ORIGINAL ARTICLE

The Effects of High-Intensity Interval Training and Curcumin Supplement on Concentrations of Antioxidant Enzyme and Oxidative Stress in Heart Tissue of Diabetic Rats

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Abstract

Objective: It was reported exercise and herbal medicine can possess synergistic effects to control of diabetes complications. The purpose of the present study was investigation of the effects of high-intensity interval training (HIIT) and taking curcumin supplement on activity levels of GPX and MDA of heart tissue in diabetic rats.

Materials and Methods: Thirty-two male Wistar rats were randomly divided to four groups of curcumin, training-placebo, training-curcumin, and control. The subjects had being trained to perform HIIT on treadmill and in the fashion of increasing manner. Diabetes was induced via injection of 50 (ml/Kg rat weight) STZ. Fifty (ml/kg rat weight) curcumin had being nourished to the animals of the curcumin groups, by using the gavage technique, during a period of eight weeks (five days a week). The rats were anesthetized via intraperitoneal injection of ketamine, 24 hours after the intervention. Then, heart tissues were separated from bodies of the rats to examine changes in GPX and MDA enzymes.

Results: Activity levels of GPX enzyme of the three groups of curcumin, training-placebo, and training-curcumin increased significantly than correspondent levels of the control group. Activity levels of MDA enzyme of the three groups of curcumin, training-placebo, and training-curcumin decreased significantly than correspondent levels of the control group. Though, no significant difference was observed between those first three groups (*P*-value≤0.05).

Discussion: The findings indicated that either HIIT or taking curcumin supplement or combination of both could solely contribute significant enhancement to activity levels of antioxidant enzymes and decreasing of Oxidative enzymes within heart tissues of diabetic mice. Although, those two interventions would not strengthen the influences of each other in decrease of MDA but they would strengthen the influences of each other in increasing of GPX.

Keywords: Curcumin, High-intensity interval training, Diabetic male rats

Introduction

Diabetes is a metabolic disorder that has involved a large population of the world. The number of patients with diabetes is estimated about three hundred millions until 2050 (1). Cardiovascular diseases have been recognized as the leading

cause of 8% of deaths in diabetic patients (2). Diabetes can progress cardiac hypertrophy and make the heart at risk of ischemic injuries, heart failure and stroke (2-4).

High blood glucose increases the oxidative stress markers which would lead to many complications of diabetes mellitus. An aspartate aminotransferase (AST) serum level and alanine aminotransferase (ALT) enzyme increase following tissues damage. Increases in levels of some markers such as Malondialdehyde (MDA) and decrease in levels of antioxidant enzymes such as superoxide dismutase (GPX), Glutathione peroxidase (GPx), and catalase (MDA) are some tissue indices in biochemistry (5).

Coronary artery disease (CAD), myocardial infarction (MI), and heart failure are the leading causes of death among diabetic patients. Acute myocardial infarction (AMI) which accompanies with CAD would cause myocardial ischemia and consequently lead to myocardial necrosis and generation of reactive oxygen species (ROS). It has been reported the drop of antioxidant performance in diabetic conditions, which might be mostly originated due to diabetes-induced oxidative stress. Markers of oxidative stress of MI (such as MDA, MDA, GPX, and GPx) would increase among diabetic patients, in comparison to those of non-diabetic patients. The results of a study have indicated the increment of antioxidants of diabetic patients might contribute to reduction of diabetes induced oxidative stress (6).

Taking new medicines or protective compounds, especially natural antioxidants that are derived from herbs have a preferential prominence. Curcumin is the principal ingredient of turmeric. Turmeric is a member of the ginger family (Zingiberaceae) and its protective effects on the liver has been defined (7). Curcumin ((1E,6E)-1,7-Bis (4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) is a bright yellow pigment that is the active principal ingredient of turmeric which is adverted as a Hindi widely used spice. Due to the minor adverse effects than synthetic medicines, curcumin (and other natural-based ingredients) can prevent from insulin resistance and consequently prohibit diabetes and its complications, in people at risk of developing diabetes (8).

In this regard, Naderi et al (2015) have stated exercise could remarkably decrease MDA level within heart tissue and blood sample (10). In addition, exercise would enhance GPX and GOx within heart tissue (25). Rovqani et al (2012) have shown diabetes would lead to significant increases in levels of MDA and nitrite and insignificant reduction in activity level of GPX, within heart. High dose of curcumin would significantly decrease levels of the both mentioned enzymes, and insignificantly reduce activity level of GPX, in heart (10-11).

Several studies have investigated effects of various training schedule, which were mostly aerobic trainings. Despite the benefits clearance of exercise on healthiness, unfortunately only few people follow those advices and perform regular exercise. Among many reasons of ignoring exercise, “lack of time” is often excused (12). The recent understanding about advantages and effectiveness of low-volume high-intensity interval training (HIIT) can aid to overcome that obstacle. Literatures have stated HIIT would significantly decrease subcutaneous fat, especially abdominal fat (14), and the body fat percentage (BFP) (15,16), increase the amount of VO_2 Max (15), and even improve insulin sensitivity (17). It has been reported HIIT consumes more calories than moderate-intensity continuous trainings, and would enhance post-exercise lipid oxidation, further. In addition, HIIT does own greater amounts of energy expenditure than continuous trainings. Investigation of such trainings on oxidative factors of heart and antioxidant enzymes certainly requires further studies.

The process of increases in the levels of antioxidant enzymes of heart is recognized as an inhibitive mechanism, to control diabetes-induced heart damages. Literatures have shown exercise could be effective about the

recent mentioned matter, especially the aerobic ones. In the other hand, taking herbs like curcumin has been recommended due to its antioxidant influences. Due to the indispensability of heart organ, tending the health affecting processes is not an option, it is an inevitable necessity. Therefore, the purpose of the present study was to investigate the effects of HIIT and curcumin supplement on oxidative properties and antioxidant enzymes, within heart tissue of diabetic rats.

Materials and Methods

The present research had been accomplished in laboratory animals of Shahid Sadoughi University of Medical Sciences and Health Services (The International Branch), in March 2016. Thirty-two adult Wistar rats, with weight range of 302 to 382 gr, were selected. Diabetes induced via single dose STZ, dissolved in normal saline (55 ml/kg rat weight and through intraperitoneal injection), into those 32 rats. In order to confirm diabetes, fasting glucose levels were measured, four days after the injection. Blood samples were taken from tails of the mice, and blood glucose levels were measured by glucometer. Blood glucose level of 300 (mmol/dl) was considered as diabetes index (19). The mice were randomly divided into four groups of curcumin, training-placebo, training-curcumin, and control. The rats were maintained in separated cages and in environmental condition of 12 hrs illumination/12 hrs dark, temperature of 22±3 °C, and relative humidity of 40 to 50%. Their nutrition had being carried out through pelleted food packages that were provided in a standard method (contain chewing seeds, consisted of calcium and phosphor) and free access water. The training-placebo and training-curcumin groups had being participated in eight weeks HIIT (five weekly sessions), which involved running on a 10 lines animal treadmill with treadmill slope of 0°, intensity of 80% VO_2 Max (after three min warm up with speed of five (m/min)), and spans sessions of 10 to 20 min (Table 1). In order to make the training groups familiar with the treadmill, those mice had being forced to run on the device, with speed of 5 (m/min), slop of 0°, and span of 5 min. The treadmill orientation training sessions had gradually increased to speed of 10 (m/min), spans sessions of 15 min, at the ending of the orientation period. Though, the treadmill slope remained 0°. The rats of the two supplementary groups had being fed on daily amount of 50 (mg/ kg rat weight) curcumin supplement (based on mean weight of the rats, with weight range from 302 to 382 gr) by using the gavage technique, during the eight week. In order to examine variation of GPX and MDA enzymes, the rats were anesthetized with ketamine/xylazine containing mixtures (80 ml ketamine to 10 ml xylazine per kg rat weight), 24 hrs after ending of the training period. Then, heart tissues were separated from the bodies, and the measurements were carried out by using ZellBio (made in Germany) kits. The inferential analysis of data was executed via two-ways ANOVA at significance level of 0.05, utilizing SPSS version 22.0. Besides, the relative diagrams were sketched by using Excel.

Results

Findings of this study showed exercise would effect on GPX concentration within heart tissue and increase its concentration significantly. Also, curcumin supplement had significant influences on increase in GPX concentration, both in the curcumin groups and the training-curcumin one (*P*-value≤0.001). Though, there is no significant

Table 1. Protocol of HIIT[ф]

Exercise Step / Exercise Element	Warm up	Main step (3 intervals) HIIT[φ]	Cool down
Span (min)	3 min	10 to 20 min	5 min
Intensity (% VO_2 Max)	50 to 60%	80% averagely (10% weekly increase)	50 to 60%

[ф]Slop of the treadmill remains 0°, during the entire steps/stages of the training

[φ]From four 2 min sets at the 1st weeks to eight 2 min sets at the 8th week, and with 1 min interval rest span between each two successive sets

difference between exercise influence and that of curcumin, on GPX concentration of heart tissue (*P*-value≤0.001). Concentration of GPX in training-curcumin group has significantly increased, which denoted the influences of training and curcumin on GPX concentration (but not a synergetic effect). The evolution of mean increments of the three groups has indicated that there was no significant difference between the three intervention groups of curcumin, training-placebo, and training-curcumin. (table 2). (*P*-value≥0.644) (*P*-value≥0.969) (*P*-value≥0.882).

In the other hand, the results of the present study indicated that training has a significant influence on MDA concentration in heart tissue, after the eight week training. Accordingly, HIIT would effect on concentration of MDA and enhance its level, significantly (*P*-value≤0.001). Also, Curcumin does own a significant influence on MDA concentration and would effect on concentration of MDA and increase its level, significantly (*P*-value≤0.001). In addition training-curcumin has a significant influence on MDA concentration. Though, there was not observed any significant difference, between the three interventions. (table 3). (*P*-value≥0.934) (*P*-value≥0.910) (*P*-value≥0.264).

Discussion

The purpose of the present study was to investigate the influences of eight weeks HIIT in companion with taking curcumin on antioxidant enzymes (GPX) and Oxidative Stress (MDA) of heart tissue. Thirty-two STZ-induced diabetic rats of the present research had been examined in the figure of division into the four groups of curcumin, training-placebo, training-curcumin, and control. Many studies showed the protective effects of regular exercise on cardiovascular diseases prevention which appear following aging, and reduction of deaths from cardiovascular disorders. Influential cellular mechanisms of

Table 2. Mean GPX levels of different intervention group and between groups, after the HIIT period, compared to the control group

Groups		*P*-value	Mean of GPX	Std. Deviation
Training	Curcumin	0.644	146	9/708
	Training+Curcumin	0.969		
	Control	0.001		
Curcumin	Training+Curcumin	0.882	122/57	11/118
	Training	0.644		
	Control	0.001		
Training+Curcumin	Training	0.969	173/57	8/921
	Curcumin	0.882		
	Control	0.001		
Control	Curcumin	0.001	64/33	2/641
	Training+Curcumin	0.001		
	Training	0.001		

Table 3. Mean MDA levels of different intervention group and between groups, after the HIIT period, compared to the control group

Groups		*P*-value	Mean of MDA	Std. Deviation
Training	Curcumin	0.934	26/78	21/118
	Training+Curcumin	0.910		
	Control	0.001		
Curcumin	Training+Curcumin	0.264	37/02	16/948
	Training	0.934		
	Control	0.001		
Training+Curcumin	Training	0.910	19/46	12/220
	Curcumin	0.264		
	Control	0.000		
Control	Curcumin	0.001	45/01	22/154
	Training+Curcumin	0.000		
	Training	0.001		

those affirmative effects have not appropriately been regained, yet. STZ-induced diabetic mice of the present study had undergone increase in blood glucose level and weight reduction, accompany with increases in urination and appetite, which was in agreement to related pervious results about diabetes effects. The induced-damage tissue with free radicals on development of diabetes complications were noticed (20). Moreover, increment in ROS and dysfunctional antioxidant situation in the body have been shown, in clinical and experimental diabetes studies (21).

The present results have indicated HIIT did own significant effects on concentration levels of GPX and MDA and would increase GPX and decrease MDA tissue enzymes. Those present results are in confirmation with those of Oláh et al (2015), Naderi et al (2015), and some others (9,10). Those recent results have indicated that the training-induced increases GPX and decreases MDA in tissue concentrations express either the glycation reduction in antioxidant enzymes or decrease in the accumulation of advanced glycation end-products (AGEs) within heart tissue, were originated from exercise (20). Furthermore, chronic effects of either resistance training or running on a rotating wheel, on decrease in oxidative stress index and increase in antioxidant activity have been reported (21). The recent mentioned issue is another approval to the cardio-protective role of exercise for handling stressful conditions of diabetes. Various mechanism mediate in those cardio-protective effects, which might partly be done through redox variations, and could consist of the induction of heat shock proteins (Hsps), development of antioxidant capacity, or enhancement in other cardio-protective molecules (22). GPX reduces the toxic effects of those radicals on secondary reactions, via transformation of superoxide anion to hydrogen peroxide (23). In addition, MDA enzyme is a determining factor for heart antioxidant status. MDA have a dominant role in erasing toxic properties of hydrogen peroxide, through deactivating superoxide ion (24). Therefore, the remarkable increases in activity levels of MDA and GPX, following training and/or curcumin interventions of the present study, might denote the effects of HIIT and curcumin supplement on reduction (or inhibition) of oxidative stress, in heart tissues of diabetic rats, through enhancement of antioxidant level. However, the present research did not investigate structural changes in the heart which is one of the limitations of the present research. Nevertheless, it can be stated that the decrease in oxidative stress, which has occurred because of the intervention, might lead to some desirable structural changes in heart tissues of the diabetic rats, due to the hand of oxidative stress on induction of structural changes in the heart, through direct damage from apoptosis induction and changes in the extracellular matrix (ECM), DNA, cellular proteins, and hypertrophic cardiomyopathy (HCM) (25).

In the other way, the eight weeks curcumin consumption would cause significant increases in capacity levels of GPX and MDA enzymes, in heart tissues of diabetic rats. The effects of curcumin on those enzymes could be addressed to its anti-diabetic and hypoglycemic influences. In this regard, Gupta et al (2011) had studied hypoglycemic effects of curcumin and have shown that long-term prescription for curcumin would enforce a prominent hypoglycemic effect (which is a desirable anti-inflammatory and anti-oxidative stress influence) on brain tissues of diabetic rats (26). Accordingly, similar result has been derived about the heart tissues. In addition, natural ingredients, like curcumin, may lead to the possible regeneration of the islets of Langerhans in conditions related to diabetes, associating with other treatments (27). Perhaps, the recent topic might likely occur, in the present study. Moreover, it has been reported curcumin operates to reduce the tissue insulin resistance, and would decrease tissue need for insulin, through intensification of the activity of glucose transporters (GLUT), within muscle tissues (28). Also, such

ingredient functions as a reducer of blood glucose level, through modulation of hepatic enzymes, which are responsible for carbohydrates metabolism, including decrease in activity level of liver phosphorylases enzyme and increases in activity levels of glucokinase and glycogen synthase (29).

It has been shown curcumin could lead to increases in levels of non-enzymatic antioxidants of the body and activity intensification of enzymatic antioxidant. In addition, curcumin would cause decreases in lipid peroxidation and oxidative stress, within tissues of STZ-induced diabetic rats (5). Another beneficial aspect of curcumin can be addressed to its hypoglycemic properties, in the present study. Accordingly, curcumin would lead to decreases in oxidative stress and its symptoms (such as MDA) within tissues (like heart tissues), through reducing AGEs.

The eight weeks high intensity interval training accompany with curcumin supplement taking do possess significant effects on concentrations of GPX and MDA. Though, the present results have indicated the training and the supplement do not strengthen influence of each other to decrease of Oxidative Stress (MDA). This means, the simultaneous exploitation of training and curcumin do not own any further significant influence, than those of the separated interventions. But those have interactive influence to increase levels of GPX enzyme.

Conclusions

Therefore, it can be concluded that both training and curcumin are effective interventions for improvement of the antioxidant performance within heart tissue, in diabetic conditions but for improvement of the Oxidative Stress have not further effects than those of separated exploitation of neither training nor curcumin.

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