DOI: https://doi.org/10.22122/arya.2022.26274 Published by Vesnu Publications

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Investigation of the effect of 8 weeks of high-intensity interval training and berberine supplementation on some echocardiography and electrocardiogram indices following myocardial ischemia-reperfusion in rats

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Original Article

Abstract

BACKGROUND: Myocardial ischemia leads to left ventricular (LV) dysfunction and cardiac arrhythmia. The present research was conducted with the aim to explore echocardiography changes and electrocardiogram parameters of the hearts of rats with ischemia-reperfusion injury (IRI).

METHODS: The study subjects included 50 male Wistar rats (8-10 weeks), which were divided into 5 groups (1: trained, 2: supplemented, 3: combined (training and supplementation), 4: sham, and 5: control). High-intensity interval training (HIIT) was performed for 8 weeks, 5 sessions per week. Rats belonging to groups 2 and 3 received 10 mg/kg berberine. Finally, after 48 hours, electrocardiogram and echocardiography were performed on all rats. Moreover, myocardial ischemia was performed by descending coronary artery ligation for 30 minutes.

RESULTS: There were significant differences between the 5 groups in terms of the volumes and dimensions of LV end-systolic dimension (LVSD), LV end-diastolic dimension (LVDD), fractional shortening cardiac output, ejection fraction (EF), stroke volume (SV), ventricular tachycardia (VT), and ventricular ectopic beats (VEBs) episodes, duration of VTs, and ECG parameters ($P \leq 0.05$).

CONCLUSION: Berberine supplementation and HIIT, as preconditioning agents, can possibly prevent the elevation of EF and fractional shortening, the reduction of cardiac output and SV, and arrhythmia improvement after myocardial IRI. Finally, these changes result in increased LV function and decreased mortality in rats with myocardial IRI.

Keywords: Berberine; Echocardiography; Electrocardiography; High-intensity interval training; Infarction

Date of submission: 11 Jan. 2021, ***Date of acceptance:*** 13 Sep. 2021

Introduction

Cardiovascular disease (CVD),[1] which is the most common cause of death, will continue to be the most important reason by 2030.[2] Myocardial ischemia causes cardiac remodeling including diastolic dysfunction, arrhythmias, and cardiomyocyte hypertrophy.[3] Timely blood reperfusion, regarded as an effective method in treating ischemia can lead to more damages in cardiomyocytes called ischemia-reperfusion injury (IRI).[1]

Echocardiography is widely used as an anatomical, hemodynamic, and functional diagnostic tool in cardiology, and systolic and diastolic functions and infarct size are obtained through this approach.[4,5] Therefore, there has always been a growing tendency to use this tool as a research instrument based on new standards and methods.[4,5] The electrocardiogram is also a reliable tool for the diagnosis of myocardial infarction (MI). MI confirmation is based on documentation showing myocardial necrosis. In general, the main criterion for detecting MI is the evaluation of electrocardiogram abnormalities and heart arrhythmia. Q wave pathologic characteristics and elevated ST segment are symptoms of ischemia of the heart.[6]

How to cite this article: Banaei P, Nazem F, Sedighi M. **Investigation of the effect of 8 weeks of high-intensity interval training and berberine supplementation on some echocardiography and electrocardiogram indices following myocardial ischemia-reperfusion in rats.** ARYA Atheroscler 2022; 18: 2363.

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Several studies have reported the protective effect of berberine on many illnesses, such as heart disease. Berberine is an alkaloid that is crystallized as yellow, needle-shaped crystals and found in plants, such as wild barberry.[7,8] To date, the role of berberine in the reduction of mortality rate after myocardial ischemia, prevention of cardiac dysfunction, decreasing of left ventricular (LV) remodeling, increasing of LV autophagy, and anti-arrhythmic effect has been investigated and it has been shown that berberine improves cardiac function.[8,9] In addition, numerous studies have reported that exercise reduces systolic and diastolic dysfunctions, and improves coronary circulation and ventricular function.[10,11] A number of researches have specifically investigated the effect of exercise on ischemic patients during the last decades.[11-13] Ranjbar et al. reported that submaximal exercise in rats improved systolic function and reduced LV dysfunction 4 weeks after heart IRI.[3] Many studies have shown that high-intensity interval training (HIIT) is more effective than continuous endurance exercise in preconditioning heart tissue.[14]

Wu et al. investigated the effect of post-IRI HIIT as a postconditioning.[15] Moreover, Rahimi et al. observed a non-significant improvement in electrocardiogram parameters after 8 days of HIIT.[14] Thus, the present research was conducted with the aim to study the impact of the mentioned interventions as preconditioning agents on changes in myocardial echocardiography and electrocardiography parameters after IRI.

Materials and Methods

Subjects: The present study subjects included 50 Wistar rats of 8-10 weeks of age (240 ± 20 g), which were obtained from the Pasteur Institute of Iran (PII). This study was carried out according to instructions of the National Institute of Health regarding laboratory animals. Moreover, the method was performed under the supervision of the Animal's Ethics Committee at Bu-Ali Sina University, Hamedan, Iran. We divided the subjects to 5 groups, each including 10 rats. They were maintained at 22 ± 2 °C, 60 ± 5% humidity, and under a 12-12 light-dark cycle. Moreover, water and food were freely available to them.

Berberine extract preparation: To prepare berberine extract, wild barberry root was obtained from South Khorasan city (Iran). The roots (1 kg) were dried at 20 ± 5 °C for 2 days, and then, they were powdered using a laboratory mill. Using a percolator, 100 grams of powder was shaken for 72 hours. It was then filtered using filter paper and prepared in solvent material (70% ethanol). Moreover, rotary was used to concentrate the extract, and then, it was kept at 4 °C. Berberine extract was obtained using a high-performance liquid chromatography (HPLC) machine. Besides, the standard berberine with over 95% purity (Berberin chloride) was obtained from Sigma-Aldrich Company (USA). The rats in the supplement and combined groups received 10 mg/kg of berberine extract for 4 days.[8]

Exercise protocol: The rats were left to rest for a week to stabilize their conditions. In order to familiarize the rats with the routine, they ran for 10 min daily for 1 week on a treadmill at a speed of 10-20 m/min. Subsequently, we separated them into the 5 groups of trained (IRI after HIIT), supplemented (IRI after berberine supplementation), combined (IRI after HIIT and berberine supplementation), sham, and control (IRI without intervention). The training protocol was performed in intervals and a rat was subjected to treadmill running for 8 weeks, 5 sessions a week. Each session included 5 minutes of warming up and cooling down, and 30-min running at 29-36 m/min (Equivalent 50%-90% VO_2 max) with 1% slope. On the 1st week, we had 5 intervals in each session, each interval consisting of 30-35 seconds of high-intensity running at 29 m/min with a 1-minute rest. It is worth mentioning that intermissions, time, and rapidity of running were slowly amplified each week without altering the rest period. Finally, the rats performed 12, 75-second intervals at 36 m/min.[16]

Measurement of echocardiography indices: Using ketamine (2.5 mL) and xylazine (1 mL) injection, the rats were anesthetized in sequence 7 days after IRI. Then, the rats' chest was shaved, and they were placed on the operating bed. In addition, echocardiography was performed using a device equipped with a 10 Hz transducer. Echocardiographic indices were obtained based on the guidelines of the American Society of Echocardiography[3] (Table 1). Subsequently, we measured the LV structural variables including LV end-systolic dimension (LVSD), LV end-diastolic dimension (LVDD), and interventricular septum thickness. LV end-diastolic volume (LVEDV) and end-systolic volume (LVSDV) were obtained using the following formula:[5,17,18]

$$\text{LVEDV} = \frac{7\times(\text{LVEDD})3}{[2.4+\text{LVEDD}]}$$

$$\text{LVESV} = \frac{7\times(\text{LVESD})3}{[2.4+\text{LVESD}]}$$

Table 1. Changes in echocardiography parameters after myocardial ischemia-reperfusion in different groups

	Train	Supplement	Combined	Sham	Control
LVDD					
Absolut (mm)	7.46 ± 0.07*	7.38 ± 0.14*	7.51 ± 0.13*	7.85 ± 0.03*	6.90 ± 0.08
Relative (mm/g)	28.06 ± 0.24*	27.18 ± 0.37*	28.29 ± 0.47*	26.67 ± 0.48*	21.66 ± 0.40
LVSD					
Absolut (mm)	4.80 ± 0.02	4.73 ± 0.05*	4.74 ± 0.09*	4.87 ± 0.30	5.02 ± 0.01
Relative (mm/g)	18.04 ± 0.14*	17.46 ± 0.44*	17.86 ± 0.42*	16.55 ± 0.31	15.76 ± 0.76
SEPD					
Absolut (mm)	1.19 ± 0.05	1.37 ± 0.13	1.20 ± 0.10	1.22 ± 0.04	1.16 ± 0.05
Relative (mm/g)	4.50 ± 0.19	5.07 ± 0.56	4.50 ± 0.41	4.15 ± 0.18	3.64 ± 0.16
LVEDV (ml)	295.30 ± 6.88*	288.77 ± 12.88#	299.77 ± 11.97*	330.85 ± 3.65*	247.80 ± 6.55
LVESV (ml)	107.53 ± 1.46	104.46 ± 2.85*	104.56 ± 4.64*	111.49 ± 1.62	119.34 ± 0.59
SV					
Absolut (mm)	187.77 ± 6.92*	184.10 ± 15.00*	195.21 ± 16.43*	219.36 ± 3.59*	128.45 ± 6.42
Relative (mm/g)	705.90 ± 23.00*	664.62 ± 47.00*	736.64 ± 60.00*	746.12 ± 19.00*	402.66 ± 0.10
SVI (ml/beat/kg)	0.70 ± 0.02*	0.67 ± 0.04*	0.73 ± 0.06*	0.74 ± 0.01*	0.40 ± 0.01
CO					
Absolut (ml/min)	70.00± 27.00*	69.00 ± 13.00*	73.00 ± 16.00*	80.00 ± 12.00*	57.00 ± 12.00
Relative (ml/min/g)	263.15 ± 90.00*	249.09 ± 16.00*	275.47 ± 47.00*	272.10 ±67.00*	178.68 ± 69.00
CI (ml/min/kg)	265.38 ± 64.00*	255.44 ± 16.00*	276.26 ± 24.00*	285.98 ± 67.00*	148.20 ± 69.00
EF (%)	63.50 ± 0.90*	63.40 ± 0.40*	64.70 ± 0.90*	66.29 ± 0.50*	51.74 ± 0.60
FS (%)	35.00 ± 0.600*	35.00 ± 0.70*	36.00 ± 0.20*	37.00 ± 0.40*	27.00 ± 0.80

Data are presented as mean ± sem.

LVDD: Left ventricular diastolic diameter; LVSD: Left ventricular systolic diameter; SEPD: Septum thickness; LVEDV: Left ventricular diastolic volume; LVESV: Left ventricular systolic volume; SV: Stroke volume; SVI: Stroke volume index; CO: Cardiac output; CI: Cardiac output index; EF: Ejection fraction; FS: Fractional shorting

The Shapiro-Wilk test, one-way ANOVA, and Tukey's test were used. *Sign of difference as compared to control group. #Sign of difference as compared to sham group. $P \leq 0.05$

Furthermore, the difference between LVESV and LVEDV was used to obtain stroke volume (SV). Cardiac output was obtained via multiplying the heart rate by SV (CO = SV × heart rate), cardiac index was calculated by dividing cardiac output by body weight, and SV index was calculated by dividing SV by body weight [19]. In addition, the following formulas were used to calculate the percentage of fractional shortening (%FS) and ejection fraction (% EF):

$$\%FS = (LVEDD\text{-}LVESD)/LVEDD \times 100,$$
$$\%EF = SV/\text{diastolic volume} \times 100$$

Cardiac echocardiographic variables were normalized in absolute and relative measures (rat body weight).[20-22] Finally, after deep anesthesia with ketamine and xylazine, the heart of the rats was removed from their chest.

Ischemia-reperfusion injury: After 48 hours of supplementation and training, subjects were anesthetized in sequence via ketamine (2.5 mL) and xylazine (1 mL) injection, reaching appropriate effect after 5-10 minutes. The rats were fixed on a surgical bed. After intubation, the subject was attached to a ventilator with a respiratory rate of 60-70 and 15 mL/kg volume. In order to fix the temperature of the body, a thermal pad was used to prepare the surgical bed at 37 ± 1 °C. The infarction incidence was monitored using Lead II, which was recorded with a PowerLab electrocardiogram (ML750 Power Lab/4sp, AD Instruments, Sydney, Australia). In order to cause the infarction, we implemented a 2 cm cross cut on the chest in the 4th left intercostal space. The pericardium was torn with small forceps, and then, the silk thread was passed under the left anterior descending (LAD) artery and 2 mm under the left atrium. Furthermore, the node stayed created to cause ischemia.[3,14] An electrocardiogram was performed to indicate the positive LAD blocking and the variations in the ECG that include ventricular tachycardia, ST-segment elevation, and premature ventricular contractions. After 30 minutes of ischemia in the LAD area, reperfusion was implemented in order to return the blood to the heart (Figure 1). Subsequently, we closed the chest via suturing with a silk thread (30 mm, USB 3.0). Moreover, tetracycline pomade was used. After surgery, the animal was placed in an oxygen chamber to regain its consciousness.[3,14] The rats in the sham group also underwent a similar surgery that included intubation and thoracotomy, but not LAD blocking.

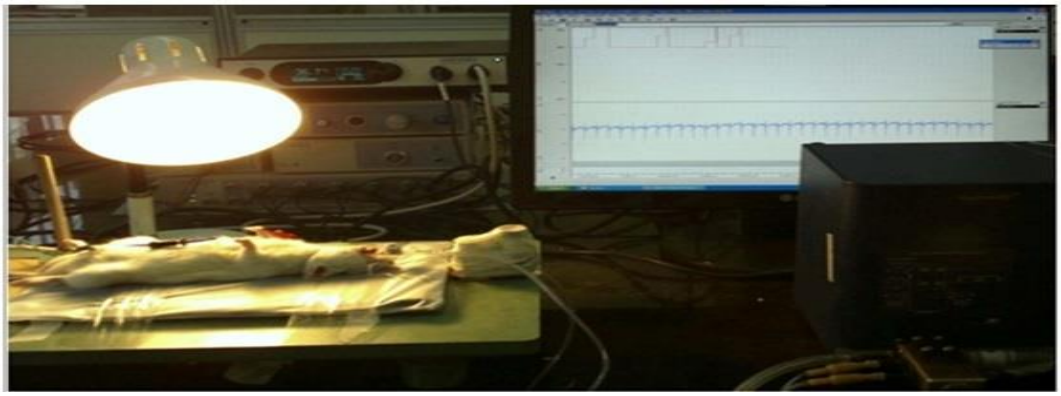

Figure 1. Myocardial ischemia-reperfusion in the studied rats on a bed equipped with a thermal pad in order to maintain the body temperature of the subject, a ventilator connection to supply oxygen, and a power lab device to evaluate electrocardiogram changes

Determination of arrhythmias and electrocardiogram variability: Using the ECG signals, ischemia-induced ventricular arrhythmias were measured consistent with the Lambeth agreements.[23] We also assessed ventricular arrhythmias for a 30-minute period. VTs can be defined as 4 or more premature ventricular beats. Furthermore, ventricular ectopic beats (VEBs) can be defined as identifiable premature QRS complexes. Mean QRS interval time, QT interval time (QTc) T and R amplitudes, and ST-segment elevations were recorded at baseline, at the end of 30-minute ischemia, and reperfusion time.

Statistical Analysis: The analysis and comparison of data were performed in SPSS software (version 23; IBM Corp., Armonk, NY, USA). The comparison of groups is presented as mean ± SD at the significant level of $P \leq 0.05$. After reaching data distribution normality via the Shapiro-Wilk test, we analyzed the data via repeated measures ANOVA and Tukey's post-hoc test.[14]

Results

Demographic changes: As illustrated in table 2, there was no difference in the heart weight of rats between the study groups, but we witnessed a significant difference between sham (384 ± 2.41) and control (368 ± 2.48) groups in terms of heart rate ($P \leq 0.05$). Moreover, a significant difference was observed in body weight at the end of the eighth week in the trained, supplemented, and combined groups (Table 2). However, heart-weight to body-weight ratio of the animals had a significant increase only in the combined and trained groups, as compared to the control group ($P \leq 0.05$; Table 2).

The changes in echocardiography parameters: Myocardial echocardiography structural variables, including LVSD and LVDD relative to body weight, had a significant increase in trained, supplemented, combined, and sham groups as compared to the control group ($P \leq 0.05$; Table 1). A significant difference was observed in LVSD in the supplemented and combined groups as compared to the control group, and in the LVSD relative to body weight in the trained, supplemented, and combined groups (Table 1; $P \leq 0.05$). Absolute and relative interventricular septum thickness after myocardial IRI showed no significant difference ($P > 0.05$). However, a significant increase was observed in LVEDV in the trained, combined, and sham groups as compared to the control group ($P \leq 0.05$). Nevertheless, LVEDV decreased significantly in the supplemented group compared with the sham group ($P > 0.05$; Table 1).

Table 2. Demographic characteristics of ischemic male Wistar rats in the study groups

	Train	Supplement	Combined	Sham	Control
Number	10	10	10	10	10
Age (week)	8-10	8-10	8-10	8-10	8-10
Heart Rate (bpm)	376.00 ± 3.02	378.00 ± 1.93	375.00 ± 2.32	384.00 ± 2.41*	368.00 ± 2.48
Heart Weight (g)	0.91 ± 0.04	0.90 ± 0.06	0.90 ± 0.08	0.85 ± 0.01	0.78 ± 0.01
Body weight (g)	266 ± 20$^{*\#}$	277 ± 50$^{*\#}$	265 ± 10$^{*\#}$	294 ± 40*	319 ± 70$^{\#}$
HW/BW	0.0030 ± 0.0001*	0.0020 ± 0.0001	0.0020 ± 0.0003*	0.0020 ± 0.0004	0.0020 ± 0.0004

Data are presented as mean ± sem. Shapiro-Wilk test, one-way ANOVA, and Tukey's tests were used. *Sign of difference as compared to control group. # Sign of difference as compared to sham group. $P \leq 0.05$

The LVSDV had also significantly decreased in the supplemented and combined groups ($P \leq 0.05$). SV, fractional shortening, and EF greatly increased in the 3 intervention groups and the sham group as compared to the control group ($P \leq 0.05$). The EF equaled 51.74 ± 0.06% in the control group and 63.5 ± 0.9, 63.4 ± 0.4, 64.7 ± 0.9, and 66.29 ± 0.5% in the intervention groups and sham group, respectively. Moreover, cardiac output (Table 1), stroke volume index (SVI) (Figure 2A), and cardiac index (Figure 2B) significantly increased after reperfusion in the intervention groups and sham group as compared to the control group ($P \leq 0.05$).

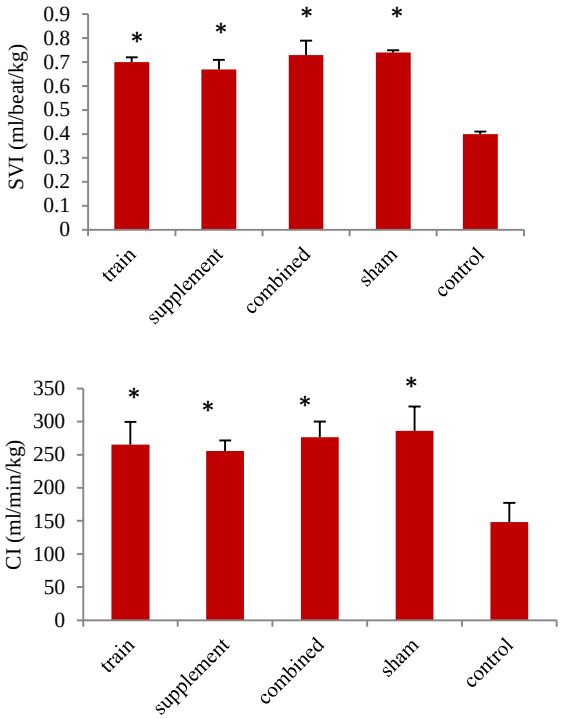

Figure 2. Stroke volume changes in different groups: (A) *Sign of difference in comparison with control group ($P \leq 0.05$); cardiac index changes in different groups; (B) *Sign of difference in comparison with control group ($P < 0.05$)

Ventricular arrhythmias: Regarding 30 minutes of ischemia, the mean number of VEBs in the combined and supplemented groups (49.83 ± 7.33 and 45.83 ± 4.56, respectively) decreased as compared to the trained and the control groups (61.83 ± 6.52 and 54.5 ± 5.08, respectively). In addition, the number of VT episodes in the combined group (10.66 ± 2.13) reduced compared to the control group (17.16 ± 2.85) (Figure 3A). The results showed that, during 30 minutes of ischemia, the total VT duration significantly decreased in the combined group as compared to the trained, supplement, and control groups (Figure 3B).

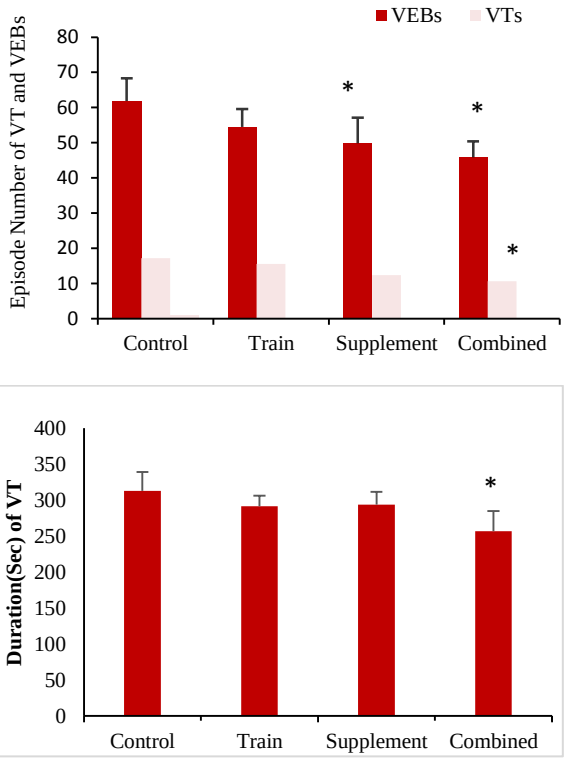

Figure 3. Ventricular arrhythmias during ischemia: (A) the total number of ventricular tachycardia and ventricular ectopic beats during 30- min ischemia in different groups; and (B) duration (s) of VT during 30-min ischemia in different groups
Data are presented as mean ± SD. *Sign of difference in comparison with control group ($P \leq 0.05$)

ECG parameters: Based on the results of repeated measures ANOVA in the QTc variable, the effect of time was significant ($P = 0.001$), but the time*group effect was not noticeable ($P = 0.09$). The results of electrocardiogram analysis showed significant differences in mean QTc shortening in trained, supplemented, and combined groups at the end of ischemic period and reperfusion compared to their baseline value ($P \leq 0.05$). Comparisons between groups showed that, at baseline, the duration of QTc was significantly lower in the combined group in comparison with the control group. When ischemia was over, QTc duration significantly reduced only in the combination and supplement groups ($P \leq 0.05$).

At the end of the reperfusion period, QTc significantly decreased in the intervention groups in comparison with the control group ($P \leq 0.05$) (Table 3).

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Table 3. Effect of training and berberine supplementation on electrocardiography parameters in the study groups

Time	Variable	Control	Trained	Supplement	Combined
Baseline	QTC (ms)	230.15 ± 10.24	220.60 ± 4.08	209.60 ± 7.93	194.60 ± 15.35$^{\#}$
	QRS (µV)	17.40 ± 0.48	19.26 ± 0.71	17.50 ± 0.40	16.60 ± 0.55
	R (µV)	592.00 ± 34.00	464.60 ± 36.01	421.20 ± 55.52	385.80 ± 56.37
	T (µV)	262.20 ± 42.76	217.52 ± 52.39	223.53 ± 22.04	247.32 ± 23.39
	ST (µV)	274.09 ± 21.48	227.33 ± 14.99	190.50 ± 19.49	184.16 ± 15.98$^{\#}$
End of ischemia 30	QTC (ms)	278.20 ± 21.65	195.00 ± 23.22*	141.00 ± 10.21$^{*\#}$	150.60 ± 12.77$^{*\#}$
	QRS (µV)	21.83 ± 2.80	17.40 ± 0.17$^{\#}$	13.30 ± 0.79$^{*\#}$	14.66 ± 0.71$^{\#}$
	R (µV)	576.20 ± 87.14	479.40 ± 93.40	434.60 ± 34.18	314.40 ± 30.70$^{\#}$
	T (µV)	235.80 ± 18.02	246.16 ± 20.74	257.71 ± 19.67	357.80 ± 38.88$^{*\#}$
	ST (µV)	324.33 ± 34.91	267.33 ± 16.39	223.33 ± 23.80$^{\#}$	212.16 ± 19.24$^{\#}$
End of reperfusion	QTC (ms)	250.40 ± 19.40	151.59 ± 15.3$^{*\#}$	146.60 ± 8.85$^{*\#}$	119.80 ± 9.33$^{\#}$
	QRS (µV)	19.59 ± 1.77	18.60 ± 1.28	16.32 ± 0.36	13.80 ± 3.88$^{\#}$
	R (µV)	540.40 ± 38.80	394.80 ± 39.20	364.60 ± 73.96$^{\#}$	251.20 ± 50.80$^{\#}$
	T (µV)	260.60 ± 17.63	220.40 ± 22.63$^{\&}$	272.40 ± 34.75	351.80 ± 32.14$^{\#}$
	ST (µV)	312.00 ± 21.63	222.73 ± 43.55	180.66 ± 28.70	124.33 ± 23.64$^{\#}$

Data are presented as mean ± standard deviation. Repeated measures ANOVA and Bonferroni test were used. *Significant difference in comparison with baseline ($P \leq 0.05$); $^{\#}$Significant intergroup differences as compared to the control group ($P \leq 0.05$); Significant intergroup differences as compared to the combined group ($P \leq 0.05$)

The repeated measures ANOVA results indicated that in the QRS variable, the effect of time ($P = 0.02$) and time*group were noticeable ($P = 0.04$). Moreover, the QRS interval was shorter in the supplemented group at the end of the ischemic period in comparison with baseline. At the cessation of ischemia, QRS amplitude decreased in the intervention groups compared to the control group ($P \leq 0.05$). At the end of the reperfusion period, QRS amplitude significantly decreased in the combined group in comparison with the control group ($P \leq 0.05$; Table 3).

According to the results of repeated measures ANOVA in the R variable, the effect of time ($P = 0.04$) and the effect of time*group were significant ($P = 0.01$). No noticeable difference in R-wave was observed at the end of the reperfusion period and ischemia compared to baseline ($P \geq 0.05$). At the cessation of ischemia, R-wave amplitude significantly decreased in the combined group in comparison with the control group. Furthermore, when the reperfusion period was over, R-wave amplitude significantly decreased in the supplemented and combined groups in comparison with the control group ($P \leq 0.05$), as indicated in table 3.

The repeated measures ANOVA results indicate that the time effect is noticeable in the T variable ($P = 0.02$), but the time*group effect is not considerable ($P = 0.09$). Additionally, T-wave significantly increased in the combined group in comparison with the baseline ($P \leq 0.05$). Nevertheless, this increase was not significant in the training and supplement groups ($P \geq 0.05$; Table 3). At the cessation of ischemia, T- wave amplitude greatly increased in the combined group in comparison with the control and training groups. As illustrated in table 3, at the end of the reperfusion period, there was a significant difference between the combined group and the training and control groups in terms of T-wave ($P \leq 0.05$).

Moreover, according to the results of repeated measures ANOVA in, time effect was significant in the ST variable ($P = 0.001$), but the time*group effect was not significant ($P = 0.08$). Furthermore, at baseline, the ST segment in the combined group was significantly lower than that in the control group ($P \leq 0.05$). At the cessation of ischemia, the ST segment decreased considerably in the combined and supplemented groups in comparison with the control group ($P \leq 0.05$). Moreover, as illustrated in table 3, at the end of the reperfusion period, ST segment elevation decreased in the combined group in comparison with the control group ($P \leq 0.05$).

Discussion

It seems that improvement was observed in some functional and structural variables of resting echocardiography of rat hearts as a result of each of the interventions (HIIT, berberine supplementation, and combination of exercise with herbal supplementation) after 8 consecutive weeks. In general, muscle shortening and EF are important in the evaluation of the systolic function.[22] Herein, the results indicated that although fractional shortening and EF were higher in the trained rats, they were lower in the control group. Therefore, it can be argued that the combination of HIIT and berberine

supplementation may relatively prevent a marked decrease in LV systolic function after MI. Zhang et al. found that a low dose of berberine (10 mg/kg) improved ventricular function including EF and fractional shortening.[8] Furthermore, Lew et al.[24] reported improved fractional shortening and EF after HIIT.

Moreover, the increase in the left ventricle internal diameter can be regarded as one of the prominent indicators of cardiac hypertrophy.[25] Furthermore, although the thickness of the interventricular septum did not show a significant difference, this index showed a tendency to change gradually. In addition, there was a significant increase in the mean relative heart weight in the intervention groups, which was accompanied by an increase in LVEDV s. These significant differences in the relative heart weight of the rats were consistent with the findings of a study by JIN et al.;[19] this is probably due to the use of a similar exercise pattern on the treadmill. However, these structural changes in the ventricular cavity were not associated with increase in myocardial hypertrophy indices. Thus, it is possible that HIIT with a defined duration and intensity along with berberine supplementation for 8 weeks did not cause significant change in the whole structure of the left ventricle in the heart of ischemic rats.

Regarding functional aspects, a previous research[3] examined exercise effects on SV and CO, and reported an increase in these parameters. In this regard, our research showed that the level of these two physiological factors was higher in the intervention groups. In this regard, scientific evidence has shown that exercise, venous return, and the Frank-Starling[25] mechanism cause the heart tissue to pump more blood and cause the greater stretching of the myocardial fibers, thereby increasing SV and CO.[25] Improved cardiac function may be related to a decrease in the impedance of LV ejection, systemic vascular resistance, and afterload.[19] Moreover, those rats which were subjected to treadmill exercise and berberine supplementation for 8 weeks showed a considerable increase in SV and cardiac indices at rest as compared to the control group, indicating that these interventions enhance cardiac function.

In addition, our results showed more significant changes in the LVDD than the LVSD. In this regard, it may be noted that the myocardial systolic phase may be affected by ketamine and xylazine, but not the diastolic phase.[26] Thus, less systolic phase changes can be attributed to the anesthesia in rats. Furthermore, some other researchers have indicated that different exercise programs increase the LVDD and LVSD in the exercise groups in comparison with the control group.[27] Thus, the difference in the type of subject may be one reason for inconsistency with the present study results. Moreover, the trained, supplemented, and combined groups showed improvements in LVSDV and LVEDV in comparison with the control group.

ECG is regarded as a fast, easy, and accessible tool for evaluating the effect of pharmacological and non-pharmacological interventions on cardiac features. In this regard, one can see a significant relationship between the risk of sudden cardiac death and prolonged ventricular repolarization.[9] However, the present research indicated that ischemic VEBs, VTs episodes, and duration of VTs decreased in the supplemented and combined groups in comparison with the control group.

In addition, Q shortening, R-wave reduction, and ST segment changes were observed in this study. Reducing QT prolongation is of great importance in the reduction of ventricular arrhythmias,[28] and the interventions of this study (HIIT and berberine supplementation) can also achieve this important goal. Moreover, the decreased in R-wave in the combined group illustrates that this intervention can decrease ventricular contractility.[28] The present study showed that the prolongation of QRS duration decreased in the supplemented and combined groups. These results are in agreement with the results of a study performed by Zhao et al.,[29] who found that injections of 60 mg/kg berberine for 4 days in mice could significantly decrease the QRS duration prolongation.[29] Moreover, in accordance with our findings, Zeng and Zeng[30] found that the consumption of berberine significantly reduced VEBs episodes.

In general, several different mechanisms have been implicated in the improvement of the LV function and cardiac arrhythmia after high-intensity training and herbal supplementation. Scientific evidence suggested that the protective role of berberine in reducing cardiac dysfunction is implemented by autophagy in the heart after MI through inhibiting the P38MAPK (p38 mitogen-activated protein kinases) pathway, activating the AKt-phospho (phosphatidylinositol 3-kinase/ Protein Kinase B) pathway, and activating autophagy proteins through the inhibition of the mTOR-signaling (mammalian target of rapamycin) pathway[8] in cardiac cells. According to various studies, the antiarrhythmic effect of berberine can

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be attributed to its positive inotropic effect, dilation of the coronary artery, inhibition of alpha-adrenergic receptors, inhibition of K^+ currents, action potential duration, the Na^+-Ca^{2+} exchange currents, and the L-type Ca^{2+} currents.[9,30]

In this respect, sarcoplasmic reticulum calcium depletion and its exchange with sodium,[31] decreased levels of ATPase activity, and fast-to-slow conversion of myosin isoforms[32] have also been reported in infarct rats, which probably lead to left ventricular dysfunction. Training, especially at intense levels, can prevent excessive calcium depletion, fast-to-slow conversion of myosin isoforms, and reduced level of phospholamban.[32] Recent studies have shown that exercise activity increases myocardial perfusion and antioxidant capacity and decreases ventricular fibrosis.[31-34] Furthermore, exercise training improves left ventricular dysfunction in ischemic rats through nitric oxide formation, vasodilatation and increased myocardial contractility with calcium access.[19] However, in our study, the trained group showed less change in the ECG variables than the supplemented and combined groups. In this regard, Rahimi et al.[14] also reported no significant changes in any of the arrhythmic variables, including episodes and duration of VT, VF, and PVC, after 8 days of HIIT on the treadmill with 95-105% VO2max. Therefore, it can be concluded that, in our study, the consumption of berberine supplementation with or without HIIT had a more positive effect on ischemia cardiac arrhythmias.

Conclusion

Herein, it can be stated that 8 weeks of HIIT, berberine supplementation, or a combination of these two independent variables, as pre-conditioning agents, can possibly prevent the reduction of SV and cardiac output, elevation of EF, and fractional shortening, and improve arrhythmia after myocardial IRI. The findings confirm HIIT and berberine use in treating heart IR disorders. However, further investigation of the role of these factors at different times of myocardial ischemia is necessary. The findings of this study could warrant future research on the mechanisms of exercise rehabilitation in patients with MI.

Acknowledgments

The investigations were performed in accordance with the National Institutes of Health Guide, and the protocol of the research was confirmed by the ethics committee of animals at Bu-Ali Sina University.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contribution

PB contributed to designing the experiments, data collection, and result interpretation. FN participated in and designed the experimental study and data evaluation, MS contributed to the laboratory work.

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