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Anti-apoptotic Effects of Interval and Continued Training and Crocin on the Muscle Tissue of the Rats with Type II Diabetes Induced by a High-fat Diet

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ABSTRACT

Introduction: Diabetes is a metabolic disease, which is directly correlated with increased physical disabilities and muscle tissue damage. The present study aimed to investigate the anti-apoptotic effects of interval and continued training and crocin on diabetic rats.

Methods: In this study, 49 adult rats aged eight weeks with diabetes inducaed by a high-fat diet and venous injection of streptozotocin were randomly assigned to seven groups, including high-intensity interval training (HIIT), low-intensity continued training (LICT), HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. The animals in the HIIT and LICT groups ran on a treadmill three sessions per week for eight weeks at the intensity of 80-85% and 50-55% of the maximum speed, respectively. The animals in the crocin consumption groups received 25 mg/kg of crocin weekly for eight weeks.

Results: HIIT and LICT could significantly increase Bcl-2 and decrease Bax and p53, as well as the ratio of Bax and Bcl-2 (P≤0.05). In addition, crocin consumption could significantly increase Bcl-2 and decrease Bax (P≤0.05). Training with crocin consumption had interactive effects on the increase of Bcl-2 and decrease of p53 and ratio of Bax to Bcl-2 (P≤0.05).

Conclusion: According to the results, continued and interval training along with the consumption of crocin exerted interactive anti-apoptotic effects on the rats with diabetes induced by a high- fat diet.

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Introduction

Type II diabetes is a worldwide phenomenon. According to the World Health Organization (WHO), the number of patients with type II diabetes increased from 108 million in 1980 to 422 million in 2014 (1). Type II diabetes is caused by increased insulin resistance due to obesity and sedentary lifestyle, which in turn leads to the reduction of insulin secretion from the beta cells (2). Strong evidence suggests that oxidative stress and elevated reactive oxygen species (ROS) are important factors caused by hyperglycemia in diabetic patients, which lead to the mutation of the mitochondrial genome and onset of cell death through necrosis or apoptosis (3).

Increased oxidative stress, the tumor suppressor protein (*p53*) activates the apoptosis process, thereby preventing the proliferation and repair of muscle cells and accelerating cell death. In this mechanism, *p53* activates caspase-9 (4, 5), and the activation of caspase-9 from the internal pathway increases the *Bcl-2*-associated X protein (*Bax*) gene as the precipitant of apoptosis and decreases the expression of *Bcl-2* as an anti-apoptotic agent (5,6).

Some researchers believe that physical exercise could decrease apoptosis through the reduction of oxidative stress (7). However, there is uncertainty regarding the effects of exercise on apoptotic markers. For instance, four weeks of endurance training at the speed of 15-18 m/min

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have been reported to significantly increase in the levels of soluble Fas and Fas/Fas ligand (FasL), while causing no changes in the level of *Bcl-2* in the cardiac tissue of diabetic and non-diabetic rats (8). On the other hand, high-intensity interval training has not been reported to cause a significant increase the *Bcl-2* levels, while it could increase the *Bax* levels in rats with myocardial infarction.

Several therapeutic approaches are used for the control and treatment of diabetes, such as the use of medicinal plants and sedentary lifestyle modifications, which are recommended to diabetic patients (9). Saffron (*Crocus sativus*) is a medicinal plant belonging to *Iridaceae* family, which contains a substance known as crocin. Researchers have assessed the beneficial effects of crocin on glycemic indices and lipid profiles (9).

With regard to the effects of inactive lifestyle on the progression of diabetes, as well as the reduction of motor ability and muscle tissue damage in diabetic patients, physical exercise plays a key role in decreasing the complications associated with diabetes, and medicinal plants could also be used for the improvement of diabetes owing to their antioxidant properties.

The present study aimed to investigate the simultaneous effects of crocin and endurance training on apoptotic markers in the muscle tissues of rats and assess the interactive effects of endurance training along with the consumption of crocin on the apoptosis markers in the soleus muscle of rats with type II diabetes induced by a high-fat diet.

Materials and Methods

This experimental study was conducted on 49 male Sprague-Dawley rats aged eight weeks, with the mean weight of 150±30 grams, which were purchased from the Animal Reproduction and Breeding Center of Islamic Azad University, Marvdasht Branch, Iran. The animals were transferred to the physiology laboratory and preserved in standard conditions.

Diabetes Induction

For the induction of type II diabetes, a combination of a high-fat diet and streptozotocin (STZ) was used. To this end, the animals received a fatty diet for eight weeks. The dietary pattern consisted of 45% total fat (derived from animal fats), containing 24 grams of fat, 24 grams of protein, and 41 grams of carbohydrate per 100 grams (10). After eight weeks, diabetes was induced via injecting a single dose of STZ (30 mg/kg*)* (10). For the confirmation of diabetes, the rats with higher glucose levels than 300 mg/dl at 96 hours after the injection were selected as the samples (10).

Animal Grouping

Based on the serum glucose, the rats were divided into seven groups, including high-intensity interval training (HIIT), low-intensity continued training (LICT), HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. Groups one and three received training in three weekly sessions for eight weeks at the intensity of 80-85% of the maximum speed for two minutes, along active rest periods (one minute). From six intervals in the first week of training, HIIT reached 12 intervals in the last week.

Groups two and four also received training in three weekly sessions for eight weeks at the intensity of 50-55% of the maximum speed. LICT was initiated during the first week at 25 minutes, reaching 50 minutes in the last week. It is notable that the total volume of the exercise (intensity, duration, and repetition) was matched between the LICT and HIIT groups (10). In addition, groups three, four, and five received 25 mg/kg of crocin (dissolved in normal saline) intraperitoneally (11).

In order to control the effects of injection on the study variables, the animals in the sham group were intraperitoneally administered with soluble crocin daily (Sigmma, Cat No. 17304). The rats were anaesthetized 24 hours after the last training session. Following that, the soleus muscle was extracted by experts, placed in liquid nitrogen in microtubes, and stored at the temperature of -70°C for further analysis.

Measurement of the Research Variables

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the research variables and investigate gene expression. The sequence of the primers used in the study is presented in Table 1.

Table 1. Sequence of Forward-Reverse Primers of Genes in Real-time Polymerase Chain Reaction

Gene	Forward (5'-3')	Reverse (5'-3')	Product Size (bp)
B2M	CGTGCTTGCCATTCAGAAA	ATATACATCGGTCTCGGTGG	244
Bax	CTGCAGAGGATGATTGCTGA	GATCAGCTCGGGCACTTTAG	147
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC	134
p53	GGCTCCGACTATACCACTATCC	GAGTCTTCCAGCGTGATGATG	104

Statistical Analyses

Data analysis was performed in SPSS version 21. Shapiro-Wilk test was used to determine the normal distribution of the data, and the changes in the weight of the animals at pretest and posttest were evaluated using paired-sample t-test. Moreover, the effects of training and crocin consumption on the study variables were assessed using two-way analysis of variance (ANOVA) and Bonferroni's post-hoc test at the significance level of P≤0.05.

Results

Table 2 shows the weight of the animals in the study groups. The levels of *Bcl-2*, *Bax*, *p53*, and ratio of *Bax* to *Bcl-2* in the rats are depicted in Figures 1-4, respectively.

Weight Changes in Study Groups

At the posttest, the weight of the rats in the control group (P=0.001) and sham group (P=0.001) significantly increased compared to the pretest. On the other hand, the weight of the rats in the LICT group (P=0.001), LICT with crocin consumption group (P=0.001), and HIIT with crocin consumption group (P=0.001) significantly reduced at the posttest compared to the pretest. However, no significant difference was observed in the pretest and posttest weight of the rats between the HIIT group (P=0.10) and crocin consumption group (P=0.09).

Effects of Training and Crocin Consumption on Bcl-2 Gene Expression

According to the obtained results, training (F=10.22; P=0.001; effect size: 0.36) and crocin consumption (F=24.76; P=0.001; effect size: 0.40) could significantly increase *Bcl-2*. Furthermore, the combination of training and crocin consumption had interactive effects on the increase of *Bcl-2* (F=5.04; P=0.01; effect size: 0.21). The obtained results also indicated that HIIT (P=0.03) and LICT (P=0.001) could significantly increase *Bcl-2*, which indicated the similar effects of HIIT and LICT on the increase of *Bcl-2* (P=0.25).

Effects of Training and Crocin Consumption on Bax Gene Expression

According to the findings, training (F=47.23; P=0.001; effect size: 0.72) and crocin consumption (F=32.01; P=0.001; effect size: 0.47) could significantly decrease *Bax*. However, training with crocin consumption had no interactive effects on the reduction of *Bax* (F=2.18; P=0.26; effect size: 0.10). Moreover, the obtained results indicated that HIIT (P=0.001) and LICT (P=0.001) could significantly reduce *Bax*, which confirmed the similar effects of HIIT and LICT on the reduction of *Bax* (P=0.99).

Effects of Training and Crocin Consumption on p53 Gene Expression

According to the findings, training (F=9.50; P=0.001; effect size: 0.34) could significantly reduce *p53*, while crocin consumption (F=2.16; P=0.15; effect size: 0.05) had no significant effect on *p53*. However, training along with crocin consumption had interactive effects on the reduction of *p53* (F=15.28; P=0.001; effect size: 0.45). Furthermore, the obtained results indicated that HIIT (P=0.001) and LICT (P=0.005) could significantly decrease *p53*, which confirmed the similar effects of HIIT and LICT on the reduction of *p53* (P=0.99).

Effects of Training and Crocin Consumption on the Bax/Bcl-2 Ratio

According to the findings, training (F=4.08; P=0.02; size effect: 0.16) could significantly reduce the *Bax/Bcl-2* ratio, while crocin consumption (F=3.63; P=0.003; effect size: 0.07) had no significant effect on the reduction of the *Bax/Bcl-2* ratio. In addition, training along with crocin consumption had interactive effects on the reduction of the *Bax/Bcl-2* ratio (F=6.78; P=0.003; effect size: 0.27). The obtained results also indicated that HIIT (P=0.006) and LICT (P=0.005) could significantly decrease the *Bax/Bcl-2* ratio, which confirmed the similar effects of HIIT and LICT on the reduction of the *Bax/Bcl-2* ratio (P=0.99).

Table 2. Pretest and Posttest Weight of Rats in Study Groups

Group	Pretest (g)	Posttest (g)	Paired-sample T-test	
	Mean±SD	Mean±SD	t	P-value
HIIT	360.64±13.12	342.12±44.11	1.91	0.10
LICT	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
HIIT with Crocin Consumption	410.47±30.87	392.41±46.52	4.24	0.005
LICT 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

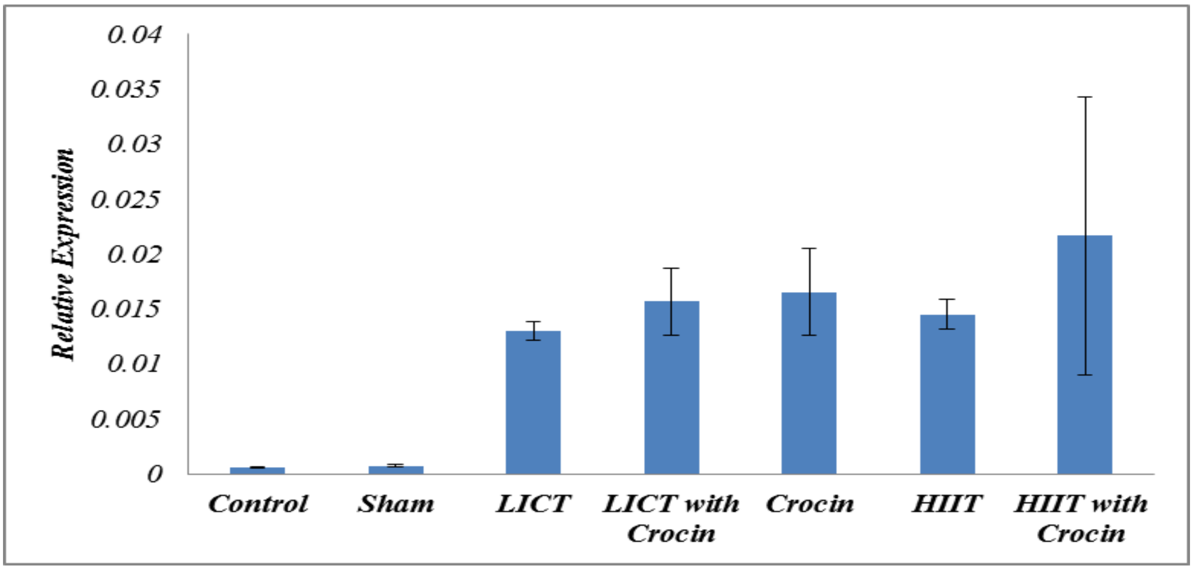

Figure 1. *Bcl-2* Gene Expression in Study Groups

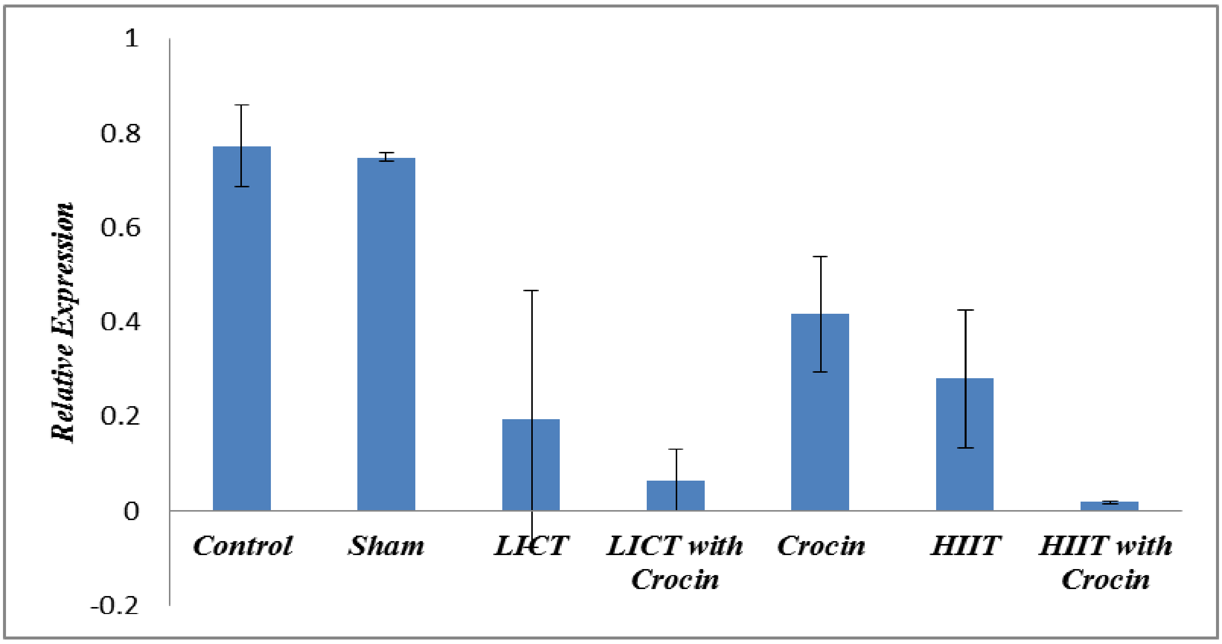

Figure 2. *Bax* Gene Expression in Study Groups

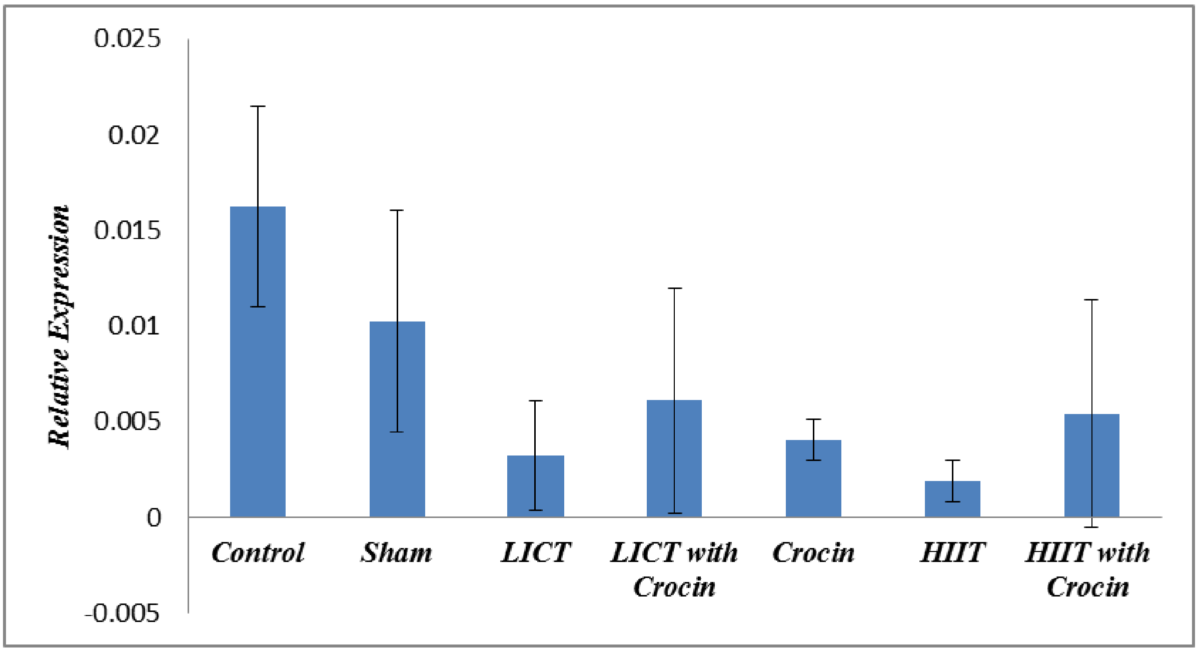

Figure 3. *P53* Gene Expression in Study Groups

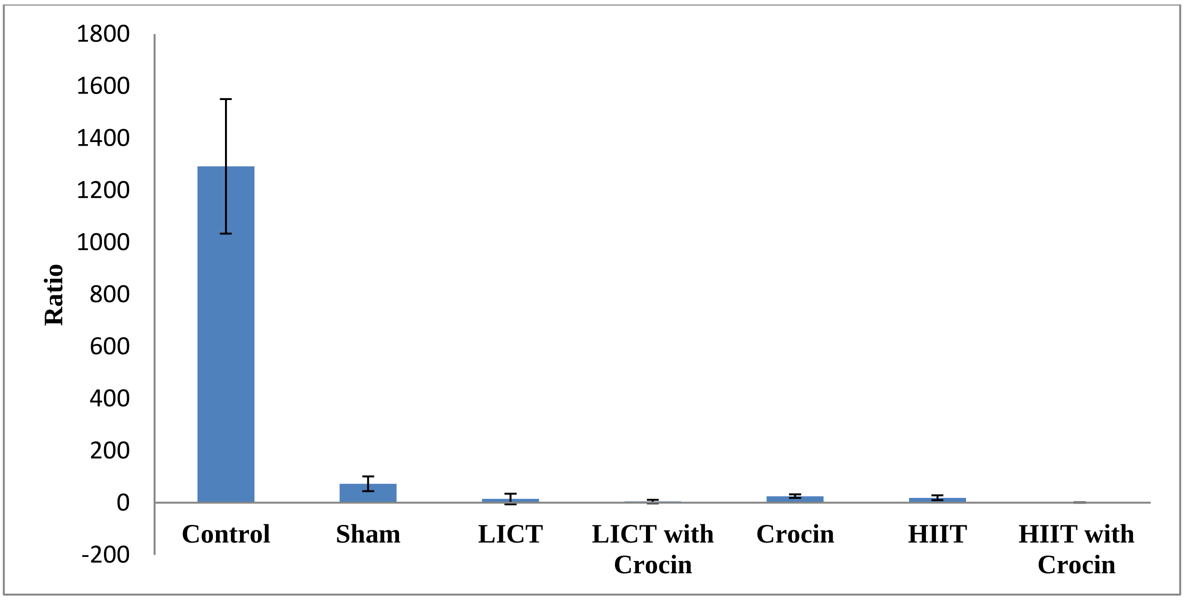

Figure 4. *Bax*/*Bcl-2* Ratio in Study Groups

Discussion

According to the results of the present study, eight weeks of HIIT and LICT could significantly increase *Bcl-2* and reduce *Bax*, *p53*, and *Bax*/*Bcl-2* ratio in the soleus muscle of the diabetic rats. Several studies have investigated the effects of physical exercise on the gene expression of *Bcl-2* and *Bax*. In line with the results of the present study, moderate aerobic training has been reported to increase *Bcl-2* and decrease *Bax* and caspase-3 in the rats with STZ-induced diabetes (12). This consistency could be attributed to the similarities in the research samples and duration and intensity of training. On the other hand, six weeks of low-intensity interval training have been reported to reduce *Bax* gene expression and increase *Bcl-2* gene expression in rats with myocardial infarction. However, HIIT has been reported to increase *Bax* gene expression, with no significant effects on the changes in *Bcl-2* gene expression in the cardiac tissues of rats with myocardial infarction (5). The discrepancies in the findings of the mentioned studies regarding increased apoptosis after HIIT could be due to the differences in the sample populations and type of the studied tissues. Correspondingly, 10 weeks of regular swimming in male rats increased the levels of anti-apoptotic proteins (Bcl-2 and Bcl-x) and decreased the levels of apoptosis-promoting proteins (e.g., BAD), followed by the phosphorylation and reduction of BAD to the *Bcl-2* ratio (13).

With respect to the association between the mechanism of the effects of HIIT and LICT, researchers have claimed that for the improvement of the mitochondrial function due to the low and high adaptation of intracellular apoptotic pathways, pre-apoptotic signal molecules, such as the pre-apoptotic proteins of the Bcl-2 family (e.g., BAK and Bax) are transmitted to the mitochondria, where they induce a series of temporary permeable pores in the external mitochondrial membrane, which inhibit the release of cytochrome C, leading to the reduction of the caspase activity (5).

According to the findings of the current research, eight weeks of crocin consumption could significantly increase *Bcl-2* and decrease *Bax* in the soleus muscle tissues of diabetic rats. However, crocin consumption had no significant effects on the reduction of *p53* and the *Bax*/*Bcl-2* ratio. A mechanism that causes crocin to decrease apoptosis may be the reduction of *p53* expression, as well as the antioxidant properties of crocin and its effects on the reduction of blood glucose and HbA1c. This mechanism depends on the anti-apoptotic effects of reducing hydrogen peroxide, H_2O_2, and caspase-3 (14).

According to the literature, crocin could inhibit oxidative stress and reduce lipid peroxidation, thereby inhibiting ROS, caspases, and *p53* and preventing apoptosis induction (15). The only study regarding the effect of crocin on *Bcl-2* and *Bax* has been conducted by Sadoughi (2017), the findings of which have indicated that the administration of 0.5 milliliter of crocin could reduce *Bax*, increase *Bcl-2* and antioxidant enzymes, and decrease lipid peroxidation (15).

According to the results of the present study, eight weeks of training along with the consumption of crocin had interactive effects on the increase of *Bcl-2*, as well as the reduction of *p53* and the *Bax*/*Bcl-2* ratio, in the soleus muscle tissues of diabetic rats. However, training with crocin consumption had no interactive effects on the reduction of *Bax*. Physical exercise could block apoptotic pathways by increasing the expression and enhancement of protein kinase B activity through the phosphorylation of the anti-apoptotic proteins of the *Bcl-2* family, inhibition of apoptotic-promoting proteins (e.g., Bax) or the direct inhibition of caspase activity (8). It seems that training and simultaneous use of crocin could also increase *Bcl-2* and anti-apoptotic processes by controlling the caspase activity and reducing the ROS (16).

Some of the limitations of the present study were failure to measure the muscle weight, muscle mass, and muscle strength in rats. Therefore, it is recommended that further investigations in this regard be conducted based on similar protocols to the present study. In such experiments, it is also suggested that factors such as muscle weight, muscle mass, and muscle strength be measured in order to confirm the positive effects of training and crocin consumption. Furthermore, it is recommended that further investigations in this regard consider the effects of resistance training and swimming along with crocin consumption on the apoptotic markers in diabetic rats.

Conclusion

According to the results, HIIT and LICT along with the consumption of crocin could exert interactive anti-apoptotic effects on the rats with diabetes induced by a high-fat diet.

Conflicts of interest:

None declared.

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