Effects of Endurance and Resistance Training and Garlic Supplementation on Cardiac Function, Cardiovascular Risk Factors and Apoptosis Indices in Rats With Metabolic Syndrome

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Abstract

Background: The purpose of this study was to investigate the effects of 8 weeks endurance and resistance training with and without garlic extract supplementation and garlic extract supplementation alone on cardiac function, cardiovascular risk factors and apoptosis indices in rats with metabolic syndrome.

Methods: A total of 48 rats with metabolic syndrome (aged 12-wk, weight range of 331.9±13.7g) were randomly divided into 6 groups of 8 each: endurance training (ET), resistance training (RT) garlic extract supplementation (GE), endurance training with garlic extract supplementation (ET+GE), resistance training with garlic extract supplementation (RT+GE) and metabolic syndrome-control (MetS-Con). Endurance and resistance training were performed 3 times per week and consisted of running on the treadmill or a rat climbing ladder with weights fastened to the tail. The supplementation consisted of 500 mg.kg-1 .day-1 of garlic extract.

Results: Results showed that BCL2-associated X protein (Bax), B-cell lymphoma 2 (Bcl2) and Bax/Bcl2 ratio and insulin resistance (IR) were significantly lower in all intervention groups compared to the MetS-Con group. And except the RT+GE group, high-sensitivity C-reactive protein (hs-CRP) and glucose were also significantly lower in all groups compared to the MetSCon group. Moreover, homocysteine (Hcy) were significantly lower in ET, ET+GE, RT+GE groups.

Conclusion: In conclusion, endurance and resistance training with or without garlic extract supplementation and the supplementation alone could improve cardiac hemodynamic, inflammation and apoptosis indices in rats with metabolic syndrome. In this regard, it would appear that the combination of garlic supplementation with endurance exercise is more helpful than the resistance exercise.

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1. Introduction

Metabolic syndrome (MetS) is a collection of cardiometabolic risk factors that includes obesity, insulin resistance (IR), hypertension, and dyslipidemia, with high socioeconomic costs [1, 2]. The risk of cardiovascular disease (CVD) increases in metabolic diseases which have a high prevalence in modern societies such as obesity, metabolic syndrome (MS), and/or type-2 diabetes mellitus [3]. Inflammation plays an important role in the development of insulin resistance (IR) [4]. There is evidence that high concentrations of high-sensitivity C-reactive protein (hs-CRP), a proinflammatory cytokine is associated with IR and MetS and may predict the onset of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD), independent of other traditional risk factors [5]. According to reports of many researchers adipose tissue in obese people, which rises free fatty acids (FFA) levels, exacerbate insulin resistance in muscle and alter hepatic glucose metabolism. On the other hand, it induces disorder in the production of adipokines which may separately affect IR and CVD [6]. Insulin resistance as the background of metabolic syndrome, is the reduction of response to insulin effects, for incitement of glucose utilization and inhibiting glucose production from the liver. 6 In addition, IR leads to reducing biological activity and increasing production of nitric oxide and endothelin-1 secretion. Reduction of the nitric oxide and oxidative stress (OS) are associated with increase of myocardial apoptosis [7]. In addition, recent research suggests a link between metabolic syndrome and myocardial apoptosis [8]. The rate cardiac muscle apoptosis is very rare at about 0.001–0.002%, can be increase internal or external factors such as irradiation, ischemia / reperfusion, various drugs, aging and physical stress (mechanical-metabolic) and provide. In this way, the background incidence of cardiovascular diseases [9]. Molecular events in apoptosis mainly due to the balance between anti-apoptotic proteins. Proteins such as Bax mitochondrial protein are involved in the formation of apoptosis and apoptosis messages [10, 11].Therefore, sports-medicine researchers are constantly seeking ways to prevent the occurrence of adverse consequences of metabolic syndrome. Physical activity and a balanced diet is a way to prevent diseases and is essential for maintaining health and improve the quality of life [12]. Available evidence suggests that using a variety of short-term and long-term supplementation and increased regular physical activity can prevent metabolic syndrome and its adverse consequences (cardiovascular disease) [12]. Recently, it has become common to use natural supplements such as garlic, because of its antioxidant compounds and its lack of toxicity [13].Garlic components include cycloolefin, S-allyl-L-cysteine, S-methyl-L-cysteine, S-acetylcysteine, S-1- proponyl-L-cysteine, S-alkyl mercapto-L-cysteine, fructose-arginine, L-arginine, L-cysteine, and L-methionine, beta-chlorogenic and can influence immune functions, cytokine release, NK (Natural Killer) cells activity and phagocytosis [13]. According to studies, there is a link between hs-CRP level and components of MetS and IR and as well as hs-CRP and IR with apoptosis in rats with MetS [6, 8]. The present study was approached with the idea of determining the effects of endurance training (ET), resistance training (RT) and garlic extract (GE) on reducing the percentage of cardiovascular risk factors induced by MetS. Thus, the main purpose of the present study was to assess and compare the effects of eight weeks ET and RT with and without GE supplementation on hemodynamic activities of the heart, Bax and Bcl2 genes expression, hs-CRP, Hcy, TAC and IR in rats with metabolic syndrome.

2. Methods

2.1.

Animals Forty-eight male Wistar rats (Rattus Norvegicus) were housed in the laboratory of Animal Physiology (Faculty of natural Sciences, University of Razi Kermanshah) at pathogen-free conditions at 22 ± 2°C, with a relative humidity of 50 ± 10%. These rats were exposed to a reverse light condition of 12 hours of light and 12 hours of darkness each day and were fed rat chow and water ad libitum throughout the study period.

2.2. Experimental design

All rats (n = 48, aged 8 weeks, weight; 160.20 ± 5.08g, Lee index; 291.01 ± 4.31) were acclimated to their new environments for 7 days. Forty-eight rats received high-calorie diet (HCD; n = 48) for 4 weeks, after a four-week highcalorie diet (HCD), rats' Lee index and weight were calculated. Then, a total of 48 rats were fed with HCD (aged; 12 weeks, weight; 331.94 ± 13.67g, Lee index; 324.33 ± 1.02) and were randomly divided into 6 groups of 8 each: 1) Metabolic syndromecontrol (MetS-Con), 2) Endurance training (ET), 3) Resistance training (RT), 4) Garlic extract (GE), 5) Endurance training + garlic extract (ET + GE), 6) Resistance training + garlic extract (RT + GE). 1) MetS-Con group, only received HCD for 8 weeks. 2) ET group performed 8 weeks of running training on a motorized treadmill. 3) RT group performed 8 weeks of resistance training consisting of a rat climbing ladder with weights fastened to the tail. 4) GE group received 500 mg.kg-1 .day-1 of garlic extract for 8 weeks. 5) ET + GE group performed 8 weeks of running training on a motorized treadmill + GE supplementation 500 mg.kg-1 .day-1 of garlic extract for 8 weeks. 6) RT + GE group performed 8 weeks of resistance training on climbing ladder with weights fastened to the tail + GE supplementation 500 mg.kg-1 .day-1 of garlic extract for 8 weeks. All exercise groups with and without GE supplementation received HCD for a long time. Rats received an HCD diet ad libitum during the 8-week intervention period. Wistar rats fed with an HCD diet generally develop metabolic syndrome, which shares many features with human metabolic syndrome. Twenty-four-hour food and water intakes for all animal groups were monitored weekly throughout the intervention period. The average 24 h food and water intake per week was noted. Energy intake (kJ/g) was calculated for the food or water consumed. Composition of 1000 g HCD diet: 150 g farina + 150 g sugar + 200 g Beef tallow + 10 g cholesterol + 8 g cholic acid + 482 g standard diet rat [14]. The amount of food consumed in each animal was weighed three times a week.

2.3. Body composition

The anthropometric data (body weight and naso-anal length) were measured weekly using an analytical balance. Body mass index (Lee index or BMI) was estimated once a month by the formula: Lee index = body mass (g) / (nasal-anal length (cm))2] [15], Lee index value higher than 310 g/cm was classified as obese.

2.4. Endurance training program

All animals were familiarized with walking on a motor-driven treadmill (10–15 m min-1, 5–10 min d-1) daily for seven days. The endurance training consisted of running on the treadmill 3 times a week (3 d wk-1) for the 8-week training period (Table 1). Electrical shocks were rarely used to motivate the animals to run. Since handling and placing the animals on a treadmill may cause non-exercise stress, sedentary control animals were also placed on the treadmill once a week to familiarize them with handling and the treadmill environment [16].

2.5. Resistance training program

Resistance training was done with the use of a 1 m high ladder inclined at 80°. There were 26 rungs evenly spaced on the ladder. The rats in the trained group were acquainted with the exercise by practicing climbing from the ladder. The rats were placed at the bottom of the climbing apparatus and were motivated to climb the ladder by touching and grooming their tail. We used electrical stimulation, forced air, food restriction/reward, and cold water to encourage the rats to perform the training and to minimize the stress. The rats rested when they reached the top of the ladder. The rats in the trained group undertook one training session per day, 3 days/week, for 8 weeks. Warming-up and cooling down consisted of 2 repetitions of climbing the ladder without weights appended to the tail, immediately pre and post each training session [17] (Table 2).

2.6. Supplementation

Garlic extract was prepared with white garlic planted in the farms of Hamedan (Iran). After harvesting, natural garlic was dried in the shade and was used after 3-month storage in the storehouse. To prepare the garlic extract, The Garlic cloves were peeled, were kept overnight in the freezer, were chopped in a blender with distilled water, and several times was passed through sterile clear cloth and the Whatman Filter. The solution obtained was centrifuged around 5,000 rpm (30 minutes/in refrigerated centrifuges) and the supernatant fluid was passed through a sterile 0·2-µm filter. Then, garlic composition was determined by of the Gas Chromatography Mass Spectrometry extract (GCMS). Garlic extract (500 mg.kg-1 .day-1) was administered orally by gavage twice a day until 8 weeks, every 8 hours (10:00 a.m and 6:00 p.m) per serving 250 mg.kg-1 .day-1).

2.7. Myocardial hemodynamic parameters

Myocardial hemodynamic parameters were recorded and monitored using a latex balloon-tipped catheter inserted through an incision in the left atrium and advanced through the mitral valve into the left ventricle and connected to a pressure transducer and a recording system (Powerlab systems, Australia). The balloon was inflated and equilibrated to give an end-diastolic pressure of 5–10 mmHg. Left ventricular systolic and diastolic pressures and time derivatives of pressures were measured during ventricular contraction (+ dP/dt) and relaxation (-dP/dt). Left ventricular developed pressure (LVDP) was calculated as the difference between the systolic and the diastolic pressures. The work index of the heart (LVDP×HR) was derived from the product of LVDP and heart rate (HR). Coronary flow (CF) rate was measured by collecting the effluent drained through the isolated heart.

2.8. Chemical analysis

Determination of circulating levels of hs-CRP, Hcy, TAC, insulin and glucose performed after 8 weeks in same conditions. All groups were anesthetized with Ketamine (90 mg/kg, Intraperitoneally (IP)) and Xylazine (10 mg/kg, IP) and were sacrificed after 12–14 hours overnight fasting and 48 hours after the last session of training and supplementation. Blood samples were taken from orbital sinus. Then coagulated samples were centrifuged for biochemical analysis. Serums were separated and thereafter values of hs-CRP, Hcy, TAC and IR were measured. Then, the rat hearts were removed. Ribonucleic acid (RNA) extraction and synthesis of complementary DNA (cDNA) was conducted, and Bcl-2 and Bax gene expression was analyzed through the Real Time-Polymerase Chain Reaction (RT-PCR). The serum hs-CRP concentration were determined by Enhanced Immunoturbidimetric assay latex-particle on a Hitachi 912 automated analyzer using reagents from Diasorin (Stillwater, MN). Total homocysteine concentration was measured by the aid of ELISA (Sandwich immunoassay technique) using commercial kit (CUSABIO, China). The ferric reduction ability of plasma (FRAP) was used as a measure of total antioxidant capacity (TAC). Rat insulin assay determinations were done by rat insulin ELISA Kit (Germany DRG-Diagnostica, GmbH). Blood glucose was determined by the glucose oxidase method, using a Spectrophotometer. The HOMA index of resistance to insulin was calculated. HOMA-IR: (Glucose × Insulin) / 405 [18].

2.9. Tissue removal

All animals were anesthetized with ketamine (90 mg/kg, Intraperitoneally (IP)) and Xylazine (10 mg/kg, IP) and killed 48 hours after the last training session and supplementation. Animals MetsCon group were killed at the same time as their partner trained animals. The cardiac tissues were carefully removed and rinsed in ice-cold physiological saline solution. The left ventricle was removed and frozen immediately in liquid nitrogen and stored at -70°C until further analysis.

2.9.1. Isolation of total RNA

Fifty milligrams of the left ventricle tissue were homogenized in the presence of 1 mL of AccuZol® (Bioneer, South Korea). After adding 0.2 mL chloroform, vigorous shaking and incubation on ice was applied for five minutes. The samples were centrifuged at 13700 g for 15 minutes at 4°C. Next, the clear upper phase containing the RNA was transferred to a new tube. An equal volume of cold isopropyl alcohol was added and after inverting the tube four to five times, samples were incubated at 20°C for 10 minutes before being centrifuged at 13700 g for 10 minutes at 4°C. The supernatant was removed and the pellet was washed in 1 ml of 80% cold ethanol followed by centrifugation at 13700 g for five minutes at 4°C. After removing the supernatant, the pellet was air-dried and re-dissolved in Diethylpyrocarbonate (DEPC)-treated water. Total RNA concentration and purity of the samples were determined by a Bio-Rad spectrophotometer (Bio-Rad, CA, USA). RNA integrity was verified by ethidium bromide staining of 28S and 18S ribosomal RNA bands on 1% agarose gel. After treating RNA with DNAse, the extracted RNA was stored at -70°C for further use.

2.9.2. cDNA synthesis

For this, 1 µL of random hexamer primer (2 µg µL-1) (Fermentas) was added to 2 µL of extracted RNA (100 ng µL-1) and a total volume of 10 µL was obtained with the addition of DEPC-water. The mixture was incubated at 65°C for five minutes and chilled on ice. Next, 4 µL of 5 × reaction buffer, 2 µL 10 mM dNTP mix, 1 µL Ribo Lock TM RNase inhibitor (Fermentas) and 1 µL of M-MuLV reverse transcriptase were added and then followed by incubation at 25°C for five minutes, at 42°C for 50 minutes and finally 72°C for five minutes. Finally, a 10-µL volume of the cDNA preparation was diluted to increase the total volume of the solution to 100 µL and stored at -20°C.

2.9.3. Real-time polymerase chain reaction and gene expression analysis

Real-time PCR was carried out using SYBR® Premix Ex TaqTM II (TAKARA) following the manufacturer's instructions. The mixture comprised of 10 µL SYBR green mix, 1.2 µL of cDNA (equivalent to 1.0 ng of total RNA with initial concentration of 100 ng µL-1), 0.4 µL PCR forward primers and 0.4 µL PCR reverse primer in 10 pmol µL-1, and millipore water was added to achieve a final volume of 20 µL. The Threshold Cycle (CT) was determined manually for each run. The PCR efficiencies for each set of primers were determined using serial 10-fold dilutions of cDNA and resulting plots of CT vs. the logarithmic cDNA dilution, using the efficiency equation (E): E = 10 (-1/slope). Melting curve analysis was performed for one cycle at 95°C for five seconds, 67°C for 25 seconds, and 99°C for zero second with a ramp rate of 0.1°Cs-1 and 55°C for 30 seconds. The quantification of mRNA was performed as a value relative to an internal reference for β-actin. Gene expression of the samples compared to the controls was calculated according to the following equation, using the REST© software 2009.

$$\text{Ratio} = \frac{(\text{E}_{\text{target}})^{\Delta c}\text{T target(control} - \text{sample)}}{(\text{E}_{\beta-\text{actin}})^{\Delta c}\text{T target(control} - \text{sample)}}$$

2.10. Statistical analysis

All data were presented as mean ± standard deviations. Normality of data was checked by shapiro-Wilk test. Then the nonparametric independent-samples Kruskal-Wallis test with pairwise comparison was performed to assess differences between the groups. cardiac action potential parameters

were analyzed using separate 1 way analyses of variance with Bonferroni post hoc comparisons. All statistical analyses were performed using SPSS statistical software version 22. $P < 0.05$ was considered as statistically significant for all analyses.

3. Results

3.1. Anthropometric status

There were no differences among groups at the beginning of this study for age, Lee index, and weight. The subjects' characteristics and anthropometric data are summarized in Table 3. During the 8 week study, all the rats of the 6 groups participated in one phase of blood sampling.

3.2. Metabolic syndrome indexs in Con-b and MetS-Con groups in the twelfth week

Rats of Con-b group were fed with a normal diet (ND). But, rats of MetS-Con group were fed with a HCD. After 12 weeks HCD, weight (t= -22.57), Lee (t= -28.74), fasting plasma glucose (t= -6.07), TC (t= -5.11), TG (t= -2.57) and LDL-C (t= -4.49) were significantly higher in MetSCon group compared to Con-b group significantly ($p < 0.05$) (Table 4).

3.3. Myocardial hemodynamic parameters

Table 5 presents SBP and HR of the experimental groups at 8 week of intervention. No significant main effects or interactions were observed for SBP, DBP, CF or HR throughout the intervention period. Table 5 presents cardiac function of the experimental groups after 8 weeks of the intervention. The ET + GE and RT + GE group's Left ventricular developed pressure (LVDP) was significantly higher compared with the MetS-Con group ($p = 0.042$ and $p = 0.036$), and the ET + GE group Ventricular Contractility Assessment (dP/dt) and cardiac work index (HR×LVDP) were significantly higher compared with the MetS-Con group ($p = 0.013$). No significant between-group differences were observed in between the intervention groups with each other.

3.4. hs-CRP concentrations

For the hs-CRP levels, the Kruskal-Wallis test showed a significant difference between groups ($p = 0.000$). The pairwise comparison showed significantly lower levels of hs-CRP after the intervention for the RT, GE, and ET + GE groups ($p = 0.000$) as well as for the ET group ($p = 0.007$) compared to the Mets-Con group. The level of hs-CRP in the RT + GE group was also considerably lower than the Mets-Con group; however, this difference was not statistically significant ($p = 0.071$). In addition, the level of hs-CRP in the RT + GE group was significantly higher than the GE ($p = 0.007$) and the ET + GE ($p = 0.035$) groups (Table 6).

3.5. Homocysteine (Hcy)

For the Hcy levels, the Kruskal-Wallis test showed a significant difference between groups ($p = 0.007$). The pairwise comparison showed significantly lower levels of Hcy after the intervention for the RT ($p = 0.05$), GE ($p = 0.007$), and RT + GE groups ($p = 0.001$) compared to the Mets-Con group. But, was not significantly different between the intervention groups with each other (Table 6).

3.6. Total antioxidant capacity (TAC)

For the TAC levels, the Kruskal-Wallis test showed a significant difference between group ($p = 0.002$). The pairwise comparison showed significantly higher levels of TAC after the intervention for the RT ($p = 0.03$), GE ($p = 0.001$), and RT + GE groups ($p = 0.001$) compared to the Mets-Con group. In addition, the level of TAC in the RT + GE group were significantly higher than the RT ($p = 0.002$) and the GE ($p = 0.005$) groups. However, the study failed to find a significant difference among intervention groups (Table 6).

3.7. Insulin resistance

For the variable of the insulin resistance, the Kruskal-Wallis test showed a significant difference between groups ($p = 0.001$). The pairwise comparison showed significantly lower mean of insulin resistance after the intervention for the ET ($p = 0.012$), RT ($p = 0.001$), GE ($p = 0.031$), ET + GE ($p = 0.000$) and RT + GE ($p = 0.006$) groups compared to the MetS-Con group. Mean of the insulin resistance in the ET + GE group was significantly lower compared to ET ($p = 0.031$) and GE ($p = 0.012$) groups. In addition, the mean of the insulin resistance in the ET + GE group was also considerably lower compared to the RT + GE group; however, this difference was not statistically significant ($p = 0.054$). The pairwise comparison showed that the glucose level was significantly lower in the ET ($p = 0.012$), RT ($p = 0.002$), GE ($p = 0.008$) and ET + GE ($p = 0.001$) groups compared to the MetS-Con group. Insulin level was significantly lower in the ET ($p = 0.024$), RT ($p = 0.001$), GE ($p = 0.053$), ET + GE ($p = 0.000$) and RT + GE ($p = 0.003$) groups that the MetS-Con group. as well as, insulin level in the ET + GE group was lower than ET and GE groups ($p = 0.023$, $p = 0.010$) (Table 6).

3.8. Bax gene expression

For the Bax gene expression, Bcl2 gene expression and Bax/Bcl2 ratio, the Kruskal-Wallis test showed a significant difference between group ($p = 0.000$). The pairwise comparison showed significantly lower levels Bax gene expression, Bcl2 gene expression and Bax/Bcl2 ratio after the intervention for the ET ($p = 0.004$), RT ($p = 0.013$), GE ($p = 0.000$), ET + GE ($p = 0.000$) and RT + GE ($p = 0.000$) groups compared to the Mets-Con group. The levels of Bax gene expression, Bcl2 gene expression in the GE group was also considerably lower than the exercise groups with and

without garlic extract supplementation; however, this difference was not statistically significant. In addition, the levels of Bax gene expression, Bcl2 gene expression in the RT group was also considerably lower compared to the ET group; however, this difference was not statistically significant (p = 0.675). Comparison of Bax gene expression, Bcl2 gene expression and Bax/Bcl2 ratio in the ET + GE and RT + GE groups showed that they were not significant difference in both groups (p = 0.844); although, Bax gene expression, Bcl2 gene expression and Bax/Bcl2 ratio were lower in the ET + GE group (Table 6).

4. Discussion

Various prospective studies have demonstrated that hs-CRP and IR independently predict future cardiovascular risk and development of T2DM, with additive prognostic information beyond that available from the Framingham risk score [5, 19, 20] Insulin resistance and inflammation (hs-CRP) are also known as two parameters tightly associated with cardiac apoptosis [19, 20]. We found that after 8 weeks of endurance training (10–22 m min-1, 10–60 min d-1) and resistance training (30–100 0 /0 body weight) programs 3 times per week with and without garlic extract supplementation (500 mg.kg-1 .day-1), hs-CRP concentration was significantly lower in ET (-42 0 /0), RT (-51 0 /0), GE (-60 0 /0) and ET + GE (-54 0 /0) groups compared to the MetS-Con group, nevertheless, the RT + GE did not have a significant effect on the hs-CRP level. The results of this study indicated that mean of the insulin resistance after 8 weeks exercise and supplementation interventions, in groups ET -75 0 /0, RT -77 0 /0, GE -73 0 /0, ET + GE -90 0 /0, RT + GE -73 0 /0 was lower compared to the MetS-Con group. Fasting glucose level in all interventions except for the combination of resistance exercise with the garlic supplementation have the relatively samepositive effect. Bax gene expression in the groups of the ET -73 0 /0, RT -74.5 0 /0, GE -78 0 /0, ET + GE -77 0 /0, RT + GE -73 0 /0 were lower compared to the MetS-Con group. And Bcl-2 gene expression in the groups of the ET + 42 0 /0, RT + 47.5 0 /0, GE + 41.5 0 /0, ET + GE + 72 0 /0, RT + GE + 69 0 /0 were higher compared to the MetS-Con group. We found that hs-CRP level in the ET and RT groups significantly decreased after 8 weeks of training. Thus, adaptation after the last of six weeks of training probably can be related to significant changes in the hs-CRP level. Davis et al. [21] and King et al.[22] confirmed the effect of intensity and duration of exercise on inflammatory markers. However, enhanced NK cell activity may confer a resistance to acute inflammation in individuals. High levels of exercise and physical fitness are associated with improved insulin resistance, lower levels of body fat and low oxidized density lipoprotein cholesterol (LDL-C(. These factors may be non-infectious triggers for elevated hs-CRP [23]. Higher physical fitness levels is related to an anti-inflammatory effect that may be a mechanism for lowering coronary heart disease (CHD) and metabolic syndrome risks [23]. In contrast, it was demonstrated that resistance training cannot affect the hs-CRP [24]. Moreover, research findings has shown that high-intensity eccentric training, can stimulate acute phase response (APS) and hs-CRP [24]. In addition, hs-CRP level in the ET + GE group was lower compared to the RT + GE group. Furthermore, hs-CRP level in the RT + GE group was higher compared to RT group. It could probably cause higher levels of glucose in the RT + GE group. Reports have shown that high glucose levels can increase levels of key proteins (hsCRP) that has links to inflammation. Interestingly, the results showed that garlic extract supplementation improved hs-CRP level significantly. These results are almost similar to those of a study by Aalami-Harandi et al. [25] that reported supplementation of garlic for nine weeks led to decreased hs-CRP. Mechanism of probable reduction of hs-CRP via garlic can be the reduction in the production of reactive oxygen species (ROS) and reduction of the damage of cells caused by the macrophage inducing a reduction in the activation of endothelial cells for gene expression of adhesion molecules and increased endothelial nitric oxide synthase. In contrast, van Doorn et al. [26] concluded that a garlic preparation has no significant effect on inflammatory parameters and endothelial function in cardiovascular diseases (CVD). The results of this study showed that amount of glucose significantly decreased after 8 weeks ET, RT and GE supplementation. Insulin levels was lower after this period compared to MetSCon group. The possible mechanism could be the improve in insulin sensitivity following regular exercise [27, 28]. In addition, the results of this study indicated that the glucose levels in RT + GE group were higher compared to RT group. Higher glucose levels may be caused by lower total antioxidant capacity in the resistance training group. Following the course of the study, merely insulin resistance was significantly improved in group ET and RT compared to the MetS-Con. In line with our study, Ho Ha et al. [29] also reported that a 12-week training protocol positively affected insulin resistance and insulin levels. In another study, 4-month walking did not have any impact on insulin resistance of diabetic patients. Thus, a long-term training may be necessary to determine whether aerobic exercises are more advantageous for improvement insulin resistance level in an experienced population [30]. Sun QY et. al. [31] reported 8 weeks of exercise intervention significantly reduced glucose. Exercise training increases the activities of phosphoinositide 3-kinase (PI3K) / Protein kinase B (PKB)/ endothelial nitric oxide synthase (eNOS) as well as the increase in phosphorylation of PI3K/Akt/eNOS in response to insulin. Interestingly, exercise training improves vasodilation in response to acetylcholine (Ach) and insulin by the same pathway of PI3K/Akt-mediated eNOS signaling cascades. Studies have indicated that expression of TNF-α and activation of p38 mitogen-activated protein kinase (p38 MAPK) markedly decreased through exercise training. In addition, the results of this study showed that endurance training with a supplement of garlic extract is more beneficial than other interventions on insulin resistance and glucose. The possible cause could be higher total antioxidant capacity and lower inflammation in endurance group taking garlic supplements. Our study showed that GE supplementation is effective in improving insulin sensitivity in rats with metabolic syndrome. Padiya R et al. [32] confirmed the effect of garlic on improving insulin sensitivity in fructose-fed rats. This probably comes from the effect of Garlic on insulin and may related to antioxidant feature of S-allyl cysteine sulfoxide in garlic. Garlic allicin can be effectively combined with compounds like cysteine and enhance serum insulin. On the other hand, garlic can act as an antidiabetic agent by increasing either the pancreatic secretion of insulin from the ß-cell [33]. Our study showed that mean of Bax and BCL2 gene expression in the groups of the ET, RT, GE, ET + GE, RT + GE compared to the MetS-Con group was lower, and there is no difference between training groups and supplementation positive effects on improvement of apoptosis index. This result was consistent with the study by the Kwak H B10 who have shown protective effects of the endurance exercise training against elevated apoptosis and lowering Bax protein expression in the aging rat heart. Moreover, Campbell K L et al [33]. have observed that exercise training induces the expression of Bax and BCL2 gene in the sedentary participants. Exercise training may increase cell-

survival proteins including Mn isoform of superoxide dismutase (MnSOD), NF-kB, extracellular receptor kinase (ERK), IGF-1/Akt pathway, and heat shock proteins (HSPs) in heart [34]. Our findings also showed changes in Bax protein expression levels in response to GE supplementation. Cheng Y C et al [35]. suggested garlic oil supplementation for 8 weeks may have protective effects on cardiac apoptosis in rats with high cholesterol intake. This could be possible via the p38 MAPK signal transduction pathway that functions to stimulate the activation of nuclear factor-kappa B, caspase-3 and − 9 [33]. Ultimately, this study hypothesized whether exercise and garlic supplementation improved hsCRP and IR in line with Bax and BCL2 protein expression in rats with MetS. In accordance with the hypotheses, the present findings demonstrated that ET, RT with and without GE supplementation have beneficial effects on reducing Bax gene expression in rats with MetS. ET alone, GE alone and ET + GE affected hs-CRP level; however, the reduction was greater in GE group. In addition, ET, RT with and without GE supplementation had beneficial effects on reducing IR. Yet, the reduction was greater in ET + GE group compared to other groups. However, more extensive research is needed before recommending exercise and GE supplementation to humans with metabolic syndrome.

5. Conclusion

In conclusion, endurance and resistance training with or without the garlic extract supplementation and the supplementation alone could improve inflammation and apoptosis indices, as well as the glycemic and insulin resistance levels, in rats with metabolic syndrome. In this regard, it would appear that there is no difference between endurance and resistance training. Besides, garlic supplementation has positive effects for improvement of apoptosis index. Moreover, endurance training with or without the garlic extract supplementation and the supplementation alone could improve total antioxidant capacity and homocysteine. However, for the insulin resistance, combination of endurance exercise with garlic supplementation can lead to the best results. For the fasting glucose level and the hs-CRP, all the interventions except for the combination of resistance exercise with the garlic supplementation have a relatively similar positive effect. Overall, performing endurance exercise with garlic supplementation would be suggested as a helpful supplementary treatment for type 2 diabetic patients. Although more studies are needed in this area to determine the best dose of supplementation and exercise intensity and volume.

Abbreviations

MetS: Metabolic syndrome; Bax: BCL2-associated X protein; Bcl2: B-cell lymphoma 2; Hcy: homocysteine; TAC: total antioxdant capacity; IR: insulin resistance; hs-CRP: high-sensitivity C-reactive protein; T2DM: type 2 diabetes mellitus; OS: oxidative stress; NK: Natural Killer; BW :body weight; Con-b: control basis; MetS-Con: metabolic syndrome-Control; ET: endurance training; RT: resistance training; GE: garlic extract; RT + GE: resistance training + garlic extract; FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment-insulin resistance; PI3K: phosphoinositide 3- kinase; PKB: Protein kinase B; eNOS: endothelial nitric oxide synthase; p38 MAPK: p38 mitogenactivated protein kinase; ROS: reactive oxygen species; MnSOD: Mn isoform of superoxide dismutase; ERK: extracellular receptor kinase; APS: acute phase response; CHD: coronary heart disease.

Declarations

Ethics approval and consent to participate

All procedures performed in this study involving animals were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards.

(Approval date, 2016-July-17).

Consent for publication

All participants were aware during the informed consent process that the results of this study may be published.

Availability of data and materials

Not applicable. Conclusions of the manuscript are based on relevant data sets available in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RA and TV contributed to data analysis and manuscript writing. RA, TV, BN, and AN contributed to discussions about the study, and to the reviewing and editing the manuscript. TV takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the final manuscript.

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Tables

Table 1

Details of the endurance training protocol used in this study

Variables	Week of training							
	1	2	3	4	5	6	7	8
Exercise duration, min d^{-1}	10	17.15	24.30	31.45	39	46.15	53.30	60.45
Treadmill grade	0	0	0	0	0	0	0	0
Treadmill speed, m min^{-1}	15	16	17	18	19	20	21	22

Table 2

Details of the resistance training protocol used in this study

Variables	Week of training							
	1	2	3	4	5	6	7	8
Exercise intensity ($^0/_0$ BW)	30	40	50	60	70	80	90	100
Exercise duration	8- week/ 3 time per week/ 5 repetitions per set, 4 periods/ per set 8 s/ between per period 3 min rest/ 2 sets 5 repetitions warming-up without weights/ 1 set cooling down without weights							
degree incline	Ladder with 110 cm/26 rungs, 2-cm between grid steps and 80° incline							

BW, body weight

Table 3

Animal characteristics

Variables	Baseline (n=48)	After 4 weeks HCD (n=48)	After 8 weeks exercise and supplementation					
			MetS-Con group (n=8)	ET group (n=8)	RT group (n=8)	GE group (n=8)	ET+GE group (n=8)	RT+GE group (n=8)
Age (years)	8 weeks	12 weeks	20 weeks	20 weeks	20 weeks	20 weeks	20 weeks	20 weeks
Weight (g)	160.20±5.08[1)]	331.94±13.67	353.09±19.62	342.95±19.88	320.49±16.65	325.29±2.9	326.5±17.99	331.8±19.66
Length (cm)	18.66±0.19	21.43±0.18	21.51±0.2	23.02±0.24	23.15±0.32	22.46±0.41	22.88±0.26	23.55±0.29
Lee index (g/cm)	291.01±4.31	324.33±1.02	328.63±0.66	300.9±0.65	295.2±0.7	305.89±0.43	300.39±0.5	293.92±0.26

MetS-Con, metabolic syndrome–Control; ET, endurance training; RT, resistance training; GE, garlic extract; RT + GE, resistance training + garlic extract; Lee, index BMI = body mass (g) / (nasal-anal length (cm))^2.

1) The data is presented as mean ± SD.

Table 4. Metabolic syndrome indexes

Variable	Con-b (n=8)	MetS-Con (n=8)
W (g)	171.29±4.17	342.5±7.9*
Lee index (g/cm)	293.19±0.7	324.5±1.2*
FBS (mg/dl)	103.87±12.82	170.12±28.05*
TC (mg/dl)	62.12±10.30	97.5±16.61*
TG (mg/dl)	28.87±5.51	56 ±29.31*
LDL-C (mg/dl)	40.97±9.54	70.92 ±16.27*

Con-b, control basis; MetS-Con, metabolic syndrome–Control; W, weight; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein-cholesterol.

***** significant difference between groups (P<0.05).

Table 5. Mean and standard deviation of the indicators in groups

Variables \ Group	MetS-Con	ET	RT	GE	ET+GE	RT+GE
Bax (Bax / ß actin)	1.73±0.31	0.46±0.29*	0.44±0.2†	0.37±0.28*	0.39±0.35*	0.46±0.44*
Bcl-2 (Bcl-2 /actin)	1.35±0.33	1.70±0.13†	1.75±0.23†	1.69±0.43*	2.05±0.27†	1.97±0.28*
Bax/Bcl2 ratio	1.27±0.17	0.12±0.05*	0.13±0.04*	0.8±0.23†	0.48±0.13*	0.54±0.11*
hs-CRP(mg/l)	4.34±0.56	2.53±0.61*	2.14±0.47†	1.74±01.01*	1.99±0.59*	2.99±0.76
Hcy (µU/ml)	21.09±6.52	7.93±2.19*	11.91±2.86	12.88±4.40	9.54±4.43*	11.35±4.70*
TAC (mmol/l)	0.61±0.15	1.25±0.12*	0.91±0.26	1.17±0.35*	1.14±0.50*	0.94±0.38
Insulin (µIU/ml)	23.77±18.19	8.13±4.49†	7.81±8.21*	8.57±3.8†	3.43±2.01*	7.35±6.4*
FBS (mg/dl)	170.12±28.05	128.75±13.75*	122.87±6.74*	128.12±22.25*	114±20*	143±34.6
IR	10.18±8.44	2.57±1.45†	2.37±2.44*	2.75±1.29†	0.98±0.66*	2.75±2.56*

Con_b, control basis; MetS-Con, metabolic syndrome –Control; ET, endurance training; RT, resistance training; GE, garlic extract; RT + GE, resistance training + garlic extract; Bax, *BCL2-associated X protein*; *Bcl-2*,B-cell lymphoma 2; hs-CRP, high-sensitivity C-reactive protein; Hcy, Homocysteine; TAC, total antioxidant capacity; FBS, fasting blood sugar; IR, insulin resistance.

*significant difference compared to MetS-Con group (P<0.01)

† significant difference compared to ET group (P<0.05)

Table 6 is not available with this version.