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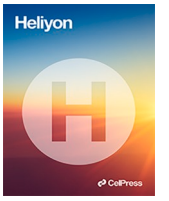

Diabetic cardiomyopathy in rats was attenuated by endurance exercise through the inhibition of inflammation and apoptosis

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ABSTRACT

Diabetic cardiomyopathy (DCM), as a ventricular dysfunction, is one of the main causes of death in diabetic patients. Former evidence revealed the beneficial effects of exercise on cardiovascular complications of diabetes. We aimed to investigate the effects of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on DCM. Male Wistar rats were divided into control, diabetic, metformin (300 mg/kg), HIIT, MICT, metformin + HIIT, and metformin + MICT diabetic groups. Serum biochemical, inflammatory, and oxidative stress indicators, gene expression of *BCL2* and *BAX*, and histopathologic changes of cardiac tissue were assessed. Our analysis revealed an increase in fasting blood sugar (FBS), creatine kinase MB (CK-MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) in diabetes. Also, the superoxide dismutase (SOD) and catalase (CAT) activity, and the total thiol were decreased, in contrast, malondialdehyde (MDA) levels increased in the cardiac tissue of the diabetic group. All of these changes were significantly ameliorated in diabetic animals treated with exercise and metformin + exercise. The level of tumor necrosis factor-α (TNF-α) and Interleukin-1β (IL-1β), as well as the infiltration of inflammatory cells, were decreased in the heart of all exercise training groups. Up-regulation of *BCL2* and down-regulation of *BAX* gene expressions were observed in the cardiac tissue of all exercise-treated groups. In conclusion, HIIT and MICT exercises are effective in preventing DCM development. Exercise training, besides improving oxidative stress and inflammation in cardiac tissue, alleviates cardiac damage by modulating the apoptotic gene expression in diabetic rats.

1. Introduction

Diabetes mellitus (DM), a serious worldwide health problem, has been assumed as a risk factor for heart disease that increases morbidity and mortality rate [1]. By 2030, the global prevalence of DM is expected to reach 439 million people [2]. According to recent research, about 15 % of people with DM are at risk of developing diabetic cardiomyopathy (DCM) [3]. DCM is known as ventricular dysfunction caused by DM, in the absence of atherosclerosis or hypertension [4]. Although the mechanisms involved in DCM are unknown, oxidative stress, inflammation, fibrosis, and apoptosis are known to be involved [2,4]. Furthermore, left

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ventricular hypertrophy and systolic and diastolic dysfunction are evident in DCM [5]. Several factors are implicated in the DCM pathogenesis such as elevation of advanced glycation end products (AGEs), renin angiotensin aldosterone system (RAAS) activity, and fatty acids consumption in cardiomyocytes. Impaired calcium homeostasis and mitochondrial failure are among other factors responsible for the development of DCM [6]. It was indicated that an increase in RAAS activity through collaboration with oxidative stress and interposing with the transforming growth factor (TGF)-β signaling pathway might lead to cardiac fibrosis and hypertrophy [5,7]. Moreover, oxidative stress caused by diabetes is known as an important factor in apoptosis induction. In this regard, the elevation of pro-apoptotic *BAX* gene expression, and reduction of anti-apoptotic *BCL2* gene expression indicate the progression of apoptosis in diabetic cardiomyocytes and the development of DCM [6,8]. On the other hand, the increased cardiomyocytes surface, followed by increased expression of brain natriuretic peptide, atrial natriuretic peptide, and β-myosin heavy chain genes, cause cardiac hypertrophy during the DCM progression [5]. As a result, the prevention and treatment of DCM are important in diabetic patients [5]. Pharmacological therapy alone are not only ineffective in the prevention and treatment of DCM, but also might be costly for patients; therefore, new treatment strategies are required [3]. Exercise, as an available non-pharmacological remedy, is recommended for diabetic people [9]. Exercise, along with controlling fasting blood sugar (FBS), improves heart function and has protective effects against DCM by intervention in different pathological pathways, such as apoptosis, fibrosis, and hypertrophy [10]. It was demonsterated that the treadmill exercise training can attenuate myocardial fibrosis in diabetic rats, through the inhibition of the TGF-β1/Smad signaling pathway [11]. In addition, exercise ameliorated cardiomyocyte contractility, mitochondrial biogenesis, and ATP production [12]. Exercise training has been shown to improve vascular complication and angiogenesis, by reducing reactive oxygen species (ROS) production and pro-inflammatory cytokines [13]. The effect of exercise on cardiovascular complications might be different based on its modality, intensity, and duration. In comparison with severe intensity or overtraining, a moderate type of exercise is more comfortable, does not require readiness, and is more suitable for diabetes management [14,15]. The previous evidence revealed that high-intensity interval training (HIIT) exercise was more effective than moderate-intensity continuous training (MICT) at attenuating cardiovascular complications in metabolic syndrome patients. Moreover, the HIIT reduced fatty acid oxidation and enhanced glucose oxidation in myocardial tissue, as well as improved mitochondrial respiratory capacity and left ventricular mechano-energetic properties in cardiac tissue, but these effects were not seen in the MICT protocol. However, less is known about the effects of either of these exercises on cardiac complications induced by diabetes [16,17]. On the other hand, the results of previous studies have shown that the exercise with different intensities decreased FBS and glycosylated hemoglobin (HbA1c) in diabetic patients, so that, there was no significant difference between high and moderate intensity [13,18]. However in other studies, the effects of HIIT on improvement of insulin sensitivity and reduction of blood sugar was more pronounced than MICT [19,20]. Therefore, in the present study, the effects of HIIT and MICT exercises on oxidative stress, inflammation, and apoptosis in the cardiac tissue of diabetic rats were investigated.

2. Materials and methods

2.1. Animals and diabetes induction

The male Wistar rats (10 weeks old with weight of 250 ± 20 g) were housed in the animal housing department in the faculty of medicine (Mashhad University of Medical Sciences) with free access to tap water and standard laboratory diet (50 ± 5 % humidity and 22 ± 2 °C temperature with 12 h light/dark cycle). Every 3–4 rats was maintained in a standard plastic cage. The Ethics Committee of Mashhad University of Medical Sciences approved this experimental protocol (IR.MUMS.MEDICAL.REC.1399.224).

Following 6 h of fasting, diabetes in rats was induced by the injection of a single dose of streptozotocin (STZ, 60 mg/kg, i.p.), and after 72 h, the animals with FBS equal or more than 250 mg/dl were assumed as diabetic samples according to our previous study [21].

2.2. Experimental protocol

Forty-nine rats were randomly divided into seven groups, with seven animals in each group (Table 1); 1. Control group; which received STZ vehicle (sterile saline, 1 ml/kg i.p.), and 6 diabetic groups in the following order; 2. Diabetes, 3. Diabetes + metformin (Metformin), 4. Diabetes + HIIT exercise training (HIIT), 5. Diabetes + MICT exercise training (MICT), 6. Diabetes + metformin + HIIT

Table 1
The animal groups of study and their treatment protocols.

Groups (n = 7)	Treatment protocols
Control	Sterile saline
Diabetic	Sterile saline
Metformin	Metformin (300 mg/kg BW)
HIIT	HIIT exercise training
MICT	MICT exercise training
Met-HIIT	Metformin (300 mg/kg BW) with HIIT exercise training
Met-MICT	Metformin (300 mg/kg BW) with MICT exercise training

Body weight (BW), Metformin: diabetes + metformin, HIIT: diabetes + HIIT exercise training, MICT: diabetes + MICT exercise training, Met-HIIT: diabetes + metformin + HIIT exercise training, Met-MICT: diabetes + metformin + MICT exercise training.

exercise training (Met-HIIT), 7. Diabetes + metformin + MICT exercise training (Met-MICT). The exercise training protocols and/or treatment with metformin were done after diabetes approval as described in previous study, and lasted for 6 weeks [21]. The metformin (300 mg/kg) as a cardiovascular protective agent was applied once a day by a gastric gavage needle. At the end of the protocol, the animals underwent deep anesthesia (xylazine (8 mg/kg) and ketamine (60 mg/kg), i.p. injection) and were sacrificed humanely. The chest wall was opened and after blood sample was gained, the heart was isolated and washed with saline, then the left ventricular tissue was segregated and kept in RNA later for gene expression assay. The residual tissue of the heart was divided into two parts, one part was kept at −70 °C for oxidative stress and cytokine evaluation, and the other part in formalin for histological examination [3,5].

2.3. Exercise protocol

The rats in the exercise groups were selected based on their ability to run and were randomly assigned to exercise groups. They were accustomed to the treadmill running at speeds of 12–15 m/min for 15 min per day for the first 5 days. After the adaptation step, the training time

was set as 40 min per day, 5 days per week for 6 weeks. The MICT group ran at a speed of 15 m/min and the HIIT group at 20 m/min at a zero-inclination angle. At the end of the first, third, and fifth weeks, performance tests were taken from rats in exercise groups (Fig. 1) [13,19–22].

2.4. Serum biochemical analysis

After centrifuging the obtained blood samples, the separated serum was used for biochemical assessments. Besides the FBS measurement, the creatine kinase muscle-brain (CK-MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), as the cardiac injury markers were investigated by using the commercial kits followed by the manufacturer's guidelines (Pars Azmoon, Tehran, Iran).

2.5. Inflammation assessment

The interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) ELISA kits for rats were used to determine the inflammation index in the cardiac tissues according to the manufacturer's instruction (Karmania, Pars Gene, Iran). The microplate reader device was used to measure the standards and sample absorbance (Biotek, USA). Then, the concentrations of TNF-α and IL-1β were defined by using a standard curve.

2.6. Cardiac oxidative stress assessment

As described in our previous study, the cardiac tissue was homogenized with an ultrasound homogenizer in a chilled phosphate buffer and centrifuged the sample at 4 °C with 5000 rpm for 10 min. The supernatants were used for survey, thiol, CAT, SOD, and MDA as oxidative stress indicators [21].

Based on the formerly mentioned protocol, for evaluating the MDA level in cardiac tissue, the homogenated tissue was mixed with a

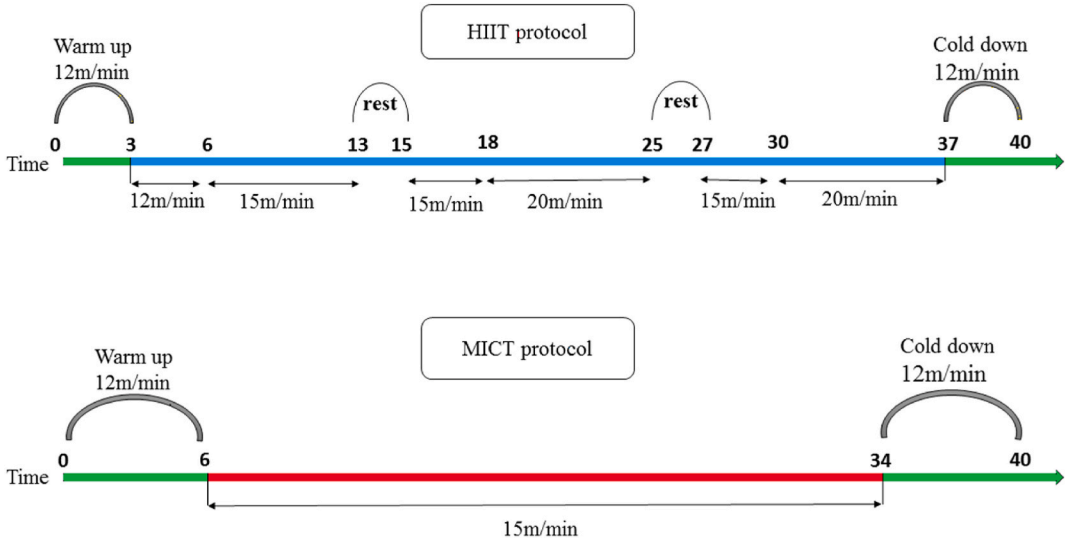

Fig. 1. Summary of experimental design. After familiarizing the animals with the laboratory environment and treadmill in the first weeks (12–15 m/min for 15 min per day for the first 5 days), the rats ran on a treadmill for 40 min per day, 5 days per week for 6 weeks.

standard mixture of trichloroacetic acid (TCA), hydrochloric acid (HCL), and thiobarbituric acid (TBA). The obtained mixture was boiled and centrifuged. Then the supernatant was separated and the absorbance was measured at 535 nm. Using an extinction value of $1.56 \times 10^5\ M^{-1}/cm^{-1}$, the MDA concentration was reported as nmol per gram weight of tissue [3,21].

To evaluate the thiol, the homogenated cardiac supernatant was mixed with tris-ethylenediaminetetraacetic acid (EDTA) buffer, and the solution absorbance was read as sample A1 at 412 nm. Afterward, the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reagent (in methanol) was mixed with sample A1, and the sample absorbance was read again at 412 nm as sample A2. Then the DTNB solution alone absorbance was also read as a blank (B). The total thiol concentration was calculated from the following equation.

$$\text{Total thiol concentration}: (\text{mM}) = (\text{A2} - \text{A1} - \text{B}) \times 1.07 / 0.05 \times 13.6$$

[3,5,21].

To assess, the antioxidant enzyme activity in cardiac tissue Aebi method was used for CAT activity and the colorimetric method of Madesh and Balasubramanian was applied for the determination of the activity of SOD [21,23,24].

2.7. Quantitative real-time polymerase chain reaction

To perform RNA extraction, the cardiac tissue was homogenized with Trizol (Yekta Tajhiz Azma Co, Iran) according to the manufacturer's guide. A nanodrop device was used to survey the quality and purity of the harvested RNA (Thermo 2000, USA). The cDNA was produced with the easy cDNA kit (Parstous, Iran) and using BioRad C1000 Touch Thermal Cycler (Bio-Rad Laboratories, USA) according to the kit guide.

The quantitative real-time polymerase chain reaction (Q-PCR) was performed with the Light Cycler System (Roche Diagnostics, Mannheim, Germany) and Real Q Plus 2x Master Mix Green (Ampliqon, Denmark) to investigate β-*Actin*, *BCL2*, and *BAX* gene expression (Table 2). The housekeeping gene of β-*Actin* was used as an internal control gene. The steps of the qPCR process were set up and performed according to its instructions. ΔΔCT method (fold changes) was used to evaluate the relative gene expression. The gene sequences were obtained from NCBI Gene. Then the primers were designed using Primer 3 software. Finally, the primers were checked and approved by NCBI Blast for their specificity, and all steps of the experiment were repeated to increase the data validity [3,5].

2.8. Histopathologic studies

After harvesting the animal hearts, the cardiac tissues were washed in normal saline and fixed in formalin for 48 h, and then embedded in paraffin. To assess inflammation, the slices were subjected to hematoxylin-eosin staining. The Nikon D5000 camera and ECLIPSE E200 light-microscope were applied to obtain the tissue photos. Also, these images were taken by a double-blinded researcher from different regions of slides. The Image J software was used for histomorphometric evaluation. The rise in the number of inflammatory cells was considered representative of inflammation development [3,5].

2.9. Statistical analysis

The results were presented as mean ± standard error of the mean (SEM) and analyzed with SPSS 20.0 software. The data were compared using One-way ANOVA followed by Tukey *post hoc* comparisons. Statistical significance was set at *P*-values less than 0.05.

3. Results

3.1. Serum biochemical parameters

The biochemical data showed that the FBS was increased in the diabetic group compared to the control rats ($P < 0.001$). FBS in all treated groups was significantly reduced compared with the non-treated diabetic rats ($P < 0.001$). Metformin as well as the HIIT, Met-HIIT, and Met-MICT groups, decreased blood sugar more significantly versus the MICT group ($P < 0.001$). Only the Met-HIIT group was able to bring the FBS level close to that of the control group (Fig. 2A). The cardiac injury markers, including CK-MB, AST, and LDH, were increased in the diabetic group compared to the control group ($P < 0.001$). In all the treatment groups, the cardiac markers decreased compared to the diabetic group ($P < 0.001$). The CK-MB was more reduced in the metformin group in comparison with the

Table 2
The list of mRNAs sequence of target genes.

Gene	Primer sequence 5′–3′	Total length amplified
β-Actin	F- CCCGCGAGTACAACCTTCT R- CCATCACACCCTGGTGCCTA	195
BCL2	F- AGGATAACGGAGGCTGGGATG R- CTCACT TGTGGCCCAGGTATG	153
BAX	F- CTACAGGGTTTCATCCAGGATC R- CCACATCAGCAATCATCCTCTG	172

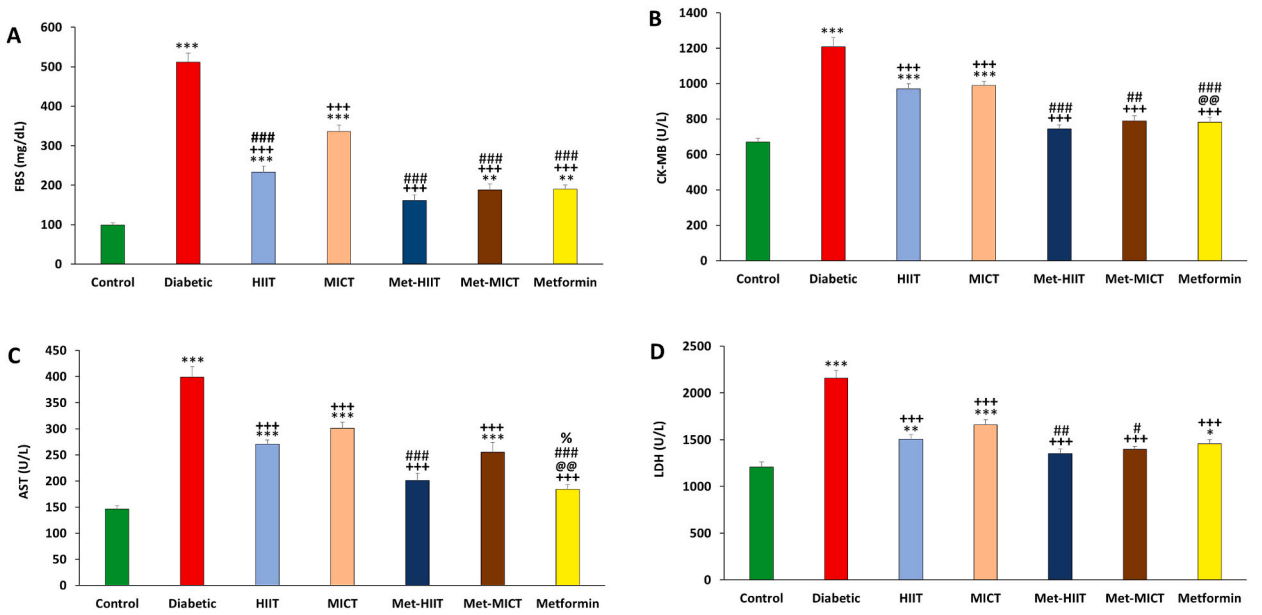

Fig. 2. Comparison of the biochemical parameters in different groups. A. Fasting blood sugar (FBS), B. creatine kinase-muscle/Brain (CK-MB). C. aspartate aminotransferase (AST), and D. lactate dehydrogenase (LDH). Data has been shown as mean ± SEM for each group (n = 7). *P < 0.05, **P < 0.01 and ***P < 0.001 vs. the control group, +++ P < 0.001 vs. the diabetic group, @@ P < 0.01 vs. the HIIT group, #P < 0.05, ##P < 0.01 and ###P < 0.001 vs. the MICT group, % P < 0.05 vs. the Met-MICT group.

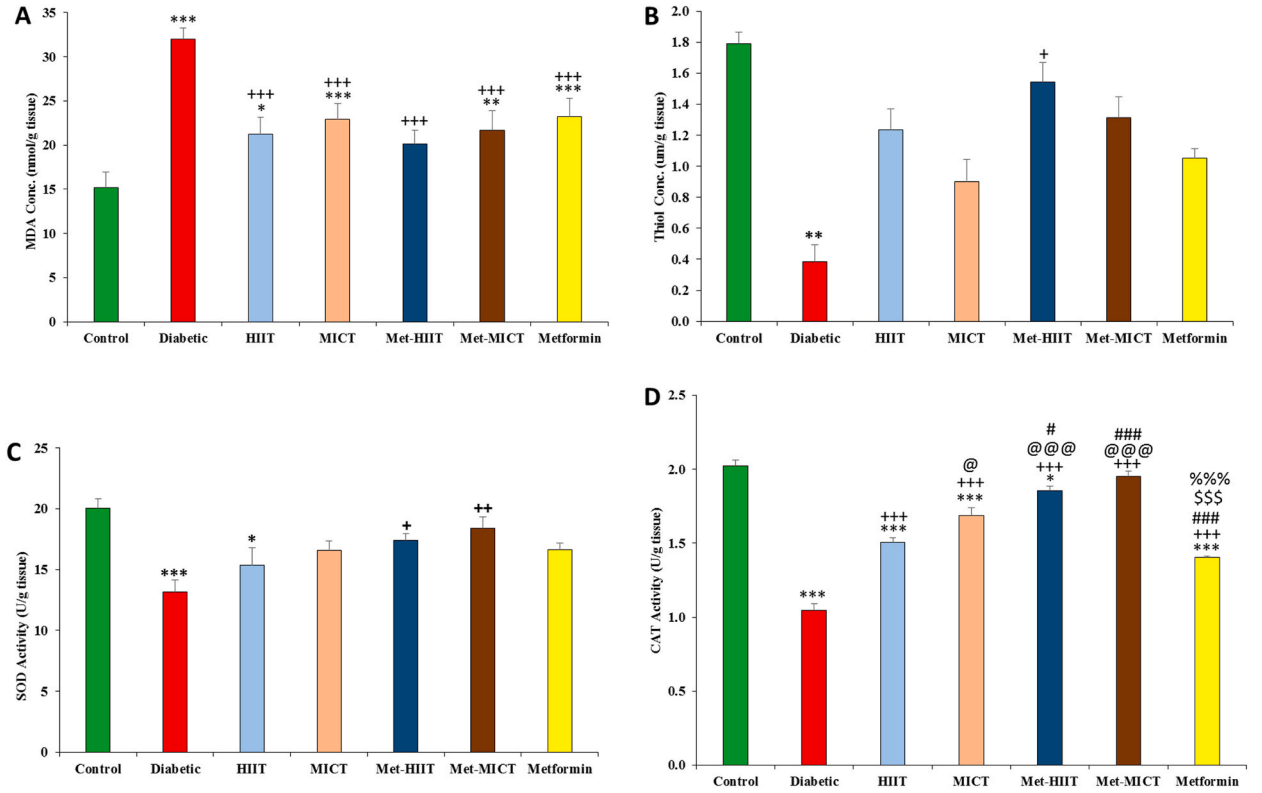

Fig. 3. Comparison of the oxidative stress parameters in different groups. A. MDA, B. Thiol. C. SOD and D. CAT. Data have been shown as mean ± SEM for each group (n = 7). *P < 0.05, **P < 0.01 and ***P < 0.001 vs. the control group, + P < 0.05, ++ P < 0.01, +++ P < 0.001 vs. the diabetic group, @ P < 0.05 and @@@ P < 0.001 vs. the HIIT group, #P < 0.05 and ###P < 0.001 vs. the MICT group, $$$ P < 0.001 vs. the Met-HIIT group, %%% P < 0.001 vs. the Met-MICT group.

HIIT and MICT groups ($P < 0.01$ and $P < 0.001$ respectively). Furthermore, the reduction of CK-MB in the Met-HIIT and Met-MICT groups was more than that of the MICT group ($P < 0.001$ and $P < 0.01$ respectively). AST level decreased in the metformin group in comparison with the HIIT and MICT groups ($P < 0.01$ and $P < 0.001$ respectively). In the Met-HIIT group also more reduction of AST was observed compared to the MICT group ($P < 0.001$). Additionally, the LDH level in the Met-HIIT and Met-MICT groups decreased compared to the MICT group ($P < 0.01$ and $P < 0.05$ respectively). As a whole, the Met-HIIT group had the greatest improvement in cardiac enzymes which was closer to that of the control group (Fig. 2B–D).

3.2. Cardiac oxidative stress

The data showed that the MDA concentration in cardiac tissue increased in the diabetic group in comparison with the control group ($P < 0.001$). The cardiac MDA level in all treatment groups was reduced in comparison with the diabetic group ($P < 0.001$) (Fig. 3A). The thiol concentration in the heart tissue of diabetic rats decreased compared to the control group ($P < 0.01$). In comparison with the diabetic group, only the thiol level in the Met-HIIT group increased significantly ($P < 0.05$) (Fig. 3B). The SOD activity in the heart tissue of the diabetes group was decreased compared to the control group ($P < 0.001$). In the Met-HIIT and Met-MICT groups, the elevation of SOD activity in cardiac tissue was observed compared to the diabetic group ($P < 0.05$ and $P < 0.01$ respectively) (Fig. 3C). CAT activity in cardiac tissue was reduced in the diabetic group compared to the control group ($P < 0.001$). However, this reduction was reversed in all treatment groups in comparison with the diabetic group ($P < 0.001$). Compared to the HIIT group, the CAT activity improved more in MICT ($P < 0.05$) as well as in the Met-HIIT and Met-MICT groups ($P < 0.001$). Moreover, in comparison with the MICT group, the intervention groups of Met-HIIT and Met-MICT had more improvement in the cardiac tissue CAT activity ($P < 0.05$ and $P < 0.001$ respectively). The cardiac CAT activity in the metformin group was lesser than that of the MICT, Met-HIIT, and Met-MICT groups ($P < 0.001$) (Fig. 3D). In the Met-HIIT and Met-MICT groups, the improvement of oxidative indices was closer to the control group.

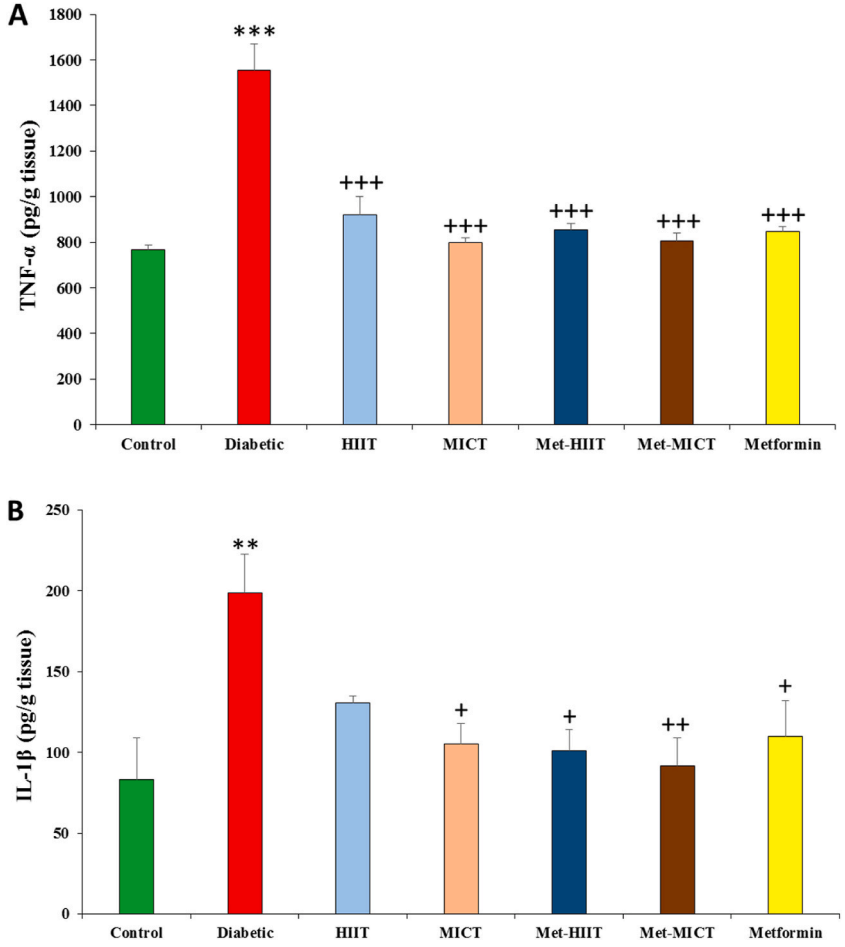

Fig. 4. Comparison of the cardiac inflammation cytokines in different groups. A. TNF-α, B.IL1β. Data has been shown as mean ± SEM for each group (n = 7). **P < 0.01 and ***P < 0.001 vs. the control group, + P < 0.05, ++ P < 0.01 and +++ P < 0.001 vs. the diabetic group.

3.3. Cardiac inflammation

TNF-α and IL-1β levels in the cardiac tissue of the diabetic group were found to be higher than that of the control group ($P < 0.001$ and $P < 0.01$ respectively). Cardiac TNF-α level in all treatment groups was lower than that of the diabetic group ($P < 0.001$). In comparison with the diabetic group, the cardiac IL-1β level was reduced in the MICT, Met-HIIT, Met-MICT, and metformin groups ($P < 0.05$ and $P < 0.01$). Although the cardiac cytokine levels in all the treatment groups were not statistically different from the control group, the Met-MICT group had the most reduction (Fig. 4A–B(.

3.4. Cardiac gene expression

BCL2 gene expression in the cardiac tissue of the diabetic group decreased significantly compared to the control group ($P < 0.001$). In all the treated groups up-regulation of *BCL2* was observed compared to the diabetic group ($P < 0.001$). In the Met-MICT and metformin groups, the expression of *the BCL2* gene was higher compared to the HIIT group ($P < 0.05$). Moreover, the *BCL2* gene was

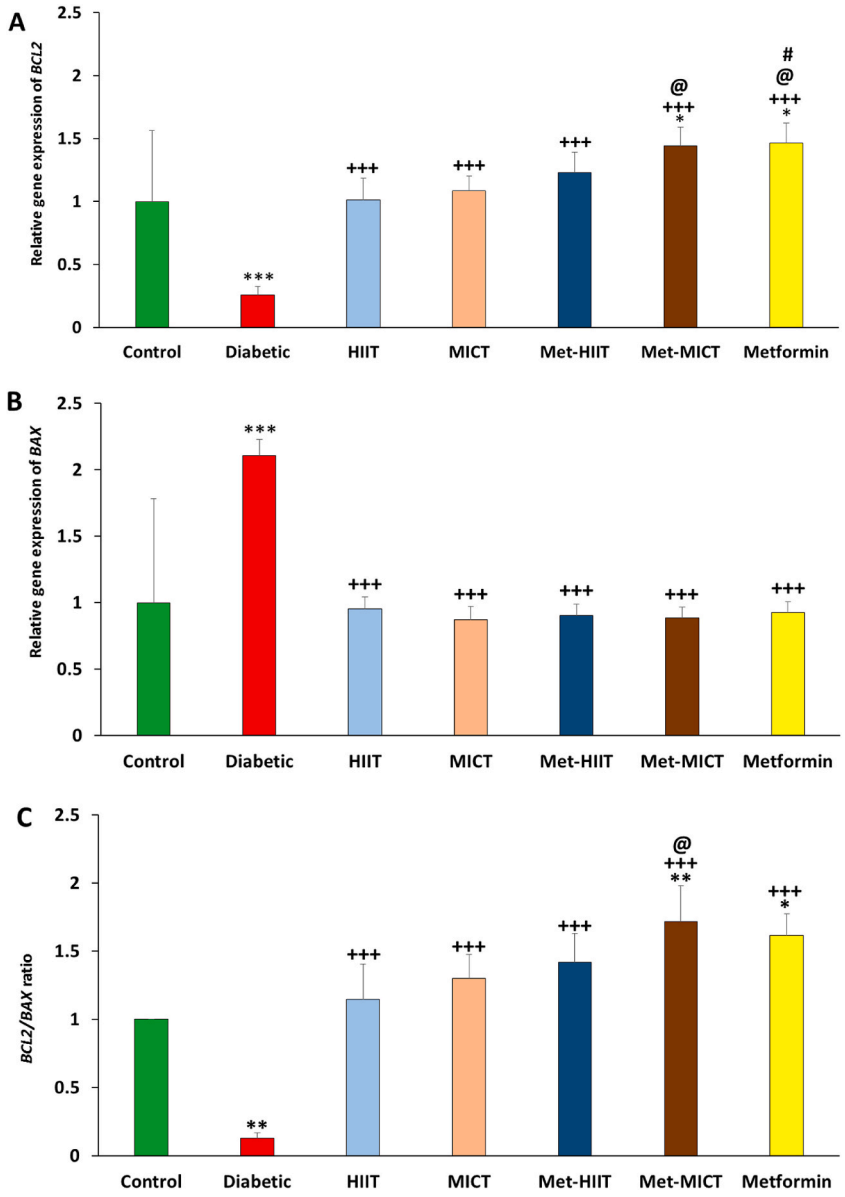

Fig. 5. Comparison of cardiac apoptotic gene expression in different groups. A. *BCL2*, B. *BAX*, and C. *BCL2/BAX* ratio. Data has been shown as mean ± SEM for each group (n = 7). **P < 0.01 and ***P < 0.001 vs. the control group, +++ P < 0.001 vs. the diabetic group, @ P < 0.05 vs. the HIIT group, and #P < 0.05 vs. the MICT group.

more expressed in the metformin group versus the MICT group ($P < 0.05$) (Fig. 5A). Cardiac gene expression of *BAX* in the diabetic group increased in comparison with the control group ($P < 0.001$) while the expression of *BAX* mRNA level in the heart tissue of all treated groups was downregulated in comparison with the diabetic group ($P < 0.001$) (Fig. 5B). The *BCL2/BAX* gene expression ratio in the diabetic group was significantly lower than in the control group ($P < 0.01$). This ratio increased in all treated groups compared to the diabetic group ($P < 0.001$). Additionally, the *BCL2/BAX* ratio in Met-MICT was increased versus the HIIT group ($P < 0.05$) (Fig. 5C).

3.5. Cardiac histopathological study

In the heart tissue of control rats, histopathological assessment (hematoxylin and eosin staining) revealed a normal structure in the ventricular muscle. The inflammatory cell number in left ventricular tissue increased in the diabetic group in comparison with the control animals ($P < 0.001$). The inflammatory infiltration of cardiac tissue has been reduced in all intervention groups compared to

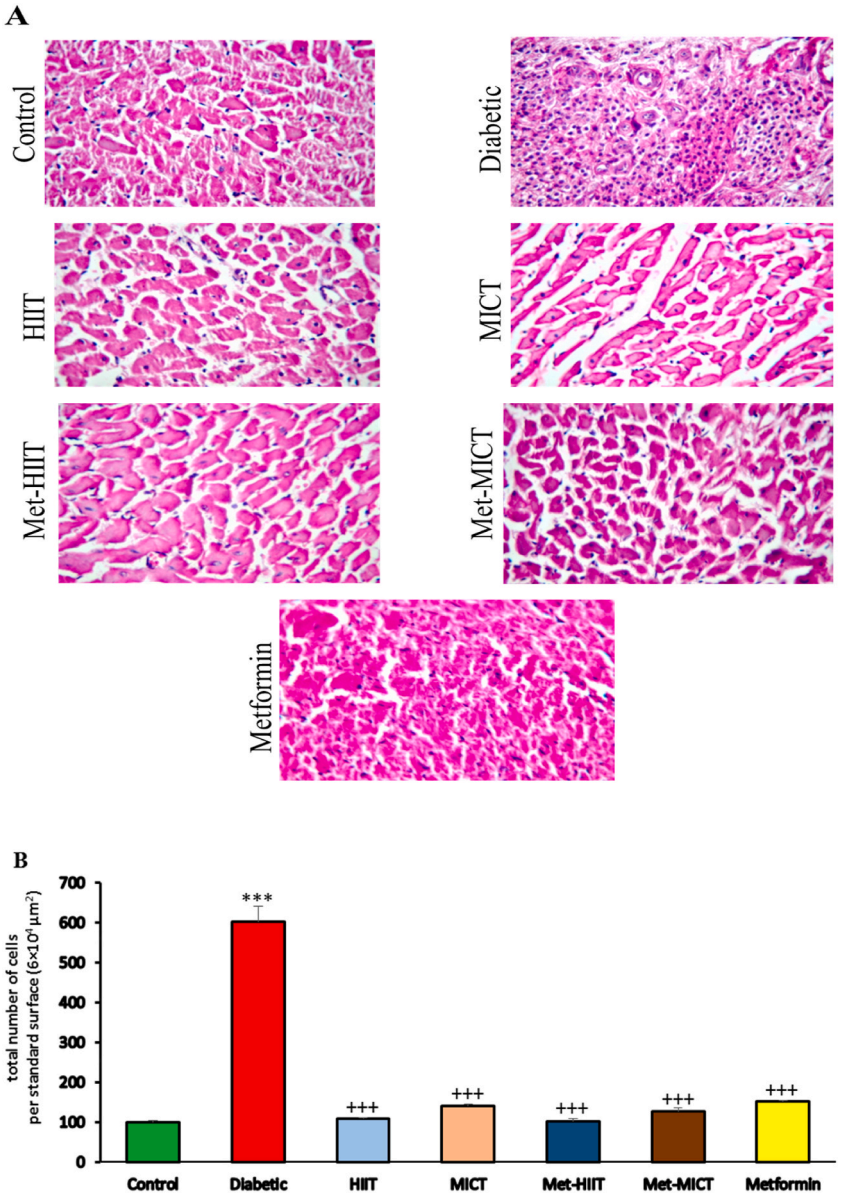

Fig. 6. Histopathologic comparison of the left ventricular tissue subjected to hematoxylin–eosin (H&E) staining between different groups. (A) Representative H&E stained myocardial sections that were used to measure inflammation. (B) The cellular myocarditis index was quantified as the number of inflammatory cells. Data has been shown as mean ± SEM for each group (n = 7). ***$P < 0.001$ vs. the control group and +++ $P < 0.001$ vs. the diabetic group. Original magnification is × 400; Bar 50 µm.

the diabetic group ($P < 0.001$). The spindle-shaped structure of the heart muscle was reserved, and a significant variation in the ventricular structure of the heart muscle has been shown in the treatment groups compared to the diabetic group. All treated groups did not show significant histopathological differences in comparison to the control group (Fig. 6A–B).

4. Discussion

DCM as ventricular dysfunction is one of the main causes of death in diabetic patients. The molecular mechanisms of DCM-induced pathways consist increase in oxidative stress, inflammation, and apoptosis, which ultimately lead to cardiac remodeling and heart failure [9,10]. Previous evidence revealed that exercise could be an effective factor in the inhibition of cardiovascular disease progression, and other causes of mortality in diabetes [25]. Exercise training is not only effective for glycemic control in diabetic patients but also protects DCM by suppressing inflammation, apoptosis, fibrosis, and hypertrophy [10]. In our study, the exercise alone or combined with metformin, besides reversing hyperglycemia, prevented oxidative stress, inflammation, and apoptotic gene expression in the cardiac tissue of diabetic rats. In all the treatment groups, exercise and metformin, FBS level was lower than diabetic ones. Likewise, the Met-HIIT group had the most reduction in blood sugar levels and reached the level of the control group. Previous experimental and clinical studies have provided conflicting results about the effect of different types of exercise on lowering blood sugar in diabetes. The results of studies revealed that although both exercise protocols, HIIT and MICT, significantly decreased FBS levels, the improvement was significantly higher in the HIIT protocol [20,26,27]. In contrast, other studies showed that the MICT exercise protocol was better in the control of blood glucose in diabetic rats [13,18]. The beneficial effects of exercise in blood glucose control also were seen in low-intensity exercise training [28,29]. However, the results of a clinical study showed that both types of exercise, HIIT and MICT, had a similar effect on FBS control [30]. However, in another clinical trial HIIT was more effective in blood sugar control than MICT exercise in type 2 diabetic patients [31]. The evidence showed that exercise might improve glycemic control by increasing the blood flow to the muscle fibers and enhancing their mitochondrial function, as well as improving insulin sensitivity, which leads to raising glucose uptake in muscles and adipocytes [22,31]. Elevated cardiac enzymes have been reported as an indicator of damage to the heart membrane, suggesting that it is caused by cardiomyocyte death or dysfunction similar to that found in DCM [3]. The six weeks of exercise training and metformin administration in our study prevented the rise of CK-MB, AST, and LDH in diabetic conditions. The CK-MB and AST reduction was more noticeable in the metformin alone or with exercise groups, although the LDH level reduction was more in the combined groups. Met-HIIT had the greatest effect in reducing cardiac biomarkers and brought the level of these enzymes to those of the control group. The results of another study revealed that the administration of HIIT exercise reduced the serum AST level, although, it was not significant in the MICT exercise [20]. Likewise, in another study, both types of exercise reduced cardiac damage by decreasing cardiac biomarkers including troponin-I, although this improvement was better in HIIT exercise [32]. Furthermore, metformin was able to reduce cardiac enzymes in diabetic rats [3,5]. Thus, exercise training due to the preservation of cell structural integrity may be able to protect against myocardial damage [20,33]. The literature shows that hyperglycemia-induced oxidative stress, ROS generation, and subsequent cardiomyocyte damage are major risk factors for the development of DCM [3]. In our study, exercise alone or in combination with metformin reduced MDA level and augmented thiol content, and SOD and CAT activities in the heart of diabetic animals. The Met-HIIT group showed better improvement in MDA level and thiol content. Both exercise combination groups raised the enzyme activity levels of SOD and CAT close to the level of the control group. In similar studies, low and moderate exercise training restored MDA, SOD, and CAT alterations [11,28,29]. In contrast, in another study, endurance exercise training in obese Zucker rats could not improve oxidative stress parameters in cardiac tissue [34]. Exercise through decreasing ROS production may improve the cardiac damage caused by oxidative stress in diabetes, which can ameliorate glucose metabolism and prevent cardiomyopathy caused by oxidative disturbance [33]. Cardiac inflammatory progress due to oxidative stress in diabetes is one of the prominent factors in heart failure promotion during DCM. Inflammation can boost ROS production and then intensify the other inflammatory cytokines development. In a diabetic situation, an increase in ROS generation, cholesterol destruction, and endothelial cell membrane damage might lead to an increase in the attracting and infiltrating of inflammatory cells. NF-κB activation provides the release of pro-inflammatory cytokines like TNF-α and IL-1β by infiltrated inflammatory cells. Here we observed the reduction of cardiac inflammatory cytokines (TNF-α and IL-1β) in the exercise training groups. In like manner, the results of former studies revealed that the different types of exercise training, particularly HIIT, decreased inflammation by reducing TNFα, IL6, and IL-1β in diabetic rats and db/db mice [27,35]. According to the previous statement, increased oxidative stress and enhanced inflammatory response during chronic hyperglycemia are important mechanisms of DCM development [3]. Thus exercise training might lead to cardiomyopathy prevention through ameliorating oxidative damage and inflammation process, which helps to recover the cardiac structure and function [9]. The apoptotic process in cardiomyocytes is a typical feature of DCM. In diabetic conditions, activation of cytochrome C in mitochondria and release into the cytoplasm, associated with caspase-3 activation leads to endogenous cardiomyocyte apoptosis [33]. Likewise, chronic hyperglycemia-induced oxidative disturbance could result in mitochondrial fission and apoptosis [3]. In the same way, inflammatory cytokines develop cardiac injury following the apoptotic cascade. The increased TNF-α and IL-1β in diabetes, activate both intrinsic and extrinsic pathways in the apoptotic process. For instance, they could enhance the apoptotic indicator, *BAX*, and in contrast, they suppressed the anti-apoptotic gene, *BCL2,* on the intrinsic pathway of apoptosis [3]. In our experiment, both types of exercise and metformin administration improved the modulation of *BAX* and *BCL2* gene expression in diabetic hearts. Moreover, the *BCL2/BAX* ratio in the cardiac tissue of all treated groups was enhanced. The metformin alone or in combination with exercises had the most protective effect on this point. In a similar study exercise training in diabetic *db/db* mice ameliorated cardiomyocyte apoptosis by reversing the increased ratio of *BAX/BCL2* [36]. The findings of other studies demonstrated that both types of exercise training protocols decreased apoptotic protein expression including caspase 3 and cleaved caspase 3, although the effect of HIIT exercise was more prominent [28,32]. In addition, it is believed that exercise could prevent DCM through inhibiting apoptosis induced by oxidative

stress in diabetic conditions. This prohibition also might be through reducing TNF-α and IL-1β and improving the inflammation process [9,33]. In our study, the histopathological assessment indicated muscle fiber disruption and inflammatory cell infiltration in the cardiac tissue of diabetic animals. The structural damage and inflammatory infiltration of cardiac tissue have been reduced in all treated groups, although the combined groups demonstrated better improvement in tissue structure. In previous studies, both types of exercises ameliorated heart tissue pathological change including myocardial cell disarrangement, fibers disruption, unequal cytoplasm distribution, and irregular nuclei, although this improvement was better revealed in the HIIT group [32]. These beneficial effects were also seen in low-intensity exercise and swimming protocol in diabetic rats [28,37]. These histological findings indicated that exercise can prevent myocardial cell membrane destruction and infiltrating of inflammatory cells which are caused by the activation of the NF-κB pathway in the course of diabetes [38].

Taken together, here we tried to compare the beneficial effect of two types of exercise alone or in combination with an anti-diabetic medication in the prevention of hyperglycemia and one of its main complications, DCM.

However, we also faced limitations in the present study. We investigated the expression of two main genes implicated in the apoptosis process, although due to lack of tissue amount and other facilities we could not evaluate other complementary and confirmative assessment methods like western blotting or tissue immunohistochemical evaluation.

On the other hand, however, the STZ-induced diabetes model is not purely a type 2 diabetes and does not mimic all of the features of this type of disease, still is the more common model in experimental investigations.

Furthermore, some echocardiographic parameters could not be investigated in small animal studies. Therefore, we used an approach that is highly specific for small animal research. Thus our findings were derived from lab animal experiments, some of the aspects of which are different from those of humans.

5. Conclusion

The data suggested that exercise training might reduce cardiac risk factors of diabetes-induced DCM. The exercise training, besides reducing FBS levels, improved oxidative stress and inflammation in cardiac tissue, which might alleviate cardiac damage by modulating the apoptotic gene expression in diabetic rats. These findings imply the protective effect of physical activity against DCM development.

Data availability statement

Data will be made available on request, and the corresponding author can be contacted if needed.

CRediT authorship contribution statement

Sadegh Shabab: Investigation, Writing - original draft. **Maryam Mahmoudabady:** Conceptualization, Supervision. **Zahra Gholamnezhad:** Writing - review & editing. **Mahtab Fouladi:** Data curation. **Ali Akbar Asghari:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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