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MYOCARDIAL DAMAGE AND THE PROTECTIVE EFFECTS OF RESVERATROL AND AEROBIC EXERCISE IN COMBINATION ON STRESS OXIDATIVE AND DELETERIOUS CARDIAC FUNCTION IN EXPERIMENTAL MODEL OF ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Introduction: Cardiovascular diseases and acute myocardial infarction have been considered as the most common alternative in hospitalized patients in developed countries. The present study aimed to evaluate the protective effect of aerobic exercise training and resveratrol supplementation on the oxidative stress and apoptosis in the rats with acute myocardial infarction due to isoproterenol application.
Methods: In this study, a total number of 50 male Wistar rats were randomly categorized into five groups (n=10): Healthy mice or Control group (C), Isoproterenol group (ISO) that received isoproterenol (150 mg/kg body weight), ISO+exercise group(ISO+Ex) that rats ran for eight weeks on treadmill, ISO+Resveratrol group(ISO+Res) that animals received 25 mg/kg/bw of resveratrol per day for eight weeks and ISO+Exercise+Resveratrol group(ISO+Ex+Res). Isoproterenol subcutaneously administrated at the end of procedure on two consecutive days. Twelve hours after the second isoproterenol injection, Electrocardiography pattern were recorded then all animals were sacrificed to samples collecting for glutathione peroxidase, malondialdehyde, and apoptosis estimation.
Results: Pretreatment of rats with resveratrol, exercise, and their combination significantly increased the levels of glutathione peroxidase activity and decreased malondialdehyde and apoptosis markers in comparison with ISO alone-induced rats ($P< 0.01$). These changes in the ISO+Ex+Res group were more than those in ISO+Res and ISO+Ex groups. ECG patterns were close to the normal in resveratrol, exercise, and resveratrol+ exercise pretreated rats administered with ISO.
Conclusion: Regular aerobic exercise and resveratrol strengthen antioxidant defense system and attenuate oxidative stress. It appears that pretreatment with a combination of these two interventions prevents the destruction of myocytes and development of apoptosis.

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Introduction

Cardiovascular disease has been one of the foremost causes of death worldwide recently [1] and according to the World Health Organization (WHO), the leading cause by 2020 will be myocardial ischemia, whose common cause is coronary heart

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disease.[2,3] Common features of cardiovascular conditions known as heart disease risk factors including hypertension as a leading cause, dyslipidemia, overweight, and diabetes mellitus (DM) and its glucose intolerance condition contribute in increasing the oxidative stress.[1] On the other hand, acute myocardial infarction (AMI) has been one of the most common diagnostic criteria in hospitalized patients in developed countries. [5]The effect of isoproterenol as a treating drug for AMI has been evaluated in an experimental animal model.[6]

Studies have suggested that MI through the generation of free radicals causes damage to the myocardial cells.[7] Free radicals may via damage to lysosome and its wall render the activation of inactive enzymes and destruction of the cells, and as a result influence many of the vital functions of cells. Ultimately, they may result in breaking the hydrogen bonds.[8] By breaking bonds between membrane molecules, membrane-bound enzymes are disabled and therefore cell activity is disrupted. Damage to DNA may also lead to cell death (apoptosis and necrosis).[9]

There is sufficient evidence on the benefits of regular exercise in the primary prevention of heart disease. A vast majority of long-term prospective researches has shown inactivity to be the main cause of cardiovascular disease.[10, 11] To diminish overall mortality related, regular physical activity has been suggested in some researches to be effective as great as fifty percent.[12]

Remarkably, the intensity of physical activity required to outcome in health benefits is less than one might expect.[13] Considerable decrease in cardiovascular disease has been noted with as little as sixty minute of walking per week.[14]Despite difficulties to separate the effects of physical activity from other lifestyle modifications, the associations observed are convincing results of the benefits of physical activity in the primary prevention of cardiovascular disease. Aerobic exercise enhances mitochondrial performance through reducing the levels of reactive oxygen species (ROS), increasing the synthesis of NO, and reinforcing the mitochondrial biogenesis.[15] Because the effect of resveratrol has been similar to the exercise training[16] and as it creates similar adaptations in skeletal and cardiac muscles, treatment with it can also be effective like the treatment of heart failure with exercise training.[17-19]Resveratrol (3,5,4´- trihydroxystilbene), as a polyphenolic extraction of grape skin/seed, the root of Polygonum cuspidatum and red wine is determined to have protective effects on multi-targets related to heart diseases.[20] The protective potential of resveratrol against oxidative damage is revealed through the upregulation of endogenous cellular antioxidant systems, compared to the direct scavenging activity of ROS. Indeed suppressing pro-oxidative genes (such as myeloperoxidase and nicotinamide adenine dinucleotide phosphate oxidase) and inducing antioxidative enzymes or substrates of these enzymes including superoxidedismutase (SOD), thioredoxin, glutathione peroxidase (GPx), and catalase by resveratrol results in the inhibition of ROS formation.[17, 20] Studies have shown that resveratrol is the chelator of transition metallic copper which is involved in free radicals' generation and lipid peroxidation.[21]

The prevention is one of the most cost-effective and practical approaches in medical sciences and currently, there is an attempt to shift the attention from the modern treatment options in cardiovascular diseases towards the prevention of these diseases. The aim of present study was the evaluation of protective effects of aerobic exercise training and resveratrol supplementation on the oxidative stress and apoptosis in the rats with AMI due to isoproterenol application. Moreover, here combinational treatments are used to increase the beneficial effects and reduce the adverse events.

Materials And Methods

Animals

Fifty male Wistar rats (8-10 weeks old and initial body mass of 180-200 g) were obtained from the laboratory animals of Tabriz University of Medical Sciences, Tabriz, Iran. They were kept in standard laboratory conditions under natural light and dark cycle. The rats were fed usual diet and water. All the experimental procedures were conducted according to the protocols approved by the Animal Care Committee of Tabriz University of Medical Sciences.

Experimental design

Animals were divided into five groups and each group consisted ten rats as follows (n= 10): Control (C), Isoproterenol (ISO), Resveratrol+ISO (Res+ISO), exercise+ISO (Ex+ISO), and Exercise+Resveratrol+ISO (Ex+Res+ISO).

Experiment 1(Pretreatment with Resveratrol in ISO+Res groups): animals were orally administered ISO+Res (Nutrabio Co., 25 mg/kg/day of body weight, for a period of 8 weeks). ISO was injected subcutaneously 24 hours after last Resveratrol administration.

Experiment 2: ISO+Ex groups were trained as mentioned in treadmill exercise protocol and ISO was injected subcutaneously 24 hours after last exercise bout.

Experiment 3 (Exercise and Resveratrol in ISO+Ex+Res group): administration of Resveratrol started on the first day of 8-week training period. The first dose of ISO was administered 24 hours after the last session of exercise and Resveratrol gavages.

Resveratrol supplementation

Resveratrol dose was selected according to previous studies.[22,23] The resveratrol produced by the American company of Nutrabio (Nutrabio, USA) was used with pharmacological grade and purity of 99.87% and in the form of aqueous solution. Resveratrol solution was administered by daily gavage, for eight weeks. The resveratrol dose of 25 mg/kg/day of body weight for each rat [22,23] was between 0.5 to 1 mL of resveratrol solution.

In order to homogenize the gavage stress, C, ISO and Ex+ISO groups received the same amount of physiologic serum via gavage.

Treadmill exercise protocol

Rats were run on a motorized treadmill, at room temperature, for eight weeks, 5 days/week, 30 min/day at a speed of 20-25 m/min up to a 5% grade.[24] The length of the training sessions was progressively increased from 15 min duration at 10 m. min^{-1} with a 5% grade, so that in the closing stages, it reached to 30 min duration at 22 m. min^{-1} (Table 1). Furthermore, before the start of exercise training, it was considered a warm-up for 5 min at 5 m. min^{-1} for rats and a cool-down at the same rate. This protocol was designed progressively, based on the scientific principles of ASCM Association.[25] In order to avoid possible stress effects, no electric shock was ever applied to animals in either group during the exercise training. All of the animals were sacrificed 48 hours after their last exercise bout.

Table 1. Regular aerobic exercise protocol

		Week							
	Adaptation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Speed (m. $min^{-1)}$	5-10	10	10	14	14	18	18	22	22
Duration (min)	10	15	15	20	20	25	25	30	30
Slope (%)	5	5	5	5	5	5	5	5	5

Induction of experimental myocardial infarction

Twenty-four hours after completion of 8-week protocol, all animals received subcutaneous injection of isoproterenol (Sigma Company) at a dose of 150 mg/kg body weight diluted in 2 mL of saline on two consecutive days with an interval of 24 hours between applications.[26] In addition, in order to standardize the infusion stress for the control group, only 2 mL normal saline was injected.

Electrocardiography

Twelve hours after the second isoproterenol injection and fasting, the rats were anesthetized by the intraperitoneal injection of a mixture of ketamine (50 mg/kg of body weight) and xylazine (10 mg/kg of body weight) at the end of experiments. The Electrocardiogram (ECG) patterns were recorded by power lab (AD instruments 4/30, Australia). The heart rate was determined using the R-R interval and to ensure the infarcted rats, ST segment elevation or depression and Q wave inversion were considered as the indicators of MI.[27,28]

Measurement of oxidant and antioxidant markers' activity

After separation of tissue samples from the non-infarcted cardiac muscle, the samples were placed in liquid nitrogen and kept at -70°C. After homogenization and centrifugation, GPx activity was measured according to the Bradford method using Randox kits.[29] The enzymatic activity of antioxidant defense systems was calculated and recorded in the units of mg protein (U/mg Protein).

After being homogenized, MDA activity was measured by Uchiyama method using the spectrophotometer.[30] MDA values were recorded in nm/mg protein.

Quantification of apoptosis

To detect the apoptotic cells, TUNEL method was conducted using the In Situ Cell Death Detection Kit, POD (Roche, Germany), according to the manufacturer's instructions with some modifications.[31] To this end, the apical cardiac muscle was isolated and fixed in 10% formalin buffered solution. After homogenizing the cardiac tissue with phosphate buffer, it was centrifuged at 1000 ×g for 15 min at 4°C. After stabilizing the samples in 10% formalin, the tissue sections (4 µm in thickness) were prepared for TUNEL staining. Briefly, the tissue sections were dewaxed and rehydrated by heating at 58°C, followed by washing in xylene and rehydrating through graded series of ethanol and double distillated water. Then, the sections were incubated for 30 min at 21°C –37°C with proteinase K working solution (20 µg/ml in 10 mmol/l Tris-Cl, pH 7.6). Ultimately, the tissue sections were stained with toluidine blue.[32, 33] For the quantitative analysis of apoptotic cells, the number of apoptotic cells was determined randomly in 5 microscopic fields and their average was expressed as ratio of apoptotic cells to the total left ventricular cells.

Statistical analysis

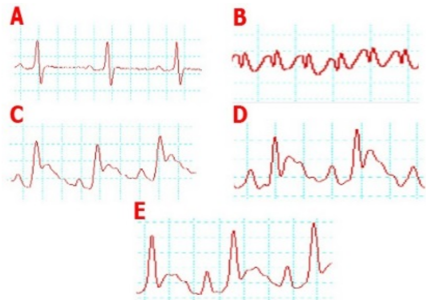
Descriptive data (means±SEM) were calculated for each dependent variable. Normality of the data was confirmed by Shapiro-Wilk test. Overall group differences were analyzed using one-way analysis of variance (ANOVA). When appropriate, post hoc analyses were conducted using Tukey test. A P value of <0.05 was considered statistically significant.

Results

Rats' physiological characteristics including age, heart rate, body weight, heart weight, and heart weight/bw are given in Table 2. The results showed that exercise and resveratrol significantly decreased heart rate and body weight in Ex and Ex+Res groups as compared to ISO group. Additionally, heart weight and heart weight /bw in ISO group significantly increased in comparison to all groups.

ECG

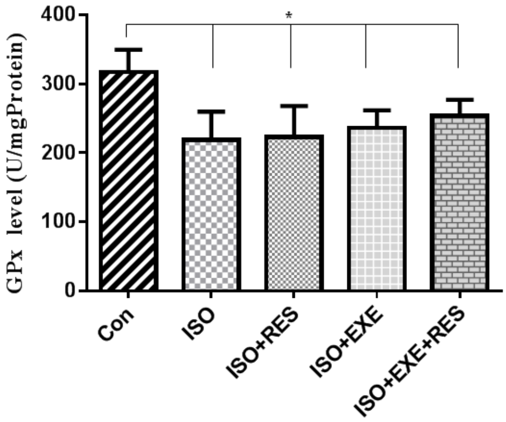
ECG patterns in different groups of rats are shown in Figure 1. Rats in the control group did not show any alteration in ECG patterns. In contrast, in treated rats with ISO (150 mg/kg), pathological Q waves along with elevated ST segments were observed. These changes were close to the normal in Res, Ex, and Res+ Ex pretreated rats, administered with ISO.



A: Control group
B: ISO group
C: Res group
D: Ex group
E: Res + Ex group

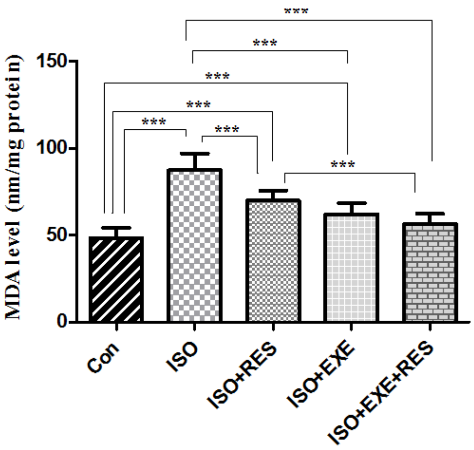
Antioxidant markers' activity

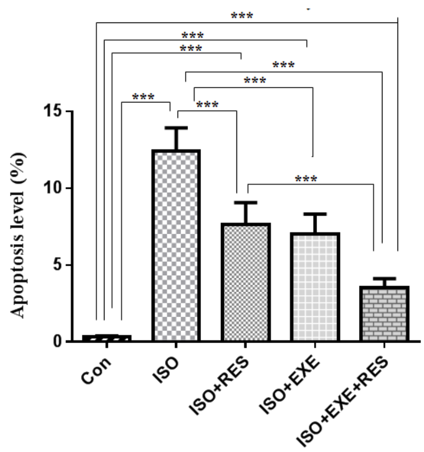
The results showed that having injected ISO, GPx activity was significantly decreased in comparison with control rats (P< 0.01). Daily pretreatment with resveratrol (25 mg/kg), exercise, and their combination for a period of eight weeks increased GPx activity compared with ISO alone-induced rats.



Stress oxidative and apoptosis

According to the results, obtained infusion of ISO after a period of eight weeks also significantly increased MDA (P< 0.01). After the oxidative stress as well, severe apoptosis was established in ISO rats which was significantly comparable with that in control rats. Rats pretreated with resveratrol, exercise, and their combination significantly showed decreased levels of these markers in comparison with ISO alone-induced rats (P< 0.01).





Discussion

Increased oxidative stress is involved with an imbalance between risen ROS production and reduced antioxidant systems. Reduced levels of antioxidant enzymes such as catalase, GPx, and superoxide dismutase lead to MI.[34] High-induced oxidative stress in the heart is one of the key events that may contribute in MI induced by isoproterenol.[35]

In the current study, subcutaneous injection of ISO (150 mg/kg) on two consecutive days with an interval of 24 hours decreased GPx and increased MDA activity and apoptosis variations. In this regard, Lobo Filho et al in 2011 showed that the induction of MI with isopropranol reduced GPx, catalase activity, and histopathological variations.[26]

The isoproterenol-induced myocardial harm has been described by several mechanisms.

It seems an unbalance in oxygen supply in cardiomyocytes leads to myocardial hyperfunction through the increase of both chronotropism and inotropism along with hypotension in the coronary arteries.[36] On the other hand, a rise in intracellular Ca^{++} overcharge is also demanded.[37] Additionally, the activation of adenylate cyclase enzyme depends on Ca^{++}.[38] Isoproterenol originated metabolic products and so oxidative stress does not mean free radicals' genesis.[39]

Moreover, mechanisms responsible for myocardial damage caused by ischemia are yet unknown. Nevertheless, numerous studies in this field indicate that several factors dependent upon each other including decreased cellular ATP, production of ROS, accumulation of hydrogen ions, producing nitrogen active species (RNS), increasing calcium, calpain and leukocyte activities inside the cell are involved in this damage. Overall, these factors increase cell damage and consequently lead to it.[40-43]

Studies have shown that factors such as age, excessive exercise, and reduced antioxidants cause increased oxidative stress in the heart and therefore possibly lead to the development of apoptosis.[44,45]

In the present study, we showed that pretreatment with exercise, resveratrol, and combination of resveratrol and exercise increased GPx activity in ISO treated rats. Moreover, pretreatment with abovementioned interventions led to a decrease in MDA activity and apoptosis variations in the rats. These findings corroborated some other studies.[46-52]

It was shown that both active lifestyles and exercise training decreased the development of heart failure in individuals with heart diseases.[53] Recently, a systematic review concluded that aerobic exercise would cause improved reverse ventricular remodeling in patients with stable heart failure and enhance their exercise capacity.[23,53,54] Furthermore, resveratrol therapy can be effective like exercise training in the treatment of heart failure, as proven in several studies.[17-19]

Kanamori et al reported that dosages of resveratrol (5 and 50 mg/kg/day) did not show any difference between the treatment groups in apoptosis index compared with the sham group after the end of protocol. Jin et al, Xu et al, and Ahmadi Asl et al also reported unchanged antioxidant defense system, oxidative stress indices, and apoptosis which were in contrast with our results.[55-58] Some possible factors such as age, sex, race, types of exercise training protocols, environment, as well as type and dose of drugs and supplements in above studies are involved in this contradiction.

The study of Kanamori et al showed that the expression of antioxidant proteins and the activity of pro-survival enzymatic pathways by resveratrol through the upregulation of AMPK and cardiac autophagy possibly was unchanged by resveratrol treatment.[57] The post-ischemic activation of Akt as the activator of survival kinase pathways and the effects of resveratrol was shown to be preconditions for the heart.[59] Moreover, cardiac NO production was likely involved since L-NAME blocked the ability of resveratrol to improve or recover the process of post-ischemic damages.[59]

ECG is gold standard for the early diagnosis of MI. The main criteria in MI diagnosis are the cardiac necrosis and the evolving pattern of ECG-abnormalities.[35]

Experimental models of MI in animals with ISO have approved ECG-changes. Determined abnormalities of ECG patterns such as risen heart rate[60] were detected in ISO-induced rats compared to normal rats. Physiopathological indicative signs of ischemia are the manifestation of Q waves and ST segment elevation related to myocardial necrosis accelerated possibly by ISO. ST elevation may be concluded by cell membrane loss.[60] Mentioned elevation of Q waves and ST segment in the region of MI was occurred in patients with severe heart disease and ischemia.[35] Prabhu and Devi in line with our results reported parallel ECG alteration in ISO induced animal models. In the mentioned study, ECG pattern approved that the combination of diosgenin and exercise were more effective.[35,61] Exercise and diosgenin treatment continuously improved the protective effects on heart; this amplified positive change may be due to the elimination of unwanted side effects. There is relatively further demand for the development of efficacy of non-toxic and safe cytoprotective remedies and drugs for the prevention of cardiovascular diseases. In vivo lipid peroxidation has been considered as one of the fundamental deteriorative reactions of cellular mechanisms in myocardial injuries.

Conclusion

Overall, this study showed that resveratrol is able to prevent cardiac functional abnormalities induced by AMI. As oxidative stress contributes in ischemic damage, resveratrol protects heart against oxidative stress production after ischemia caused by isoproterenol by reducing the production of superoxide and lipid peroxides, and activating antioxidant defense system. Beside resveratrol administration, exercise is one of the hopeful protective elements of cellular function for progressing defense mechanisms against oxidative stress in MI condition.

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Conflict of interests:
The authors declare no conflict of interests.

Table 2. Characteristics of rats in different groups

	Groups				
	C	**ISO**	**ISO+Res**	**ISO+Ex**	**ISO+Ex+Res**
N	10	10	10	10	10
Age (week)	8-10	8-10	8-10	8-10	8-10
Heart Rate (b.p.m)	336±22	382±27$^{\#}$	370±24$^{\#}$	357±23$^{\#*}$	352±15$^{\#*}$
Body weight (g)	345±32	364±33$^{\#}$	352±35	342±28*	338±31*
Heart weight (g)	1.14±0.15	1.22±0.19$^{\#}$	1.19±0.23	1.12±0.25	1.09±0.17*
Heart weight /BW	0.003±0.002	0.003±0.01$^{\#}$	0.003±0.001	0.003±0.002	0.003±0.002

Results are expressed as Mean±SEM for (n= 10) each group. $^{*}P< 0.05$ when compared with ISO, $^{\#}P< 0.05$ when compare to C. Based on the table, exercise and resveratrol decreased physioanatomical characteristics in Ex and Ex+Res groups.

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