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Effects of high-intensity interval training on the expression of microRNA-499 and pro- and anti-apoptotic genes in doxorubicin-cardiotoxicity in rats

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ABSTRACT

Background: Because clinical use of doxorubicin (DOX) in chemotherapy is limited due to cardiotoxicity, finding new strategies to alleviate DOX burden and improving patients' health are necessary. Due to positive cardiovascular impacts of high-intensity interval training (HIIT), here we have investigated the effect of HIIT on DOX-induced cardiotoxicity by evaluating the myocardial apoptosis mechanism as well as microRNA-499a-5p expression.

Methods: Male Wistar rats (250–270 g) were randomly allocated into four groups: control, HIIT, DOX, and HIIT +DOX. HIIT was performed as 7 sets of alternative intervals of high and low trainings for 1 h a day, 5 days a week for 6 weeks using a rodent treadmill. After the last session of HIIT, the trained and time-matched control rats received intraperitoneal injection of DOX (20 mg/kg). Three days later, the left ventricular samples were obtained to determine the expression of microRNA and genes and proteins regulating apoptosis via real-time PCR. Myocardial apoptosis was also evaluated using TUNEL staining method.

Results: DOX administration significantly increased the expression levels of Bax and caspase-6 mRNAs, Bax protein and Bax/Bcl2 ratio, while reduced the expression levels of Bcl2 mRNA and protein in comparison to control group ($P < 0.01$). Pre-treatment of DOX-received rats with HIIT significantly up-regulated the Bcl2 and reduced the Bax, Bax/Bcl2, and caspase-6 expression profiles toward control values ($P < 0.05$), not affecting GSK-3β expression. In addition, DOX toxicity significantly overexpressed microRNA-499, comparing to control rats ($P < 0.01$). HIIT significantly reversed this overexpression and also reduced TUNEL-positive apoptotic cells in DOX-received rats ($P < 0.05$).

Conclusions: The data suggested that prior training of rats with HIIT had protective effect on DOX cardiotoxicity through reversing the expression profiles of pro- and anti-apoptotic factors and microRNA-499 and reducing myocardial apoptosis.

Introduction

Cardiovascular toxicity with doxorubicin (DOX) is the most important limiting factors for the universal usage of this drug in treatment of various cancers [1]. Mechanisms of action of DOX in killing cancer cells have been thoroughly identified; however, the mechanism of its complications in healthy tissues, including heart, has not been completely ascertained. Determining these interfering mechanisms can propose new therapeutic targets and open a promising perspective to reduce the side effects and increase the efficacy of this drug in eliminating the cancer outcomes. The concomitant management of cancer therapy and DOX-induced cardiomyopathy may substantially prevent or minimize the risks of DOX treatment [2,3].

Apoptosis or other forms of cell death contribute to DOX-induced cardiac toxicity, a phenomenon leading to the loss of myocytes function or irreversible cardiac injury [4]. Both intrinsic and extrinsic pathways of apoptosis are involved in DOX-cardiotoxicity. Overproduction of free radicals and oxidative stress, mitochondrial injury, intracellular

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calcium dysregulation can mediate DOX-induced apoptosis in cardiomyocytes [5]. It has been suggested that myocardial apoptosis is a common mechanism of acute and chronic cardiomyocyte loss, in which DNA damage and fragmentation, mitochondrial dysfunction, cytoskeleton derangement and other cellular imbalances can contribute to deteriorating contractile force and cardiac function [5,6]. Therefore, anti-apoptotic approaches can be considered as the concomitant therapy in DOX challenges.

Furthermore, microRNAs (miR) are the conserved non-coding RNAs that modulate the mRNA translation rate and thereby hinder the protein expression of their target genes (7.8). It has been revealed that development of certain cardiovascular disturbances are related to the dysregulation of miRs [7], suggesting the possibility that specific miRs would modulate the damage pathways and increase the cardiomyocyte tolerance to DOX toxicity.MiR-499-5p is preferentially expressed in the myocardium and has the protective effects against cardiomyocyte ischemic injuries via it anti-apoptotic impacts [8,9]. Recent studies also suggest the measuring of circulating miR-499 as a potential biomarker for acute myocardial infarction [10].

Different types of exercises have long been subjected for treatment or prevention of many diseases, especially cardiovascular disease. The duration and intensity of exercise are two important factors that play a critical role in tolerance to exercise among patients with cardiac injury [11]. The high-intensity interval training (HIIT) is described as repeated bouts of high intensity exercise with intervals of rest or low intensity exercise periods as recovery [12]. It has recently been reported that HIIT has a considerable protective effects on different tissues in comparison to normal endurance exercise [11]. In other words, this kind of short-term, high-intensity exercise not only have no negative effects, but in most cases, including in pathologic conditions, it induces a more protective phenotype. For example, a previous study showed that HIIT improves aerobic capacity more effectively than does continuous endurance exercise in cardiac patients [13]. Additionally, it has been reported that HIIT exercise can improve myocardial hemodynamic parameters and significantly contribute to the protection of myocardium against ischemia-reperfusion injury [14]. Thus, these findings point to this hypothesis that this exercise can be prescribed to patients who do not tolerate a longer period of exercise.

The identification of mechanisms of action of this interval exercise could help to find the promising therapeutic targets for the prevention of cardiac disorders, especially in pathologies such as DOX-induced toxicity. Increasing the tissue tolerance to pathological stresses and decreasing the cell apoptosis and death are the main ways of aerobic exercise efficiency [15], but the contribution of these phenomena has not been demonstrated in the effectiveness of HIIT exercise in DOX-cardiotoxicity. Considering the potentials of HIIT exercise in developing a protective phenotype in the heart tissue, the aim of this study was to investigate the preventive effect of this type of exercise on DOX-induced cardiac toxicity and the levels of tissue apoptosis and expression of miR-499a and genes regulating apoptosis in the heart of rats.

Materials and methods

Animals and chemicals

Twenty four male Wistar rats in the weight range of 250–270 g were prepared and housed in Animal house of the Tabriz University of Medical Sciences. The animals experienced a constant temperature of 23 ± 2 °C with a controlled cycling of 12 h of light and 12 h of darkness. They were fed with normal food and water ad libitum. To reduce the adverse effects of any stress on rats, they were kept in their cages for one week and then the interventions were started [16]. DOX, ketamine, xylazine, kits and other reagents were obtained in their highest quality. This study passed the ethical approval of the local animal research committee and all procedures were performed according to the standard guidelines.

Study design and animal grouping

Animals were randomly divided in the following groups, in which 6 rats were allocated in each group:

1) Non-treated healthy group (control);
2) HIIT exercise group (HIIT);
3) DOX-receiving group (DOX); and
4) HIIT exercise with DOX-receiving group (HIIT+DOX).

HIIT protocol lasted for 6 weeks with 10 days of habituation period before exercise and the rats in other groups spent the same time in their cages without any interventions. Doxorubicin hydrochloride (20 mg/kg) was intraperitoneally injected to the DOX-receiving groups at the end of the HIIT exercise period. This dosage has been frequently used in the previous studies to induce cardiac injury [17]. A similar amount of normal saline was injected to the animals of DOX-untreated groups. After 72 h of DOX challenge, the blood and tissue of all rats were sampled under general anesthesia.

Protocol of HIIT

The HIIT exercise was performed as described previously [18,19]. Before starting exercise protocol and in order to get familiarized to the environment, the rats moved on a motor-driven rodent treadmill for 10 days at a speed of 10 m/min and with a gradual increase in duration of training from 10 to 50 min. After two days of rest, the rats entered to the main HIIT protocol in which the animals first spent 10 min of warming period with 40–50% VO2max on treadmill, then they experienced 49 min of alternative running with high and low intensity in 7 cycles. Each cycle lasted for 7 min and included 4 min of high intensity running (with an approximate intensity of 85 to 90% VO2max) and 3 min of slow running or active rest (approximately 65 to 75% VO2max). At the end of last cycle, the rats spent 6 min of cooling period with an approximate intensity of 50 to 60% VO2max. This program was implemented for 5 consecutive days a week, 60 min each day and totally for 6 weeks.

Tissue sampling

Seventy-two hours after DOX challenge, the animals were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). Then, 3 ml of blood was attained from portal vein and the heart was immediately removed from the body and the left ventricle was divided in two parts; one part was dissolved in 10% formalin for TUNEL study and the second part were placed in RNase Later solution (Qiagen, Germany) in order to preserve RNA from digestion. This sample was used for assaying pro-apoptotic and anti-apoptotic genes expression.

RNA extraction, cDNA synthesis, and quantitative real-time PCR

The left ventricular samples immersed in RNase Later solution were firstly underwent the steps of RNA extraction process. Approximately 100 mg of samples was homogenized and then the Trizol method was used for RNA extraction, according to the manufacturer's instructions (Roche, Germany). RNA yield and purity were determined using a NanoDrop spectrophotometer at 260/280 nm (NanoDrop ND-2000C, Thermo Fisher Scientific, USA). First-strand complementary DNA (cDNA) was synthesized from the RNA of samples using the Exiqon cDNA Synthesis Kit, according to the manufacturer's instructions. Briefly, 1 μl of isolated RNA (30 μg) were firstly mixed with 1 μl of random hexamer primer and 6 μl RNASe-free H2O and then incubated at 65 °C for 5 min. Subsequently, micro-tubes were chilled on ice, and a mixture of reaction buffer 4 μl, RNase inhibitor 1 μl, dNTP mix 2 μl and reverse transcriptase 1 μl was added to each sample. Samples were

immediately incubated at 25 °C for 5 min followed by 42 °C for 60 min; the reaction was finally terminated by heating at 70 °C for 5 min. Reverse transcription was performed with the final volume of 20 μl pre tube.

Light Cycler 96 Roche device was used to evaluate the expression of miR-499a-5p and pro-apoptotic genes Bax, caspase 6 and glycogen synthase kinase3beta (GSK-3β), and anti-apoptotic gene Bcl2. Primers were produced by the custom oligonucleotide synthesis service Metabion (Martinsried, Germany). Primers were designed using Primer-3 software and in order to check the specificity, all of them were blasted by Basic Local Alignment Search Tool on the NCBI website (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers have been listed in Table 1. The purity of each amplified product was confirmed using melting curve and analyzed to ensure the identity of the specific PCR product. Relative quantification of miR-499a and target mRNAs was calculated using $2^{-\Delta\Delta CT}$ formula (Livak method). Relative amounts of the microRNA and mRNAs were normalized to U6 and GAPDH transcript levels, respectively.

Western blotting

Left ventricular samples (approximately 50 μg) were subjected to electrophoresis to separate proteins on 12.5٪ SDS-PAGE gels and transfer them to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA). The membrane was then blocked by a 5% non-fat skim milk in phosphate buffer saline containing 0.1% Tween-20 for 60 min, followed by incubation with monoclonal antibodies against anti-Bax (1:2000), anti-Bcl-2 (1:2000), and anti-β-actin (1:2500), (from Cell Signaling Technology, USA), at 4 °C overnight. After washing with Tween-20-containing Tris-buffer saline for 10 min, the membrane was incubated with secondary antibody (1:2500, horseradish peroxidase conjugated, Cell Signaling) for an hour at room temperature, with gentle shaking. Then, the membrane was rinsed again, and the protein bands were developed using the enhanced chemiluminescence method (ECL, GE Healthcare) and the optical densities were visualized by a visualizing machine and ImageJ 1.6 software (National Institutes of Health, Maryland, USA). The intensity of β-actin bands in each sample was used for normalization of the protein intensities.

Determination of myocardial apoptosis

Myocardial apoptosis was assessed by TUNEL staining, as previously described. TUNEL staining was performed with fluorescein UTP according to manufacturer instructions (In Situ Cell Death Detection Kit; Roche Diagnostics) for apoptotic cell nuclei and 49,6-diamidino-2-henylindole (DAPI) (Sigma) stained all cell nuclei. All assays were performed in a blinded manner.

Statistical analysis

Quantitative data was demonstrated as mean ± standard error (SE) of mean. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, La Jolla California USA). One-way ANOVA were performed to compare the quantitative data between different groups. The difference between groups was followed by Tukey post-hoc test. Statistical deference was considered significant when $P < 0.05$.

Table 1
Primers.

Genes	Forward primer	Reverse primer
Bax	AAGTGCCCGAGCTGATCAGAA	TGGGGGTCCCGAAGTAGGAAA
Bcl2	ACCCCTGGCATCTTCTCCTTC	TGCAGCTGACTGGACATCTCT
Caspase 6	TGGAGGCCGACTTCCTGTATG	GATGAACCATGACCCGTCCCT
GSK-3β	GGGTCAACGGGAGAGGTGAAG	GCAAAACGCGTTTTATAGCGG
GAPDH	CCCATCACCATCTTCCAGGAG	GAAGGGGCGGAGATGATGAC

Results

Bax and Bcl2 genes expression levels

Administration of HIIT for 6 weeks to healthy rats significantly reduced the mRNA level of Bax ($P < 0.05$) without any significant effect on Bcl2 gene expression level, as compared with those of control group (Fig. 1A and B, respectively). In addition, DOX toxicity induced a

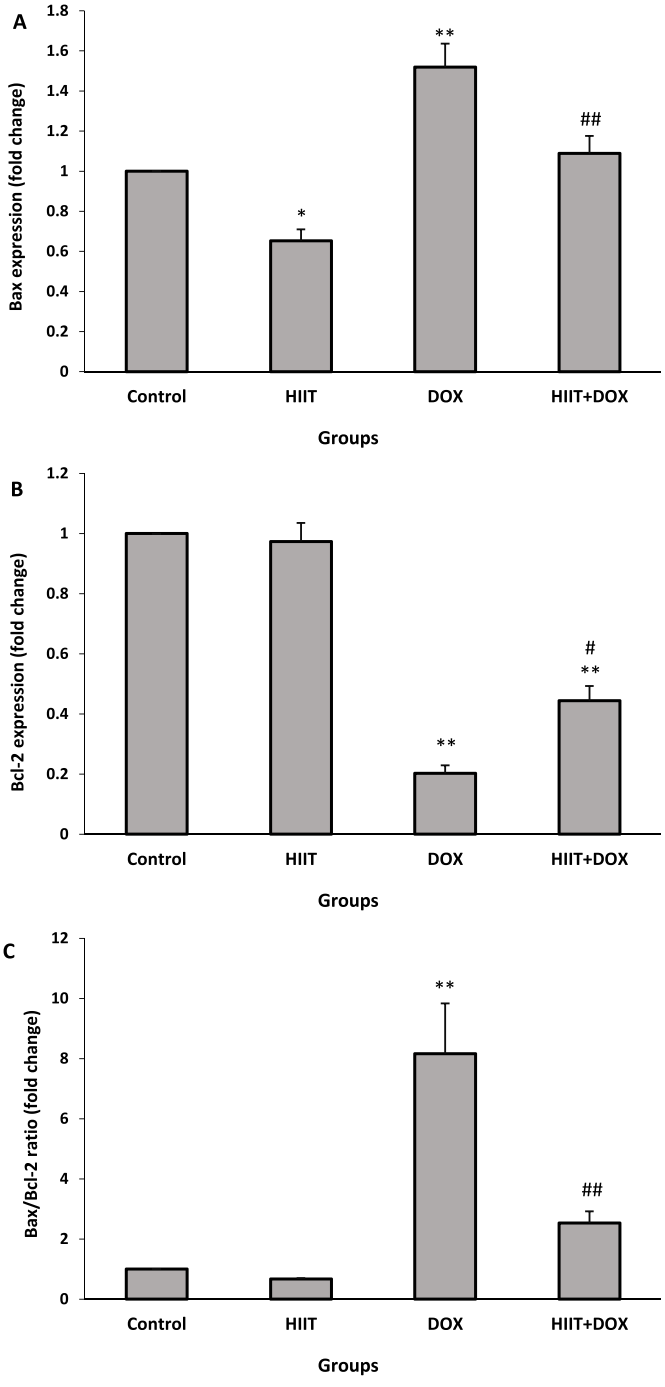

Fig. 1. The expression profiles of Bax and Bcl2 genes and Bax/Bcl2 ratio in experimental groups. Mean + SE. $n = 6$ in each group. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared with control group and $^{\#}P < 0.05$ as compared with DOX group. HIIT: high intensity interval training, DOX: doxorubicin.

significant increase in Bax expression level ($P < 0.05$) and Bax/Bcl2 ratio ($P < 0.01$) (Fig. 1C) and a significant decrease in Bcl2 expression level ($P < 0.01$) in comparison to control group. However, pre-treatment of rats with HIIT significantly reversed the DOX-induced alterations in genes expression, so that the mRNA level of Bax and Bax/Bcl2 ratio was significantly lower and mRNA level of Bcl2 was higher than those of DOX group ($P < 0.05$, all) (Fig. 1 A,B,C).

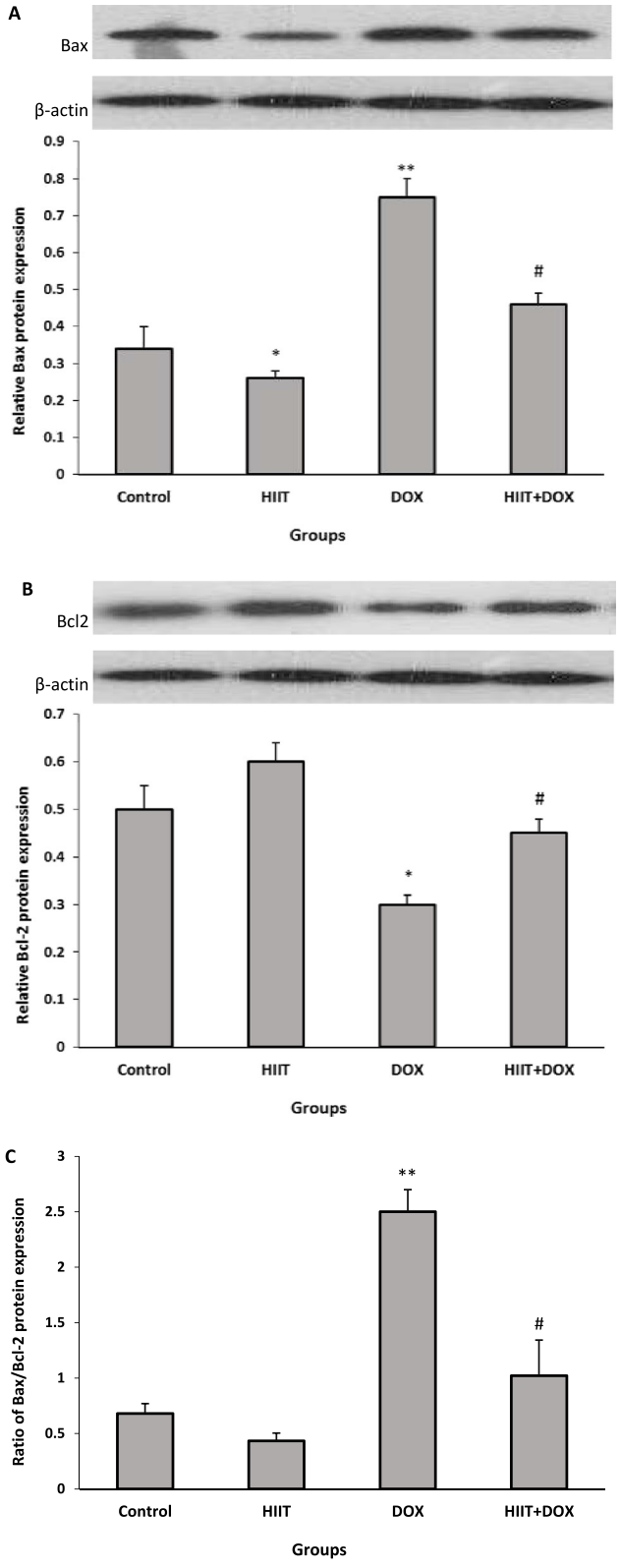

Fig. 2. The protein expression profiles of Bax and Bcl2 and Bax/Bcl2 ratio in experimental groups. Mean + SE. $n = 6$ in each group. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared with control group and $^{\#}P < 0.05$ as compared with DOX group. HIIT: high intensity interval training, DOX: doxorubicin. β-actin gene expression was used as internal control.

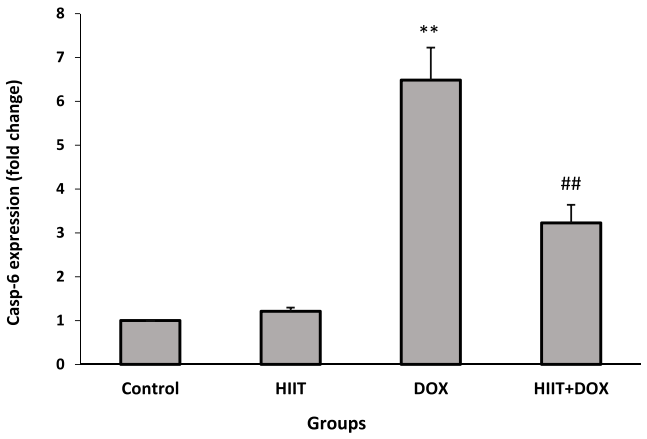

Fig. 3. The expression profile of caspase-6 gene in experimental groups. Mean + SE. $n = 6$ in each group. $^{**}P < 0.01$ as compared with control group and $^{\#}P < 0.05$ as compared with DOX group. HIIT: high intensity interval training, DOX: doxorubicin.

Bax and Bcl2 protein expression levels

HIIT significantly reduced the level of Bax protein as compared with the control group ($P < 0.05$) (Fig. 2A). Bcl2 protein level ($P < 0.05$) was significantly reduced and Bax protein level ($P < 0.01$) as well as Bax/Bcl2 ratio ($P < 0.01$) were significantly increased in DOX-treated rats compared with the controls (Fig. 2A, B and C). Prior administration of HIIT to DOX-receiving rats significantly reversed the expression levels of Bax and Bcl2 proteins and their ratio toward control values (all $P < 0.05$) (Fig. 2).

Caspase-6 gene expression level

Our experiments demonstrated that HIIT could not significantly affect the mRNA of caspase-6 gene in comparison to the control group (Fig. 3). Nevertheless, the mRNA of caspase-6 gene in DOX group demonstrated a significant up-regulation, comparing to the control group ($P < 0.01$). Moreover, administration of HIIT to DOX-treated rats significantly reduced the expression level of caspase-6 gene in comparison to the DOX group ($P < 0.05$) (Fig. 3).

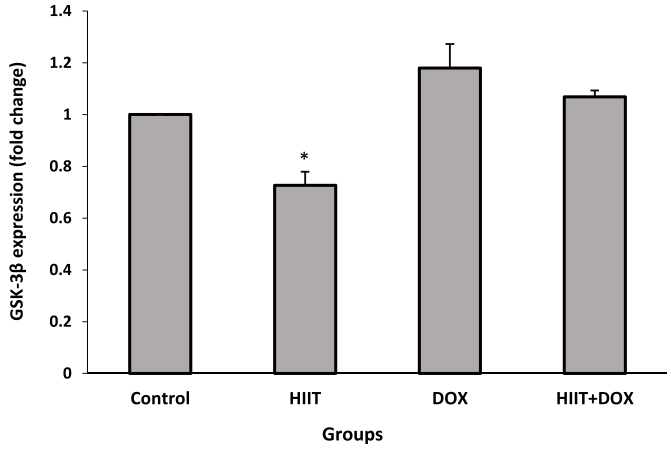

Fig. 4. The expression profile of GSK-3β gene in experimental groups. Mean + SE. $n = 6$ in each group. $^{*}P < 0.05$ as compared with control group. HIIT: high intensity interval training, DOX: doxorubicin.

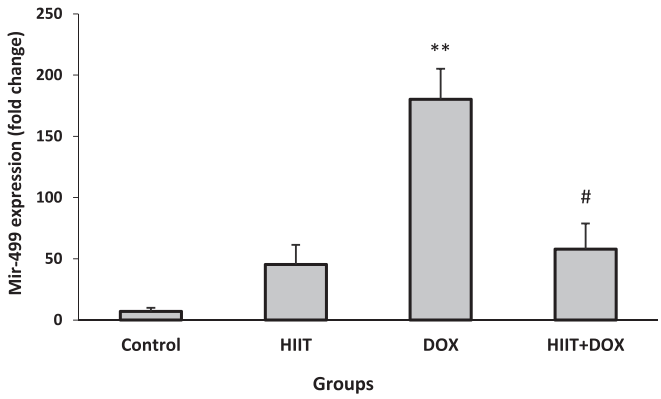

Fig. 5. The expression profile of miR-499a-5p in experimental groups. Mean + SE. $n = 6$ in each group. $^{**}P < 0.01$ as compared with control group and $^{\#}P < 0.05$ as compared with DOX group. HIIT: high intensity interval training, DOX: doxorubicin.

GSK-3β gene expression level

The expression profile of GSK-3β gene has been shown in Fig. 4. The expression of this gene was significantly down-regulated in HIIT group in comparison to control group ($P < 0.05$). However, the mRNA levels of GSK-3β was not statistically significant between DOX and control groups or between HIIT+DOX and DOX alone groups (Fig. 4).

Expression level of miR-499

Furthermore, the expression of miR-499a-5p was increased in HIIT group as compared with the control rats ($P < 0.05$) (Fig. 5). However, administration of DOX to rats also increased considerably the levels of this miR, comparing to control group ($P < 0.01$). Interestingly, pre-training of DOX-treated rats with HIIT significantly prevented the over-expression of miR-499a-5p induced by DOX toxicity ($P < 0.05$; Fig. 5).

Myocardial apoptosis

The histopathological micrographs of TUNEL staining for myocardial apoptosis are shown in Fig. 6. HIIT group showed a similar feature of TUNEL staining in comparison to control group; the TUNEL positive cells and stained nuclei were very low in both groups. However, there was a numerous apoptotic cells and nuclei in DOX group. In addition, administration of HIIT considerably reduced the apoptotic cells in DOX-treated rats (Fig. 6). These results indicated the protective and anti-apoptotic effects of HIIT in DOX-induced cardiotoxicity.

Discussion

In the present study, we evaluated the effects of six weeks training of rats with HIIT exercise regimen on the expression levels of genes and proteins regulating cell apoptosis as well as miR-499 level in the myocardium of healthy and DOX-treated rats. HIIT training in healthy rats could significantly decrease the expression of pro-apoptotic genes, Bax and GSK-3β. Additionally, HIIT exercise significantly reduced the DOX-induced cardiotoxicity by preventing the down-regulation of Bcl2 mRNA and protein and up-regulation of Bax gene and protein as well as Bax/Bcl2 ratio, and caspase-6 gene. In addition, DOX toxicity led to the overexpression of miR-499a-5p, while preconditioning with HIIT in DOX-treated rats significantly inhibited this overexpression. Moreover, HIIT considerably reduced the DOX-induced histological changes and TUNEL-positive cells indicative of myocardial apoptosis. These findings together suggest that HIIT exercise has beneficial impacts against cardiotoxicity of DOX treatment.

Apoptosis is involved in both anticancer effects and side effects of DOX, via distinctly different mechanisms [16]. In the pathophysiology of DOX-cardiomyopathy, several factors such as overproduction of reactive oxygen species (ROS), intracellular calcium dysregulation, endoplasmic reticulum stress, and mitochondrial dysfunction play critical roles, all of which can lead to the initiation of apoptotic processes in myocytes [5,6]. DOX-induced apoptosis in cardiomyocytes leads to the loss of normal mitochondrial function and destruction of the cellular

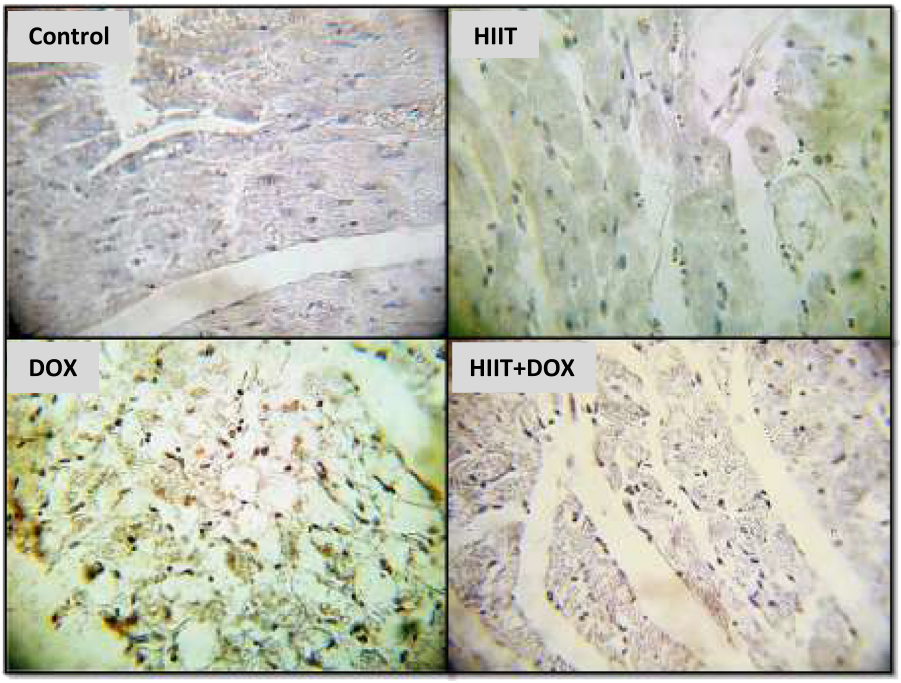

Fig. 6. Micrographs of myocytes indicating the apoptosis in experimental groups. Myocardial apoptosis was detected by TUNEL staining. HIIT: high intensity interval training, DOX: doxorubicin.

DNA which are necessary for physiological genes expression and myocytes survival [17,18]. Also, following the onset of apoptosis, other types of cell death, including necrosis and autophagy are activated [19], and eventually the phenotype of DOX-induced cardiomyopathy is manifested. For this reason, finding better interventions for the impediment of DOX-cardiotoxicity and cell apoptosis is an important clinical issue. Therefore, it seems reasonable to use such a therapeutic approach that increases the effectiveness of DOX in treating cancerous tissue and, at the same time, prevents drug toxicity in non-cancerous healthy tissues.

Previous studies have shown that the administration of exercise can independently cause the regression of the growth of tumor cells and treat cancers; thus, it is frequently recommended as a part of cancer treatment, for all cancer patients [20]. Aerobic endurance exercise, as one of the most effective adjuvants, has been able to double the antitumor effect of DOX and impose the beneficial effects against cardiotoxicity of DOX [15,21,22]. On the other hand, recently the researchers' attention has been focused on short-term, high-intensity exercises, which are more easily accepted by patients and have considerable advantageous in comparison with long-term endurance exercises [12,13]. However, the efficiency of this type of exercise on the side effects of DOX in myocardium and underlying mechanisms has not been studied yet. Based on the results of the present study, the administration of HIIT before DOX challenge significantly reduced DOX-cardiotoxicity and apoptotic cells via influencing the expression of Bcl-2, Bax, Bax/Bcl-2 and caspase-6 at gene or protein levels. Both protein and mRNA levels of Bcl-2, as an anti-apoptotic factor, were increased by HIIT in DOX-treated group, suggesting the involvement of Bcl-2 in the cytoprotective mechanism of HIIT against DOX insult. In addition, HIIT simultaneously reduced the expression of Bax mRNA and protein following DOX treatment. Although Bax and Bcl2 regulate cell apoptosis independently, there is also a competition between them [23]. Thus, the Bax/Bcl2 ratio can determine the fate of cell survival. In view of that, this ratio was significantly increased by DOX treatment and recovered by HIIT. The proteins of Bcl2 family maintain the integrity of outer mitochondrial membrane and regulates the release of cytochrome-C and other apoptotic factor like Apaf1 into the cytosol, preventing the development of apoptosome [24,25]. By reducing the Bax/Bcl2 ratio, HIIT may reduce the mitochondrial permeability and inhibit the progression of apoptotic response induced by DOX. Additionally, Bax expression following apoptosis can increase the expression of caspases like caspase 3 and 6. Caspase-6 is located at the downstream of caspase activation pathway and is involved in the execution of apoptosis by two ways [26]. First, increased expression of caspase-6 in DOX-toxicity can degrade Lamin B which is required for the normal integrity of nuclear and cellular morphology and thereby lead to the accumulation of the condensed chromatin into apoptotic bodies. Moreover, caspase-6 promotes caspase-8 cleavage, as an initiator caspase to promote cytochrome-C release. By reducing caspase-6 expression, HIIT can also inhibit these signals of apoptosis.

GSK-3β is another player in increasing the mitochondrial permeability [24]. The expression level of this gene did not change after DOX treatment. This finding suggests that GSK-3β probably has no or only little involvement in the apoptotic effects of DOX. Also, HIIT could significantly reduce the expression of GSK-3β in control rats but its reducing effect was not statistically significant in DOX-receiving rats. This implies that other negative effects of DOX (such as inflammatory responses and oxidative stress) may block the possible effect of HIIT on GSK-3β expression.

It has been reported that DOX can negatively affect the expression profile of miRs, altering the normal expression of pro-survival genes and proteins [27]. Under physiological conditions, MiR-499a-5p is abundantly present in cardiomyocytes *and* is believed to have the protective effect against apoptosis induced by myocardial infarction [9,10]. In addition, restoration of the expression of miR-499 has reduced the severity of muscular dystrophy via improving mitochondrial function and muscular energetics [28]. Therefore, increasing the expression of this miR following HIIT regimen may activate the cellular anti-apoptotic pathway and thereby increase the heart's ability to adapt against DOX toxicity. Contrary to our presumption, the level of miR-499 has also increased substantially following the DOX challenge. However, pre-training of DOX-receiving rats with HIIT prevented the sharp increase in this miR, and this effect was in the same direction of myocardial anti-apoptotic effects of HIIT. It is possible to conclude that the expression of miR-499 is underwent a compensatory increase in response to DOX toxicity in order to counterbalance the negative pathological effects of DOX, by the mechanisms that need to be identified in future studies. To test this hypothesis, one should block the DOX-induced pathological alterations such as apoptotic pathways, and then assess the direct effect of DOX on miR-499 expression. By modifying the apoptotic alterations of DOX in the present study, administration of HIIT prevented, to some extent, the development of this secondary response and inhibited the sharp increase in miR-499 expression, although HIIT itself also had an incremental (protective, not compensatory) effect in healthy rats.

In this study, the HIIT regimen was used as a preventive protocol to oppose the DOX-cardiotoxicity. In clinical practice, patients who are at early stages in cancer diagnosis and yet can tolerate this type of exercise before starting DOX treatment can benefit from the positive impacts of HIIT. However, it is still not clear whether concomitant HIIT administration during cycles of DOX treatment also has a protective effect in cancer survivors exposed to DOX. Many preclinical studies have reported that the aerobic regular exercise administered before, during or after DOX treatment has been able to reduce DOX-cardiotoxicity [29], and thus, it seems that by optimizing the intensity, timing and duration of HIIT regimen, the translatability of its concomitant administration in the treatment plan of a specific category of patients would be achievable. Additional studies can be helpful for getting better conclusion in this regard.

In conclusion, training of rats with HIIT regimen prior to DOX treatment had cardioprotective effects against DOX-toxicity via increasing the expression of anti-apoptotic Bcl2 factor, reducing the expression of pro-apoptotic factors Bax and caspase-6 and myocardial apoptosis and restoring miR-499a expression. Thus, HIIT might be a promising therapeutic strategy for the treatment of DOX-induced cardiac injury.

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Disclosure of conflict of interests

None.

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