigation at -70 ° C. RNA extraction was performed based on the RNA extraction kit instructions of *Yekta Tajhiz* Company, using extraction kit solutions and proposed protocols of the manufacturer. For this purpose, 30 milligrams of the desired heart tissue were dried in liquid nitrogen and placed in a 1.5 tube after hitting in a sterile piston. The first step in extracting RNA from animal cells was to destroy the cell wall with the help of a lubricating buffer (here RB Buffer). 350 µl of RB buffer was added to the sample (centrifuge cellular deposition); (Previously, 10 µl of mercaptoethanol-β per 1 milliliter was added to buffer) and was placed at room temperature for 5 minutes. Next, the filter column was placed inside the collection tube and the sample mixture was transferred to the filter column and centrifuged at 14000 rpm for 2 minutes. After centrifuge, the clear solution was transferred from the collection tube to a new microcentrifuge tube. Then the same amount as its volume, ie 350 µl, ethanol 70% was added to it; then it was well

vortexed. The RB Mini Column was placed inside the collection tube and transferred to the RB Mini Column, to which ethanol was added, centrifuged at 14,000 rpm for 1 minute; and the solution in the collection tube was discarded. In the next step, 500 µl wash buffer1 was added to the RB mini column and centrifuged at 14000 rpm for 1 minute and the solution in the collection tube was discarded. This step was repeated twice and then centrifuge was performed for 3 minutes at a speed of 14000. The RB mini column was then inserted into the elution tube; 50 µl RNase-free ddH<sub>2</sub>O was added to the RB mini column and 1 minute spent and after 1 minute centrifuged for 2 minutes at 14000 rpm. The solution in elution tube contained extracted RNAs stored at 70 ° C. Synthesis of cDNA was carried out according to the instructions in the kit formantase (K1622). Reverse transcription reaction was performed using RevertAid™ M-MuLV Reverse Transcriptase enzyme. When preparing cDNA from a purified sample, following retrieval, a volume of 1000 ng RNA was removed. Then 0.5 µl of Random Hexamers (oligodeoxyribonucleotide used as a primer to initiate cDNA synthesis), 0.5 µl oligodT primer and up to 12 µl of water in the kit was added; transferred to a temperature of 65 ° C for 5 minutes and then placed on ice for 2 minutes. In the next step, µl4 5X reaction buffer, µl 2 dNTP Mix, µl1 RiboLock RNase inhibitor and µl1 RevertAid RT was added to the previous compound, which was placed at 65°C for 5 minutes. The mixture was then first placed at 25 ° C for 5 minutes. After that, it was placed at a temperature of 42 for 60 minutes. In the end, in order to disrupt the RT enzyme, the reaction tubes were placed at 70 ° C for 5 minutes. Prepared cDNA for RT-PCR was used or stored for storage at -20C°. After optimizing the reaction, the cDNA of the tested groups was subjected to RT-PCR

reaction according to the 2-7 timetable. The principles of performing RT-PCR were similar to those of PCR, except that the MasterMix containing Cybergren (Takara) was used instead of normal MasterMix. The binding temperature for all primers is 60 ° C. The sequence of primers used is also shown in Table 1. After completing the activity of the device and observing the diagrams for increasing the number of desired pieces and the amount of fluorescence emission calculating  $\Delta\Delta Ct$ , the change in the desired gene expression compared to B<sub>2</sub>m and the control state without distinct environments was measured.

$$\Delta Ct = C_{t \text{ interets}} - C_{t \text{ GAPDH}}$$

$$C_t = \Delta C_{t \text{ Treat}} - \Delta C_{t \text{ Un Treat}} \quad \Delta\Delta$$

Then, using the formula  $\Delta\Delta Ct$ -2, its expression was calculated. To determine the normal distribution of the findings, the Shapiro-wilk test was used; also paired sample t-test, Independent sample t-test and Two-way ANOVA with bonferroni post hoc test in SPSS software (version 21) were used ( $p \leq 0.05$ ).

## Results

Weight of rats in seven groups presented in table 1 also gene expression levels of Bcl- 2, Bax and P53 in heart tissue of rats presented in figure 1- 3. The results of paired sample t- test in table showed that weights of control ( $p=0.001$ ) and sham ( $p=0.001$ ) groups in post-test significantly increased rather than pre-test; weights of low intensity continued training ( $p=0.001$ ), low intensity continued training with crocin ( $p=0.001$ ) and high intensity interval training with crocin ( $p=0.001$ ) groups in post- test significantly reduced rather than pre- test nevertheless there were no significant differences between weights of high intensity interval training ( $p=0.10$ ) and crocin ( $p=0.09$ ) groups in pre-test and post- test. For review the effect of crocin solvent on research variables the results of independent sample t- test showed that there were no significant different in gene

expression levels of Bcl- 2 ( $t=0.42$  and  $p=0.67$ ), Bax ( $t=-1.73$  and  $p=0.10$ ) and P53 ( $t=0.92$  and  $p=0.37$ ) between control and sham groups. The results of two way ANOVA test showed that endurance training ( $F=2.65$ ,  $p=0.08$  effect size 0.11) and crocin consumption ( $F=2.92$ ,  $p=0.95$  effect size 0.06) had no significant effect on Bcl-2 gene expression of heart tissue in diabetic rats but training with crocin consumption had significant interaction effect on increase of Bcl-2 gene expression of heart tissue in diabetic rats ( $F=13.14$ ,  $p=0.001$  effect size 0.38); endurance training ( $F=6.84$ ,  $p=0.003$  effect size 0.24) had significant effect on reduction of Bax gene expression of heart tissue in diabetic rats nevertheless crocin consumption ( $F=1.25$ ,  $p=0.27$  effect size 0.02) had no significant effect on Bax gene expression of heart tissue in diabetic rats also training with crocin consumption had no significant interaction effect on reduction of Bax gene expression of heart tissue in diabetic rats ( $F=0.24$ ,  $p=0.78$  effect size 0.01), the results of bonferroni post hoc test showed that high intensity interval training ( $p=0.005$ ) and low intensity continued training ( $p=0.01$ ) had significant effect on reduction of Bax gene

expression of heart tissue in diabetic rats also high intensity interval training and low intensity continued training had equally effects on reduction of Bax gene expression of heart tissue in diabetic rats ( $p=0.99$ ). The results of two way ANOVA test showed that endurance training ( $F=4.24$ ,  $p=0.02$  effect size 0.16) and crocin consumption ( $F=5.74$ ,  $p=0.02$  effect size 0.12) had significant effect on reduction of P53 gene expression of heart tissue in diabetic rats also training with crocin consumption had significant interaction effect on reduction of P53 gene expression of heart tissue in diabetic rats ( $F=3.28$ ,  $p=0.04$  effect size 0.13); the results of bonferroni post hoc test showed that high intensity interval training ( $p=0.01$ ) had significant effect on reduction of P53 gene expression of heart tissue in diabetic rats but low intensity continued training had no significant effect on reduction of P53 gene expression of heart tissue in diabetic rats ( $p=0.68$ ) nevertheless high intensity interval training and low intensity continued training had equally effects on reduction of P53 gene expression of heart tissue in diabetic rats ( $p=0.30$ ).

**Table 1.** Sequence of the forward- reverse primers of the genes desired for real-time PCR reaction

Gene	Forward (5'-3')	Reverse (5'-3')	Product size (bp)
B2m	CGTGCTTGCCATTCAGAAA	ATATACATCGGTCTCGGTGG	244
Bax	CTGCAGAGGATGATTGCTGA	GATCAGCTCGGGCACTTTAG	147
Bcl- 2	ATCGCTCTGTGGATGACTGA GTAC	AGAGACAGCCAGGAGAAAT CAAAC	134
P53	GGCTCCGACTATAACCACTAT CC	GAGTCTTCCAGCGTGATGAT G	104

## Discussion

The results of the study showed that exercise training had not significant effect on Bcl-2 gene expression; However, high intensity interval training and low intense continued training had significant effect on reducing the Bax expression of the heart tissue of diabetic

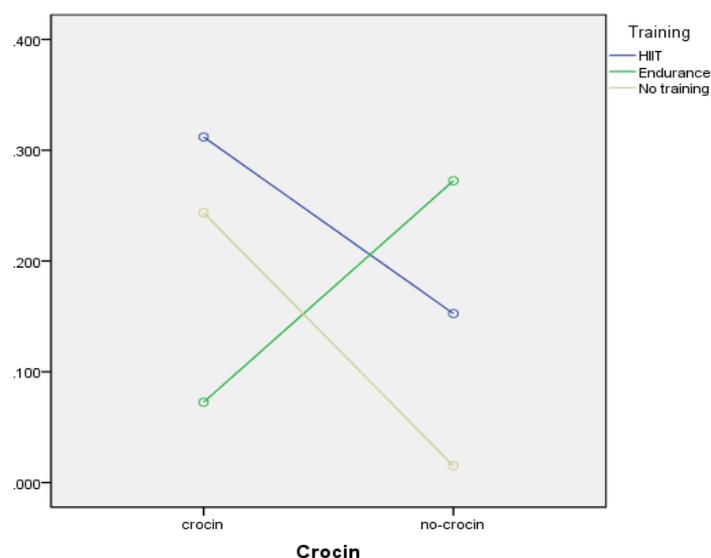
rats, Also, high intensity interval training had significant effect on the reduction of P53 gene expression in the heart tissue of diabetic rats. T2DM is caused by development of cellular resistance to insulin combined with insufficient insulin production (22).

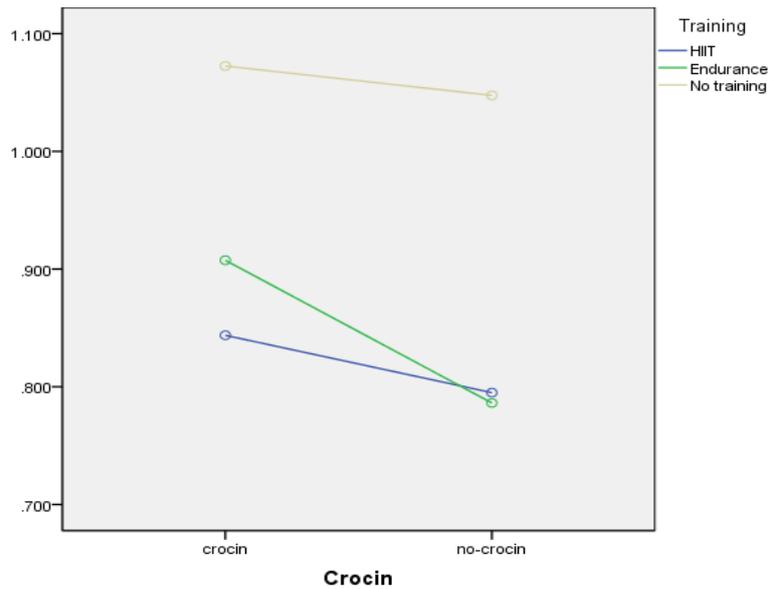
**Table 2.** Pre-test and post-test weight of rats in seven groups of research

Group	Pre- test (gr)	Post- test (gr)	Paired samples t- test	
	Mean±STD	Mean±STD	t	Sig
High intensity interval training	360.64±13.12	342.12±44.11	1.91	0.10
Low intensity continued training	375.12±33.18	352.11±17.18	-21.71	0.001
Crocin consumption	345.25±44.08	364.12±13.10	-1.97	0.09
High intensity interval trainings with crocin consumption	410.47±30.87	392.41±46.52	4.24	0.005
Low intensity continued training with crocin consumption	394.88±25.66	354.22±18.12	17.47	0.001
Sham	390.59±42.33	409.62±45.17	-21.71	0.001
Control	384.64±50.41	420.88±62.14	-10.01	0.001

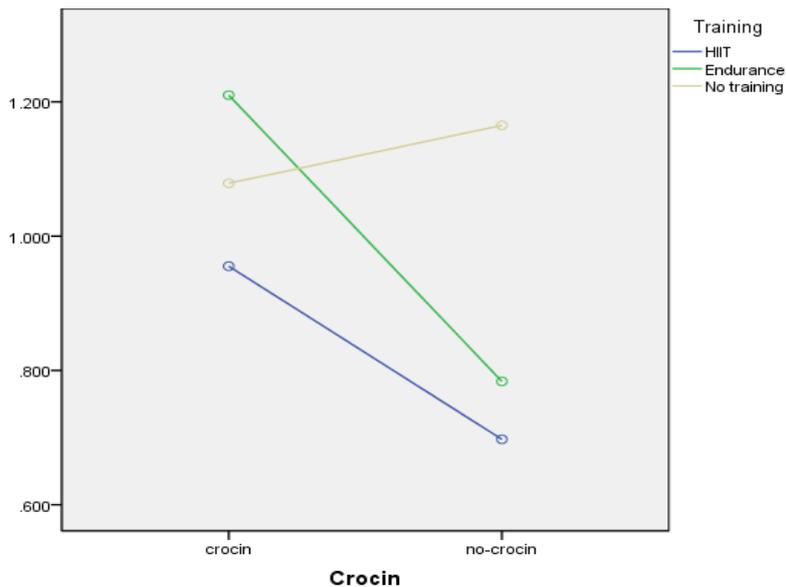
**Table 3.** The results of two-way ANOVA to study the effects of high intensity interval and low-intensive continued trainings with crocin consumption on Bcl-2, Bax and P53 gene expression levels in heart tissue of diabetic rats

Variable	Factor	Sum square	of df	F	P	Effect size
Bcl- 2	Training	0.086	2	2.65	0.08	0.11
	Crocin	0.047	1	2.95	0.09	0.06
	Interaction of training and crocin	0.42	2	13.14	0.001	0.38
Bax	Training	0.02	2	6.84	0.003	0.24
	Crocin	0.55	1	1.25	0.27	0.02
	Interaction of training and crocin	1.70	2	0.24	0.78	0.01
P53	Training	0.70	2	4.24	0.02	0.16
	Crocin	0.47	1	5.74	0.02	0.12
	Interaction of training and crocin	3.48	2	3.28	0.04	0.13

**Figure 1.** Bcl-2 gene expression levels in heart tissue of rats in seven groups of research



**Figure 2.** Bax gene expression levels in heart tissue of rats in seven groups of research



**Figure 3.** P53 gene expression levels in heart tissue of rats in seven groups of research

Apoptotic death of  $\beta$ -cells has been observed in response to diverse stimuli, such as glucose, cytokines, free fatty acids (FFA), leptin, islet amyloid polypeptide, endoplasmic reticulum (ER) stress, and sulfonylureas (23, 24). It was also found that in T2DM, in addition to its established negative effect on insulin secretion induces  $\beta$ -cells apoptosis through increased release of interleukin-1 beta (IL-1 $\beta$ ) and decreased release of IL1Ra. Other cytokines

released by adipocytes such as TNF- $\alpha$  and IL-6 may also modulate survival. Hyperglycemia activates NADPH oxidase resulting in elevation of reactive oxygen species (ROS) formation through the polyol pathway, hexosamine pathway, and diacylglycerol/protein kinase-C (DAG/PKC) pathway. It also interrupts the mitochondrial electron transport chain at complex III resulting in increased oxidation of molecular

oxygen by coenzyme Q yielding superoxide anion, nitric oxide, and subsequent activation of NOS. This attributes to the production of peroxynitrite ONOO causing oxidative stress and nitrosative stress events to the cytokine production, reducing in Bcl-2 and also induce proapoptotic members Bid, Bik, Bax and caspase-3 and as a result apoptosis of cells (24, 25). On the other hand, exercise training may promote cell-survival proteins including Mn isoform of superoxide dismutase (MnSOD), Nuclear factor kappa light chain enhancer of activated B cells (NF-kB), extracellular receptor kinase (ERK), IGF-1/Akt pathway, and heat shock proteins (HSPs) in heart, which may be potential upstream regulators of diabetic-induced apoptosis in the heart (26). Probably one of the other mechanisms of protective functions of the exercise training in diabetic patients is increased protein kinase B. One of the factors contributing to stress and stimulation signaling is protein kinase B. Protein kinase B acts as the main agent in the signaling pathway of phosphatidylinositol -3 kinase, which plays a role in cellular processes, including cell survival, metabolism, cell growth and proliferation. Increasing the expression and enhancement of protein kinase B activity through phosphorylation of the anti-apoptotic proteins of the Bcl-2 family, disabling the apoptotic promoter protein such as Bax and direct inhibition of caspase activity can block the apoptosis pathways. Also, regarding the relationship between the mechanism of the effect of high intensity interval training and low intensity continued training, researchers believe that improving mitochondrial function due to low and high adaptation of intracellular apoptotic pathways, pre-apoptotic signal molecules (e.g., pre-apoptotic proteins of the Bcl-2 family such as BAK And Bax) are transmitted to the mitochondria and induce a series of temporary permeable pores in the external mitochondrial membrane, which inhibit cytochrome C release and lead to a decrease in caspase activity (27). However, exercise training did

not significantly affect the expression of Bcl-2 in the heart tissue, In justifying this finding, researchers believe that the existing evidence, exercise intensity, duration and frequency is the most critical factor effective on the reduction of apoptosis (27, 28). Many studies have examined the effects of exercise on Bcl-2, Bax and P53, For example, long-term moderate aerobic exercises (29, 30), intense interval training (27) and swimming training (31); decreased Bax, Caspase 3, BAD in streptozotocin- induced diabetic rats (19); obese Zucker rats (31). And in some studies had no significant effect on the changes of Bcl-2 in heart tissue of rats with myocardial infarction (27); also reduced Bcl-2 levels in skeletal muscle of elderly (32); high blood pressure (30); muscle tissue in rats (33); The reasons for consistency of results can be the sameness in the population, intensity of exercise type of exercises, long duration of the training period. On the other hand, inconsistent with the present study, in a study cardiac muscle cell apoptosis after anaerobic exercise and detraining of caspase 3 expression increased in the 4-week training group compared to the 4-week control group and 12-week training compared to 12-week control. Detraining caused a significant decrease in caspase 3 expression. As a result, anaerobic exercise can increase apoptosis by increasing the expression of caspase 3 in the left ventricle of the rat heart (34); For reasons of inconsistency, one can point out the difference during the training period in these two studies; Eight weeks of aerobic exercise with a 60% maximum oxygen consumption at a speed of 5% and a gradient of 5% and duration of 30 minutes per day increased the levels of TNF- $\alpha$ , ROS, P53, Bax levels in skeletal muscle of elderly rats (32); The difference in the statistical population is another reason for the inconsistency of the two studies. It can be noted that the statistical population of the present study was diabetic rats, but elderly rats were evaluated by Ziaaldini *et al.* On the other hand, eight weeks

of optional activity (rotary wheel) had no significant effect on Bcl-2 and Bax changes in rats (35); high intensity interval training increased Bax expression in the heart tissue compared to the control group and had no significant effect on the changes of Bcl-2 in heart tissue of rats with myocardial infarction (27). Among the reasons for the inconsistency of this research, the statistical population and the differences in the evaluated tissue can be pointed out. The results of present study showed that eight weeks of crocin consumption had no significant effect on increase of Bcl-2 gene expression and reduction of Bax gene expression in heart tissue of diabetic rats. However, the use of crocin had significant effect on reducing of P53 gene expression in the heart tissue of diabetic rats. High levels of blood glucose lead to inflammation and increase oxidative stress; as a result, an increase in reactive oxygen species accelerates cell death (apoptosis) from both the signal pathway within the cytosolic and mitochondrial regions. One of the possible mechanisms of crocin to reduce apoptosis is the reduction of P53 expression levels, as well as the antioxidant properties of crocin and the effects of reducing blood sugar and HbA1c. In other words, this mechanism is dependent on the anti-apoptotic effects of reducing hydrogen peroxide, reducing  $H_2O_2$  and caspase 3 (36). In fact  $Ca^{2+}$  plays a very important role in the onset and induction of apoptosis. The expression level of Bcl-2 decreases with increasing  $Ca^{2+}$  release from endoplasmic reticulum (37). Crocin inhibited NF- $\kappa$ B activation, the levels of NO, TNF- $\alpha$ , IL-1 $\beta$ , and intracellular reactive oxygen species (ROS) release from cultured the rat brain microglial cells induced by LPS. It was indicated that  $\alpha$ -crocin reduced the effect of TNF- $\alpha$  in cells and also blocked the TNF- $\alpha$ -induced expression of Bcl-XS and LICE and ameliorated the cytokine-induced decrease of Bcl-XL mRNA expression in cells (28). Although in the present study, the use of Crocin did not have a significant effect on

increase of Bcl-2 and decrease Bax expression but decreased the expression of p53. Nevertheless, it seem, the anti-apoptotic effects of crocin are dependent on its dosage (14, 15). Regarding the effect of crocin on apoptosis, the only study found was done by Sadoughi (2017) in which it was shown that receiving 0.5 ml of crocin reduced Bax and increased Bcl-2 and antioxidant enzymes, and decreasing Lipid peroxidation reduces apoptosis. However, Crocin has a protective effect on the performance of kidneys of diabetic rats; it has been shown that crocin-dose administration can reduce serum malondialdehyde levels in diabetic rats (38). Crocin was also reported to inhibit oxidative stress and reduce lipid peroxidation and thereby reducing renal damage caused by diabetic nephropathy (39); It was reported that a single week of aqueous extract of saffron consumption caused a significant increase in superoxide dismutase, catalase, glutathione peroxidase and decrease in malondialdehyde in young male rats (40). Saffron consumption of 100 mg/kg of rat body weight increased the tissue content of superoxide dismutase and glutathione peroxidase, as well as the reduction of the malondialdehyde tissue in rats in a temporary localized cerebral ischemia model (41). 80 and 40 mg of hydroalcoholic extract of saffron per kg of body weight of rats increased total antioxidant capacity and decreased lipid peroxidation in cisplatin-affected rats (42). The aforementioned studies were consistent with reducing the oxidative stress and anti-apoptotic effects, which can be explained by the molecular mechanism of the saffron plant and its anti-inflammatory properties. Endurance training with crocin consumption had interaction effect on increase of Bcl-2 gene expression and reduction of P53 gene expression in the heart tissue of diabetic rats. However, endurance training and consumption of crocin had no interaction effect on reduction of Bax gene expression in diabetic rats. Research to find a method to reduce apoptosis caused by diabetes with

exercise can prevent excessive cell loss. Increasing the ROS caused by diabetes with the signal pathway activating the intracellular dual pathways, reducing the expression of antioxidant enzymes and other proteins protect the apoptosis. Crocin may inhibit the activation of ROS by reducing the lipid peroxidation and thereby inhibit the growth of caspases and P53 and prevent induction of apoptosis (13). Also crocin inhibited NF- $\kappa$ B activation, the levels of NO, TNF- $\alpha$ , IL-1 $\beta$ , and intracellular ROS. Researchers believe that  $\alpha$ -crocin reduced the effect of TNF- $\alpha$  in cells and also blocked the TNF- $\alpha$ -induced expression of Bcl-XS and LICE and ameliorated the cytokine-induced decrease of Bcl-XL mRNA expression in cells (28). But the exercise training by increasing the expression and enhancement of protein kinase B activity by phosphorylation of the anti-apoptotic proteins of the Bcl-2 family and inhibition of the apoptotic promoter protein such as Bax, or by direct inhibition of caspase activity can block the pathways of apoptosis (13). Studies have shown, exercise training and crocin consumption from two distinct signal pathways improve the process of diabetes-induced apoptosis in the heart tissue. The most important point is that exercise activity and the simultaneous use of crocin in both interventions, can reducing the ROS (17). In this regard, the use of aqueous extract of saffron and endurance training for period of one week has interactive effects on reducing malondialdehyde and increasing levels of superoxide dismutase, catalase and glutathione peroxidase in young male rats after an acute exercise session and one week of sports activity (17). 100 mg of saffron per day for 14 days followed by a running session with 70% maximal oxygen consumption increased superoxide dismutase levels, but had no significant effect on malondialdehyde and catalase reduction in young active men (43). Eight weeks of training with rotary wheel (optionally) and the simultaneous use of saffron extract at 50 mg / kg per day caused a

significant decrease in P53 and HbA1c (36). Regarding the results of this study, the lack of investigation of apoptosis extracellular pathways is one of the limitations of this study. Therefore, it is recommended that other apoptosis pathways be investigated in future studies. Considering the fact that the protocol of the present study showed that endurance training and crocin consumption did not have a significant effect on improvement of some apoptosis markers in the heart tissue, it seems that the intensity, type and duration of exercise, as well as the dosage of crocin, had a beneficial effect on The process of apoptosis is recommended, therefore, it is suggested that in future studies, the duration of exercise and the dose of crocin in the changes in apoptosis in heart tissue in diabetic rats can be investigated. It is worth noting that the inability of crocin as a potent antioxidant is related to drug interactions, which has received special attention in medical science. Interactions that cause not only a medication to have a positive effect, but also sometimes exacerbate negative effects. In this regard, it is suggested that in the future studies, researchers investigate the effects of crocin consumption after the end of the training period.

### Conclusion

With regard to the findings of the present study it seems that interval and continued trainings with crocin consumption in high- fat diet and streptozotocin induced diabetic rats have interaction anti-apoptotic effects.

### Ethical issues

Not applicable.

### Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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