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Effect of High Intensity Interval Training with Curcumin on Gene Expression of Bax, Bcl- 2, and Caspase- 3 in Aged Female Rat Hepatocytes

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Abstract

Introduction: Apoptosis is a type of cell death that is essential for homeostasis. Findings on the impact of physical activity on apoptosis are contradictory. The aim of this study was to investigate the effect of high intensity interval training with curcumin on gene expression of Bax, Bcl- 2, and Caspase- 3 in aged female rat hepatocytes.

Methods: In this experimental study, 35 aged female, postmenopausal Wistar rats (2-year-old), were randomly assigned to five groups including control, curcumin, high intensity interval training (HIIT), curcumin with HIIT and sham. Curcumin was given at 30 mg/kg by gavage in experimental groups 3 days a week. The HIIT protocol consisted of three sessions of high intensity treadmill training per week for 8 weeks. Forty-eight hours after the last training session and gavage, the Bax, Bcl- 2, and Caspase-3 genes expression were measured in the hepatocytes.

Results: The expression of Bax (P = 0.0003) and Caspase- 3 (P = 0.0006) genes increased significantly due to HIIT, while curcumin reduced this increase (P≤0.05). Bcl- 2 gene decreased due to HIIT (P = 0.001), and Curcumin with HIIT increased Bcl- 2 (P≤0.05).

Conclusion: HIIT and curcumin had an antagonistic effect on expression of apoptosis-regulating genes in hepatocytes.

Keywords: Curcumin, Training, Apoptosis, Hepatocyte

Introduction

Ageing is a major risk factor for chronic diseases. As the age increases, the disease develops and progresses (1). The liver plays an important role in the ageing process due to the combination effects of energy metabolism pathways and the detoxification of drugs and toxic substances (2). As the age increases, liver function declines and liver dysfunction associated mortality increases, indicating that liver ageing increases the predisposition to diseases (3). The underlying cause of age related diseases can be increased oxidative damage to DNA. Increased DNA damage can accelerate ageing, lower metabolic rate, change daily calorie intake, reduce bioavailability, decrease longevity, and lead ultimately to cell death (4). Apoptosis is a type of programmed cell death that occurs in physiological environment and is essential for maintaining tissue homeostasis and primary growth and development. Inhibition of apoptosis is one of the major causes of tumor formation. However, apoptosis upregulation leads to infectious diseases, autoimmune diseases, and neurological diseases (5). Therefore, control of apoptosis is an important therapeutic target for the treatment of apoptosis related diseases (6, 7). Several genes play an important role in the production of apoptosis, including Bcl-2, P53, Bcl -XL, Bax, Bak, Bad, Bim and Mcl- 1. In the liver of the older people, apoptosis increases and leads to an increase in the incidence of age related liver diseases (8), alcoholic fatty liver (9), and

increased side effects of drugs (10). It has been established that physical activity leads to adaptive responses including weakening of increased ROS production, lipid peroxidation, inflammatory factors, and other harmful, age related factors (11). Hallsworth *et al*. (2012) observed a significant decrease in body fat, intrahepatic liver (IHL) levels, and circulating liver enzymes in people with non-alcoholic fatty liver disease (NAFLD) after twelve weeks of high-intensity training (12). On the other hand, the active ingredient of turmeric, i.e., curcumin, has an anticancer effect on different types of malignant cells (13). Curcumin affects its effects on various cell processes, including the activation of pro-apoptotic signaling pathways (14- 17). Curcumin has a protective effect on liver (18-20). Zang *et al.* (2016) reported curcumin-induced suppression of oxidative stress and inhibition of hepatocyte apoptosis in rats (21). Considering that liver Bax and Bcl- 2 levels change with increasing age and increase apoptosis, physical activity and curcumin appear to inhibit the process by affecting the initiation proteins of apoptosis. However, the simultaneous effect of high intensity interval training (HIIT) and curcumin on hepatocyte apoptosis has not yet been studied. Therefore, this study is conducted to examine the effect of training and curcumin on markers of hepatocyte apoptosis in older female rats.

Methods

In an experimental study, 35 female, (2-year-old), postmenopausal Wistar rats with a mean baseline weight of 250- 300 g (Table 1) were purchased from the Pasteur Institute of Amol and randomized to five groups; control, curcumin, HIIT, curcumin with HIIT and sham. The dynamic physiological changes simultaneously may also play a critical role in the discordant findings. Therefore, aged female rats were selected in this study. All rats were kept under appropriate laboratory conditions, a light/dark cycle of 12 hours of light and 12 hours of dark, and an average temperature of 22 ± 2°C and (50 ± 5) % humidity. After being transferred to the laboratory, rats underwent a 5- day treadmill training adaptation program consisting of 5-min treadmill training a day. The rats were kept in transparent polycarbonate cages of 30× 15× 15 cm. The rats' food was procured from Karaj Animal Feed Co. per 100 g/bw, 10 g of food was given to rats in cages based on weighing conducted once every three days. Water was provided ad libitum in a 500 ml bottle for laboratory animals. It should be noted that this study was designed and implemented in accordance with the Ethics Guide for Animal Research adopted by the Ministry of Health and Medical Education Policy Planning Board. The rats in curcumin and curcumin with HIIT groups were given 30 mg/kg of curcumin (Sigma Co., Germany) by gavage. In the sham group, the rats were given the same volume of saline. For HIIT protocol, before the start of the original protocol, the rats underwent a 5- day treadmill training adaptation program consisting of 5- min treadmill training a day. The HIIT protocol consisted of three sessions per week for 8 weeks. Each session consisted of 10 sets of 1-min training at an intensity of 70 % exhaustion with a 2- min rest between sets. The treadmill training started at a speed of 20 m/min in the first week with addition of 2 m/min to the speed per week, so that the speed in the eighth week reached 34 m/min. The rats were allowed 5 min before training to warm up and 5 min after training to cool down. Table 2 shows the intensity of training per week in the rats of different groups. Forty-eight hours after the last training session and injection, after 10- to 12-hour fasting, rats were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (5 mg/kg) at 5/2 ratio. The liver tissue (the lower part of the right lobe) was quickly removed and placed inside a microtube containing liquid nitrogen. RNA extraction from the liver tissue was performed by using Qiazol (Kit Qiagen, Germany) according to the manufacturer's instructions.

An RNase-free DNase was used to eliminate the potential contamination of DNA with RNA. Required values were determined based on the concentration of extracted RNA. The concentration of RNA was determined by spectrophotometry (UV Eppendorff, Germany). For all the genes studied, the reference gene, i.e., GAPDH, was used to obtain an appropriate annealing temperature gradient. To investigate the efficiency of primers, a specific standard curve was plotted for each gene (diluted DNA series). The melting curve was also evaluated for the accuracy of PCRs specifically for each gene in PCR procedure by using a negative control curve to investigate the presence of contamination in each PCR. Reference genes were approximately equal. The expression of the target gene was normalized to the reference genes using the ΔΔCt and $2^{-\Delta\Delta Ct}$ formulas, and at each stage, the blastocyst gene expression of the control group was considered calibrator. All data were presented as mean (standard deviation). Two-way analysis of variance was used to determine the main effect of HIIT and curcumin, and HIIT-curcumin interaction effect in different groups. If a significant difference was observed, then Scheffe post hoc test was used to conduct inter-group comparisons ($P \leq 0.05$).

Results

HIIT significantly increased the expression level of Bax gene (P = 0.0003), while curcumin reduced its expression (P = 0.035). The combination of HIIT and curcumin significantly reduced Bax expression (P = 0.002). The combination effect of these two interventions on the expression of this gene was antagonistic. The pattern of expression changes of the Caspase- 3 gene was similar to that of the Bax gene so that the expression of this gene increased significantly due to HIIT (P = 0.0006). Curcumin significantly decreased the expression of the Bax gene (P = 0.002). Curcumin with HIIT, led to a significant reduction in the expression of the Caspase- 3 gene (P = 0.0005), indicating an antagonistic interaction between the two interventions. Expression of Bcl- 2 gene significantly decreased by HIIT (P = 0.01). Curcumin had no significant effect on the expression of Bcl- 2 gene (P = 0.145). However, HIIT and curcumin significantly increased Bcl- 2 gene expression (P = 0.001).

Table 1. Mean and standard deviation of rats weight in the studied groups (In grams)

Group	Mean± Standard deviation
Control	255.52±1.4
Sham	268.44±56.17
Curcumin	243.67±27.63
HIIT	262.41±32.43
HIIT with Curcumin	264.39±33.66

Table 2. Training intensity in each session in HIIT groups

Time	Running Speed (m/ min)
The first week	20
second week	22
The third week	24
Forth week	26
Fifth week	28
Sixth week	30
Seventh week	32
Eighth week	34

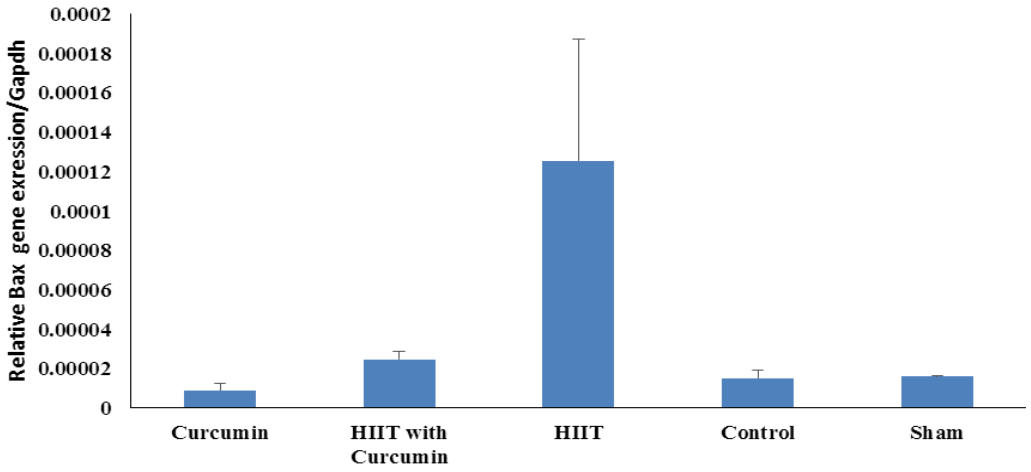

Figure 1. Bax gene expression in study groups

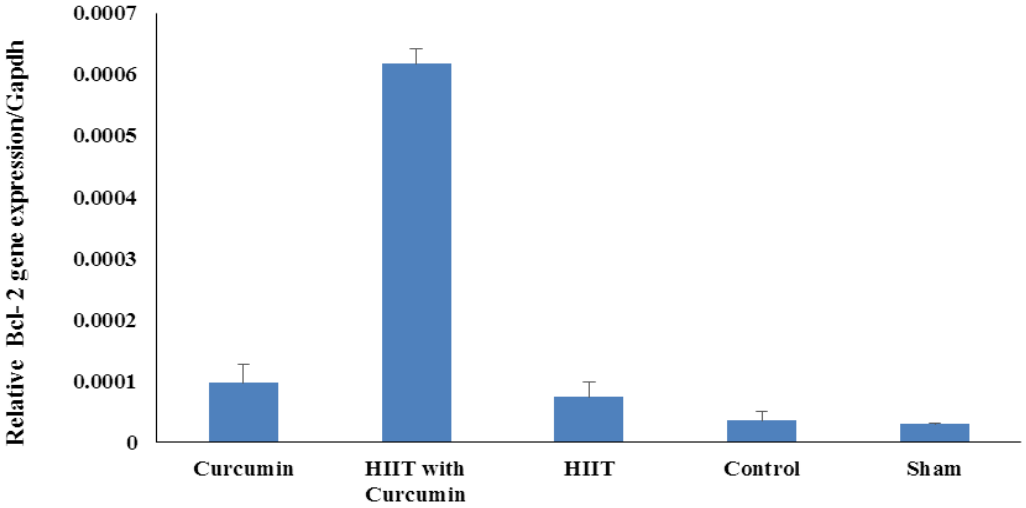

Figure 2. Bcl- 2 gene expression in study groups

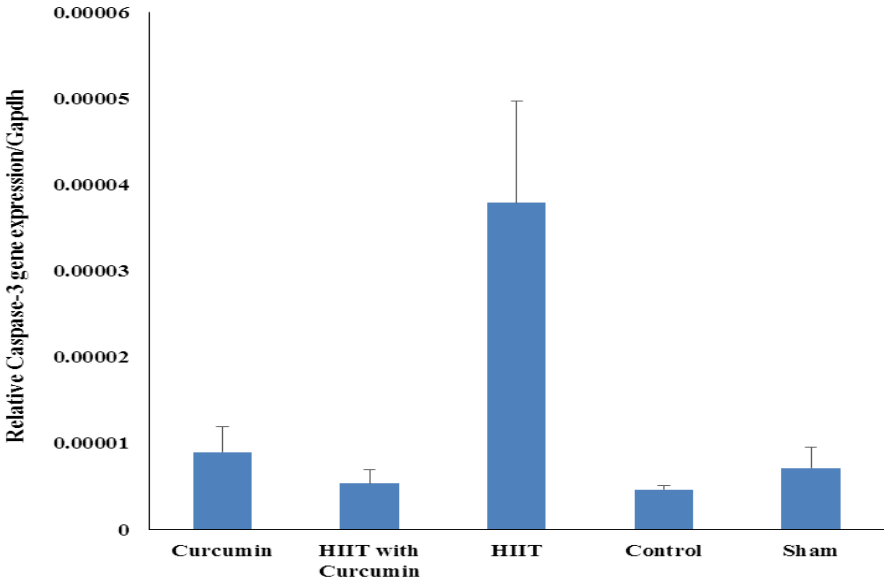

Figure 3. Caspase- 3 gene expression in study groups

Discussion

The results of this study showed that eight weeks of HIIT with curcumin could have different effects on hepatocytic apoptosis in older female rats. So far, few studies have been done on the role of physical exercise on the expression of apoptotic pathway-regulating genes in hepatocytes, which can provide valuable information on the effect of physical training and curcumin on stimulation or inhibition of hepatic apoptosis markers. The most important finding of this study was that HIIT was an important factor in the induction of apoptosis, as the expression of the Bax and Caspase-3 pro-apoptotic genes increased during HIIT. However, the expression of the Bcl- 2 anti-apoptotic gene did not significantly increase during HIIT. On the other hand, curcumin alone reduced the expression of the Bax and Caspase- 3 pro-apoptotic genes and increased the expression of the anti-apoptotic Bcl- 2 gene. Training and curcumin interaction was observed to have an additional effect on the expression of these genes. Ageing increases levels of oxidative stress in various organs, including the liver, and it seems that mitochondrial dysfunction plays an important role in this process (22), which is consistent with the study of Mach *et al*. (2015), such that in older rats, Bax and Caspase-3 were observed to tend to increase non-significantly (23). Physical activity is a physiological stress that affects the concentration of cytokines, growth factors and oxidative status. All of these factors are known as potential factors for speeding up cell death or prolonging cell life. Physical activity-dependent apoptotic signals depend heavily on the duration of training, which affects the balance between pro-inflammatory factors and protective factors of the cells (24). Fernandes *et al*. reported similar results (25). Regarding the signaling pathways, there is evidence that physical activity stimulates the intrinsic and extrinsic pathways of apoptosis. After activity, apoptosis is associated with mitochondrial membrane depolarization, cytosolic cytochrome C increase, and a significant reduction in Bcl- 2 protein (26). High-intensity activity affects the balance between the production of free radicals and their elimination by antioxidant defense mechanisms. Reactive oxygen species (ROS) affect certain apoptotic pathways and cause the depolarization of the outer layer of the mitochondrial membrane. This phenomenon can contribute to the mitochondrial membrane instability and the release of cytochrome C. (27). After high physical activity, the production of ROS and nitrogen reactive species in the liver, their byproducts, and their susceptibility to damage due to their production have been reported (28, 29), which is in agreement with the study of Ascensão *et al*. (2013) (30).

Conclusion

Based on the results of this study, HIIT promoted the expression of pro-apoptotic genes and reduced the expression of the anti-apoptotic hepatocyte genes. On the other hand, curcumin modifies these changes and, by inhibiting the expression of pro-apoptotic genes, exerts a protective effect on hepatocytes in the elderly.

Ethical issues

This study was designed and implemented in accordance with the Ethics Guide for Animal Research adopted by the Ministry of Health and Medical Education Policy Planning Board.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

Acknowledgments

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