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Effects of Combined Therapy with Resveratrol, Continuous and Interval Exercises on Apoptosis and Lipid Profile in the Liver Tissue of Rats with Nonalcoholic Fatty Liver Disease

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Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder. In this study, combined therapy with resveratrol supplementation alone or in combination with interval and continuous exercise trainings was considered on apoptosis and lipid profiles in the liver tissue of rats with NAFLD.

Methods: 56 rats were divided into two groups including control and NAFLD. The NAFLD rats were then randomly divided into seven experimental groups including, patient, saline, resveratrol (RSV), continuous exercise (CT), interval exercise (IT), continuous exercise + RSV (CT+RSV), and interval exercise + RSV (IT+RSV). Apoptosis biomarkers, including liver Bax and Bcl- 2 levels, and lipid profiles including High-density lipoprotein (HDL), Low-density lipoprotein (LDL), total cholesterol (Cho) and triglyceride (TG), were measured using specific ELISA kits. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test at a significant level of 0.05.

Results: Resveratrol supplementation alone or in combination with exercise trainings significantly decreased the serum LDL, Cho and TG levels ($p<0.05$), while this combined therapy significantly increased the HDL level ($p<0.05$). RSV alone or in combination with interval and continuous trainings significantly decreased Bax level ($p<0.001$), but significantly increased the Bcl- 2 level ($p<0.001$).

Conclusion: NAFLD is strongly associated with liver cells apoptosis and abnormality in lipid profiles. Although resveratrol alone has an anti-apoptotic and lipid profiles modulating properties, combined therapy with interval and continuous trainings can be more effective.

Keywords: Apoptosis, Non- Alcoholic Fatty Liver, Lipid Profiles, Trainings, Resveratrol

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver illness which increases with age. The disease involves a range of disorders that are associated with fat deposition in hepatocytes and includes steatosis, nonalcoholic steatohepatitis (NASH), fibrosis, and hepatic cirrhosis (1). Given the very central role of the liver in the body and the fact that fatty liver may be associated with severe liver dysfunction, it is important to prevent and find treatment strategies for people with NAFLD. There is currently no definitive treatment for NAFLD. Therefore, it is essential to find new and effective therapies for replacing or helping the existing fatty liver treatments. Recent studies have shown that increased levels of oxidative stress due to overproduction of free radicals and inflammation are mechanisms associated with increased cell death, decreased antioxidant levels, and impaired lipid profile, leading to progression of NAFLD (2). Therefore, oxidative stress control through reducing apoptosis and improving lipid profile can be further studied as a possible therapeutic method. Antioxidants and regular physical activity are important factors that today have attracted many researchers in controlling and treating NAFLD (3). Exercise and some physical activities are among the most effective treatments for some disorders and diseases. Regular physical activity plays an important role in the treatment and prevention of low back pain, arthritis, obesity, heart disease, hypertension, osteoporosis, respiratory disorders, and liver disease, especially NAFLD (4). Studies have also shown that exercise can positively affect the complications of fatty liver, promote the health, and improve physical and mental problems (5). Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is an herbal compound of the polyphenols group, which has many beneficial effects as a potent antioxidant (6). This supplement not only slows the aging process and increase longevity, but also regulates insulin pathway through regulating metabolic pathways and hence prolongs cell life (7). Recent studies have shown that resveratrol controls blood pressure and prevents lipid elevation (8). Since increased levels of oxidative stress and inflammation are currently considered important factors in the progression of NAFLD, it seems that antioxidants along with regular exercise can help in treating and reducing the severity of NAFLD. Given the antioxidative and antiinflammatory role of resveratrol, no study has been performed into the use of this compound along with exercise in patients with NAFLD. In this regard, the present study aimed to investigate the effect of 8 weeks of regular exercise along with resveratrol supplement consumption on apoptotic markers of the liver tissue (including Bax and Bcl- 2 proteins) and lipid profile (including HDL, LDL, triglyceride, and total cholesterol) in the serum of elderly rats with NAFLD.

Methods

In this experimental study, 56 male Wistar rats (40 to 50 weeks old) with an average weight of 250-300 g were selected from the Laboratory Animal Center of Islamic Azad University of Sari. Four rats were kept per cage made of polycarbonate (15×15×30 cm), under controlled weather conditions (22±2 °C, 50±5% humidity, and 12:12 day/night cycle), and standard diet and water. This study was approved by the Animal Ethics Committee of Sari University. At first, the rats were divided into two groups of control (n= 7) and NAFLD (n= 49). Rats in the control group were subjected to standard diet (including 12% fat, 57% carbohydrate, 28% protein, and 3 % other) for 6 weeks, while those in the NAFLD group were subjected to a high-fat diet (22 % fat, 50 % carbohydrate, 24% protein, and 4% other) to induce NAFLD for 6 weeks (Table 1). Subsequently, the rats in the NAFLD group were divided into 7 subgroups (7 rats in each group) including: patient, saline, resveratrol (RSV), continuous exercise (CT), interval exercise (IT), continuous exercise + RSV (CT+RSV), and interval exercise + RSV (IT+RSV). Before beginning the main exercise, the rats in the exercise groups were get familiar with the treadmill; to this end, they walked on a zero-degree slope at a rate of 8-10 m/min for 5 minutes in 5 sessions in a week. The main exercise program was performed for 8 weeks. In the continuous exercise group, the rats ran at a rate of 15 m/min in the starting week for 5 min; the exercise rate and duration increased 1- 2 m/min and 1- 2 min per week, respectively, so that the rate reached 20 m/min and the duration 60 min in the fourth week. The exercise was performed 5 sessions a week (9, 10). Resveratrol was prepared based on our previous study (11). For each administration of resveratrol, a 100 μL solution of 7 % ethanol or 10 % DMSO with water was prepared for each rat, and resveratrol was suspended in the solution and administered. To reduce the error rate for all subjects, the solution was prepared simultaneously and injected intraperitoneally 20 mg/kg body weight in the supplement and supplement plus exercise groups. This process was performed for 8 weeks. At the end of the study, all animals were anesthetized after 12-14 hours fasting and 48 hours after the last exercise. The liver tissue and blood samples were taken and stored at -80 C for further analysis. To determine the apoptosis rate, the levels of Bax and Bcl- 2 proteins in liver tissues of all animals were measured using the ELISA specific commercial kits based on the manufacturer's instructions (ZellBio, Germany). The results were presented in nanograms per gram of tissue. The levels of lipid profile, including HDL, LDL, Cho, and TG, were measured in serum samples of all rats using specific kits (Rat HDL: R910100112; Rat LDL: R910100123; Rat Cho: R910600110; Rat TG: R910480132) purchased from Pars Azmoon Company. The results were expressed in mg/dL. The quantitative data were described using central dispersion indices such as mean and standard deviation. The Shapiro-Wilk test and the Levin's test were used to determine the normal distribution of data and the consistency of the variances, respectively. In addition, the significant difference among different groups was evaluated through one-way analysis of variance, and if a significant difference was observed, the intergroup difference was determined through Tukey's post hoc test in ANOVA. The significance level for all calculations was considered $p<0.05$. All statistical operations were performed with SPSS 20.

Results

The mean and standard deviation of the apoptosis indices are shown in Table 2. According to the results of intra group differences, the highest mean level of Bax, compared to other groups, was observed in the patient group (18.09±3.19 ng/mg protein) and the saline group (18.59±2.34 ng/mg protein) ($p<0.0001$), whereas the level of Bcl- 2 in the patient group (1.13±.0.37 ng/mg protein) and the saline group (1.2±0.34 ng/mg protein) were significantly lower than that of other groups ($p<0.001$). Then, mean level of Bax was significantly reduced and the mean level of Bcl- 2 was significantly increased in the liver tissue of rats in the RSValong with CT, IT groups compared to the patient and saline groups (both $p<0.0001$) (Table 2). Based on the results of tukey's post hoc test, there was no significant difference in the mean levels of Bax and Bcl- 2 between RSV, CT, IT, CT+RSV and IT+RSV. According to the results, a regular interval or continuous exercise plus resveratrol resulted in the highest decrease in the Bax level and the highest increase in the Bcl- 2 level in elderly rats with NAFLD. The mean and standard deviation of lipid profile are shown in Table 3. According to the results of intra group differences, the patient and saline groups had the highest mean serum levels of LDL, total cholesterol, and triglyceride ($p<0.0001$), while the serum levels of HDL in the patient group (23.24±5.41 mg/dL) and the saline group (22.98±5.5 mg/dL) were significantly lower than other groups ($p<0.001$). Administration of resveratrol along with interval or continuous exercise significantly decreased serum levels of LDL, cholesterol, and triglyceride and significantly increased the serum levels of HDL compared to the patient and saline groups (both $p<0.0001$). Based on the results of tukey's post hoc test T, there was no significant difference in the mean levels of LDL, cholesterol, and triglyceride between RSV, CT and IT groups. There was no significant difference in the mean serum levels of HDL between RSC, IT, CT+RSV and IT+RSV groups. According to the results, a regular interval or continuous exercise plus resveratrol led to the highest decline in the levels of LDL, triglyceride, and cholesterol and to the highest increase in the levels of HDL in elderly rats with NAFLD (Table 3).

Table 1. Component of high-fat and standard diet

Component / Diet	Fat (%)	Carbohydrate (%)	Protein (%)	Other (%)*
Standard Diet	12	57	28	3
High-fat Diet	22	50	24	4

* Vitamins and Minerals

Table 2. The variation of apoptosis biomarkers in different groups(M±SD)

Group	Bcl- 2(ng/pgtissue)	Bax(ng/pg tissue)
Control	0.59 ± 2.26	1.51 ± 8.11
patient	0.37 ± 1.13	3.19 ± 18.09
saline	0.34 ± 1.2	2.43 ± 18.59
RSV	0.29 ±1.93	2.68± 11.79
CT	0.37 ± 1.79	2.08± 12.57
IT	0.25 ± 1.88	3.05 ± 12.11
CT+ RSV	0.46 ± 2.01	2.03 ± 10.41
IT +RSV	0.59 ± 2.13	2.56 ± 9.96
P value	<0.001	<0.001

Table3. The variation of lipid profile in different groups (M±SD)

Variable / Group	TG (mg/dl)	Cho (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	17.6 ± 104.64	10.25 ± 81.91	5.76 ±36.54	5.27 ± 23.64
patient	20.02 ± 228.18	10.8 ± 124.67	5.41 ± 23.24	8.94 ± 48
saline	18.94 ± 227.42	13.23 ± 125.38	5.5 ± 22.98	9.32 ± 50.7
RSV	25.7 ±149.9	14.03 ± 98.98	4.98 ± 31.15	7.01 ± 34.24
CT	20.87 ± 164.92	21.11 ± 96.94	5.48 ± 28.52	4.91 ± 39
IT	19.24 ± 156.07	15.29 ± 94.18	6.04 ± 30	6.21 ± 37
CT + RSV	15.09 ± 140.61	12.55 ± 90.34	5.58 ± 33.32	7.57 ± 31.31
IT + RSV	13.02 ± 133.92	13.18 ± 87.94	7.58 ± 34.57	6.93 ± 29.57
P value	<0.001	<0.001	<0.001	<0.001

Discussion

In this study, the effect of resveratrol alone or along with interval and continuous exercise on apoptotic biomarkers of liver tissue and serum lipid profile was investigated in NAFLD rats. Our results showed that the mean levels of Bax and Bcl- 2 were significantly increased and reduced respectively in the liver tissue of NAFLD rats compared to the control group. Previous studies have shown that an increasing ratio of Bax to Bcl- 2 is associated with an increase in cell apoptosis (12), while a decreasing ratio of Bax to Bcl- 2 reduces apoptosis through inhibiting cytochrome C (13). Therefore, increasing the Bax level and decreasing the Bcl- 2 level in the liver tissue of NAFLD patients is indicative of an increase in the rate of cell apoptosis. The results of this study indicated that hepatocytes in NAFLD rats suffer from severe apoptosis. In addition, in this study, there was a significant increase in the mean serum levels of LDL, TG, and cholesterol in NAFLD rats compared to control group, while serum levels of HDL decreased significantly. The results of this research are comparable to previous studies in which the rate of hepatocyte apoptosis and the levels of TG, cholesterol, LDL, and VLDL were elevated and the levels of HDL reduced in patients with NAFLD (14, 15). Although extensive studies have shown the increased rate of hepatocyte apoptosis and impairment of lipid profile in patients with NAFLD, the actual mechanism of these abnormalities is still not well known. It seems that increased oxidative stress caused by free radicals and inflammation is one of the main mechanisms involved in the pathogenesis of NAFLD. Recently, some studies have shown the increased levels of oxidative stress caused by free radicals and decreased levels of enzymatic and non-enzymatic antioxidants in the liver tissue of patients with NAFLD (16). For example, in a recent study, we showed that liver tissue in NAFLD rats has significant structural damage, such as increased inflammation and oxidative stress (11). In addition, a significant decrease was observed in our previous study in the mean activity of antioxidant enzymes such as catalase and superoxide dismutase in the liver of rats with NAFLD (11). Edin *et al.* (2014) showed a significant decrease in the activity of antioxidative and non-antioxidative enzymes such as superoxide dismutase (MDA), glutathione peroxidase(GPX), glutathione reductase(GR), and reduced glutathione (GSH) in the liver of rats with NAFLD (17). Ding *et al.* (2016) observed a significant increase in the levels of MDA (lipid peroxidation biomarker) and Reactive oxygen species (ROS) in the liver tissue of rats with NAFLD, while the activity of superoxide dismutase (SOD) was significantly reduced (18). In a recent study by Haji Qassim *et al.* (2018), there was a significant increase in Tumor Necrosis Factor-α (TNF-α) levels in the liver of rats with NAFLD, while the level of Interleukin-10 (IL-10) was significantly decreased (11). Inflammation in rats with NAFLD may arise from the decreased levels of IL-10 and increased levels of TNF-α in their liver because IL-10 has an anti-inflammatory role and TNF-α plays an important role in inflammation. Similarly, Edin *et al.* (2014) observed an increase in the levels of inflammatory mediators such as TNF-α and Tumor Necrosis Factor- β (TNF- β) in the liver of rats with NAFLD, which was associated with increased infiltration of inflammatory cells and a significant increase in serum levels of liver enzymes, leptin, cholesterol, triglyceride, and MDA (17). Therefore, these results indicated that oxidative stress induced by ROS and inflammation is the main cause of NAFLD pathogenesis, which can ultimately lead to apoptosis and death of hepatic cells. According to the results of the present and previous studies, the use of antioxidants or drugs that protect liver cells against oxidative stress can reduce the disease severity and apoptosis of hepatocytes. In this regard, the effects of resveratrol alone or in combination with continuous exercise on the severity of NAFLD was investigated in the present research. The results of this study showed that liver damage in resveratrol receiving rats was significantly decreased in comparison with the patient group. This effect was associated with a significant increase in the serum levels of HDL and a significant reduction in the serum levels of LDL, TG, and total cholesterol. On the other hand, the level of Bax protein in the liver tissue decreased significantly, while resveratrol along with exercise significantly increased the Bcl- 2 level. Although resveratrol or exercise alone improved lipid profile and decreased apoptosis, resveratrol along with interval or continuous exercise had a stronger therapeutic effect. Several studies have been performed regarding the efficacy of resveratrol so far. For example, we studied the effect of resveratrol alone and along with exercise on the number of apoptotic hepatocytes and oxidative and inflammatory stress markers in our previous research (11). The results showed that resveratrol, especially in combination with interval and continuous exercise, was associated with a significant increase in the activity of SOD and Catalase

(CAT) enzymes as well as IL-10 levels, while the level of TNF-α, the rate of lipid peroxidation, and the number of apoptotic hepatocytes decreased significantly (11). In a recent study, Wang *et al.* (2018) showed that resveratrol reduces inflammation and oxidative stress through decreasing the production of ROS and expression of inflammatory cytokines, as well as increasing the levels of anti-inflammatory mediators and antioxidants (19). In addition, Palacs-Verubel *et al.* (2017) showed that resveratrol increases the expression of IL-10 gene, while decreasing the expression of TNF-α gene (20). Zhang *et al.* (2017) showed that resveratrol increases the expression of anti-inflammatory cytokines such as IL-10, while it significantly reduces the transcription of pro-inflammatory cytokines (21). The increased expression of anti-inflammatory cytokines, such as Interleukin- 4 (IL- 4), was also reported by several studies (22). The protective effect of resveratrol on other inflammatory mediators such as Interleukin- 6 (IL-6), Interleukin- 1 (IL-1), Interleukin-1 β (IL-1 β), Interferon gamma (IFNγ), Interleukin- 5 (IL- 5), Interleukin- 33 (IL- 33) (23), nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and matrix metalloproteinases (MMPs) (24) was also reported in previous studies. Many studies have also shown that resveratrol protects hepatic cells through its high antioxidative properties (25). Elbe *et al.* (2017) indicated that resveratrol reduces the acetaminophen-induced hepatotoxicity through reducing MDA, iNOS activity, and increasing the content of SOD, CAT, and GSH in the liver tissue (26). Several studies have already examined the effects of resveratrol along with a variety of exercises on improving liver function in patients with NAFLD. For example, in a clinical trial, Faghihzadeh *et al.* (2014) showed that resveratrol (500 mg per day for 12 weeks) along with physical activity significantly reduced the levels of alanine aminotransferase (ALT), inflammatory cytokines, nuclear factor-κB activity (NFKB), serum levels of cytokine- 18 (IL- 18), and hepatic cirrhosis (27). In another study, Tang *et al.* (2015) showed that the combination of resveratrol and exercise increased the activity of several antioxidants including SOD, CAT, GPX, GR, glutathione-s-transferase (GSTs), thyroxine reductase, NADH cytochrome B5-reductase, and NAD (P) H-quinone oxidoreductase receptor (28). Joong *et al.* (2015) showed that moderate and mild exercise can inhibit macrophage permeation, while resveratrol alone cannot reduce macrophage permeation and activation (29). Another study showed that combination therapy with resveratrol and exercise had anti-aging properties and can increase the activity of GSH, GPX and GSTs in elderly animals (30). Therefore, according to the results of this research and previous studies, we suggest that treatment with resveratrol along with exercise, especially interval exercise, can improve NAFLD probably through reducing oxidative stress caused by increased free radicals, improving antioxidants, and decreasing inflammation and apoptosis.

Conclusion

The findings of this study showed that NAFLD is associated with increased apoptosis of hepatic cells and impairment of lipid profile. Although resveratrol can be used as a compound with high antioxidative and anti-inflammatory properties in NAFLD patients, resveratrol along with interval or continuous exercise can improve lipid profile and NAFLD possibly through reducing oxidative stress, inflammation, and the number of apoptotic cells.

Ethical issues

Not applicable.

Authors' contributions

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

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