The effect of endurance training and hydroalcoholic extract of *Anethum Graveolens* L. (dill) on biochemical cardiovascular risk factors in obese male rats

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Abstract

Introduction: Obesity is a chronic disease and is a known risk factor for development of cardiovascular diseases (CVD), and its risk can be independently decreased through lifestyle modification. The aim of this study was to assess the effect of endurance training and hydroalcoholic extract of dill on biochemical cardiovascular risk factors in obese male rats.
Materials and methods: Thirty-two obese male rats (weighing 350-400 g) with aged 12 weeks were randomly divided into four equal groups (n=8) including endurance training (ETr: 10 weeks, 5 sessions per week at 75%VO_2max), dill extract (DEx: 300 mg/kg body weight via gavage), endurance training+dill extract (ETr+DEx), and control (Ct). Eight rats (weighing 240-280 g) were also in the non-obese control (NCt) group. Fasting plasma lipid concentration was measured 48 hours after the last intervention session. Data were analyzed with one way ANOVA at $P < 0.05$ significance.
Results: The result showed a significant increase in the levels of TC, TG, LDL-C, VLDL-C, TC/HDL-C, and LDL-C/HDL-C in the Ct group compared to the NCt group ($P < 0.05$). There was a significant decrease in the plasma levels of LDL-C, VLDL-C, TG, TC/HDL-C, and LDL-C/HDL-C in the ETr+DEx group and TC/HDL-C ratio in the ETr group compared to the Ct group ($P < 0.05$). There was no significant difference in the levels of HDL-C among groups ($P > 0.05$).
Conclusion: The results of this study showed that endurance training combined with the dill extract improved the plasma lipid profile. Therefore, it can be more effective in obesity complications reduction than exercise training or dill extract alone.

Keywords: Endurance training, Hydroalcoholic extract of dill, Cardiovascular risk factors, Obese male rat

Introduction

Obesity is the result of imbalanced energy intake and expenditure, which is associated with cardiovascular disease (CVD) and 75% of all cardiovascular-related deaths worldwide are linked to atherosclerosis. The obesity provide the groundwork for atherosclerosis, a clustering of interrelated and coincident risk factors which include increased total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), and diminished high-density

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lipoprotein (HDL-C) (1, 2). Diminished HDL-C and increased obesity-related inflammatory conditions, activate endothelial cells in the arteries which causes an increase of adhesion molecules on the surface of activated endothelial cells, it leads to monocytes attached to the endothelium (3). These adherent cells finally transmigrate through the endothelium and accumulate in the layers of the artery wall, where they differentiate into macrophages and in the presence of certain factors, into foam cells (the main component of a fatty streak) (3). Multiple lines of evidence indicate that LDL and its oxidized form (oxLDL) play a key role in endothelial dysfunction and CVD. Low-density lipoprotein can be oxidized by vascular endothelial cells, smooth muscle cells, and macrophages. Then oxLDL binds to receptor LOX-1 in endothelial cells and uptake of oxLDL by macrophages transform them into foam cells (3-5).

The previous study has shown that a 1 mg/dl increase of HDL-C levels led to decreased CVD risk. HDL-C acts as an anti-atherogenic agent and is also an effective antioxidant (6, 7). Also, a meta-analysis study indicated that a reduction of 2 to 3 mmol/L LDL-C by statins could reduce the risk of CVD by 50%. However, this means that there is still a considerable risk of CVD despite statins being widely used to lower levels of LDL-C (8).

Today, the use of non-pharmacological strategies such as regular exercise training and herbal supplements is prevalent to lower blood lipid levels (9). Several studies have shown that regular moderate-intensity endurance training is associated with lower TC, TG, LDL-C, and higher HDL-C, so it could result in a reduction in cardiovascular disease risk and increased life expectancy in men and women (10-12). Kodama et al. (2007), showed that endurance exercise training resulted in a 2 to 3 mg/dL increase in HDL-C level (12). Also, irregular exercise training was associated with significant improvements in lipid profiles following eight weeks of training in obese subjects (13).

The side effects of fat-reducing drugs are high and affect the gastrointestinal and gastric secretions. Fat-reducing drugs users may experience nausea, flatulence, diarrhea or constipation, an increase in liver enzymes production, and memory loss or impaired consciousness (14). Various chemical drugs such as lovastatin, clofibrate, and cholestyramine are used to lower LDL-C levels and increase HDL-C levels. In this regard, tending to use herbal medicine not only in the treatment and preventing various diseases, but also in preventing the toxic effects of other drugs has dramatically increased (14, 15). Fortunately, recent researches in the field of medicinal plants have had promising results. Dill is one of these plants which is the scientific name *Anethum graveolens (A. graveolens)* a one-year-old herbaceous and aromatic plant. Its medicinal product is mainly in the form of seed with 43-63% D-caron and the rest is D-limonene, ketone, and a fatty substance (14). Hydroalcoholic extract of dill also contains flavonoids such as quercetin, isorhamnetin, anethole, and dillapiole. Anethole and dillapiole have biological properties, including reduction in cholesterol, LDL-C, and TG, and increasing HDL-C (15). Also, dill has properties antibacterial, antifungal, anticonvulsant, antioxidant, anticancer (16).

According to the results of a few studies, consumption of hydroalcoholic extract of dill decreases serum lipids concentration (14, 17, 18). Among them, shown 30 days of hydroalcoholic extract of dill consumption (250 and 500 mg/L) resulted in decreased significantly TC, TG, LDL-C and also increased significantly HDL-C in male rats (17). Daily intake of 100 and 200 mg per kg of body weight of hydro-alcoholic extract for 30 days in rats fed with high cholesterol diet, decreased lipid profile, blood glucose, and liver enzymes (14). In addition, a study showed that six weeks of hydroalcoholic extract of dill

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consumption, swimming training, and their combination, had a significant effect on lowering LDL-C, VLDL-C, TG, and cholesterol and increasing HDL-C levels in diabetic rats (18). However, a study was performed on normal and obese diabetic mice showed that the dill extract was not effective in reducing lipid profile in obese diabetic rats (19). Also, the study carried out on 150 obese patients with hyperlipidemia was indicated that the *A. graveolens* could not improve plasma lipid profile in obese patients (20).

According to the above studies and inconsistent results for an exact comment about the dill, it seems that doing more comprehensive studies on the dill effect on blood lipids and their mechanism is required. Also, there are few studies on the effect of endurance exercise training and hydroalcoholic extract of dill on cardiovascular risk factors. Therefore, the aim of the present study was to assess the effect of endurance exercise training and hydroalcoholic extract of dill on biochemical cardiovascular risk factors in obese male rats.

Materials and methods

Experimental design

Male Wistar rats (n=40, 12 weeks old) were purchased from Pasteur Institute of Karaj, Iran. Thirty-two obese (weighing 350-400 g, 60% kcal energy from fat) and eight non-obese (weighing 240-280 g, 10% kcal energy from fat) male rats were housed in a well-ventilated facility and kept under controlled environmental conditions with 12 hours light/dark cycle at 22-24 °C, relative humidity in the range of 40–45% and allowed to take water and food ad libitum. After acclimatization, obese rats were assigned into four equal groups (n=8) including endurance training (ETr: 10 weeks, 5 sessions per week at 75% VO_2 max), dill extract (DEx: 300 mg/kg body weight via gavage), endurance training+dill extract (ETr+DEx), and control (Ct). Eight non-obese male rats were also assigned to the non-obese control (NCt) group. All experimental protocols were performed according to guidelines from the Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1397.179).

Measurement of lee obesity index

Lee's obesity index was determined in this animal study. It is an index to confirm obesity whereby its value of more than 310 is considered as obese. It was calculated based on the formulae whereby the cube root of body weight (g) was divided by nose-to-anus length (cm) of the rat (21). Body weight was monitored weekly, to carefully characterize weight gain.

Standard and high-fat diet

Standard chow diet and high-fat diet comprised of standard pellet, which was purchased from the Royan Institute for Animal Biotechnology (RI-AB), Isfahan, Iran. The obese rats (n=32) were fed high-fat diet (60% calories from fats, 20% from proteins, and 20% from carbohydrates; D12492; Research Diets) (22) and, none-obese rats (n=8) were fed standard diet of the following caloric composition: 10%, fat; 20%, protein; 70%, carbohydrates (D12450B; Research Diets) (22).

Endurance exercise training protocol

The exercise training program consisted of a 10 weeks endurance exercise training on a treadmill, five days/week. After being familiarized with a motor-driven rodent treadmill for one-week (15 min/day, speed of 15 m/min, and 0% slope), animals ran on a treadmill at 27 m/min, 0% slope for 20 min/day during the first week. The intensity and duration of the exercise training were progressively increased over 5 weeks to 27 m/min and 60 min/day until at weeks 5-10 the animals were running at 27 m/min, 0% slope for 60 min/day (23).

Preparation and consumption of *A. graveolens* extract

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Dried A. graveolens (dill) seeds were purchased from the near local market of Birjand, Iran. These seeds were identified and authenticated by the expert of the Department of Plant Pathology, Faculty of Agriculture, University of Birjand, Birjand, Iran. The extraction was performed with 100 grams of macerating powder seeds in 96% ethyl alcohol for one day at ambient temperature, filtered through Whatman No.1 filter paper. Then, the residue was extracted with ethanol (70%) for 12 hours at ambient temperature, filtered through filter paper. Then, the resulting macerate was collected in a clean conical flask and was evaporated using a rotatory evaporator (Heidolph, Schwabach, Germany) at a temperature of 50 °C and rotation speed of 70 rpm up to one-third of the original volume (17). Obtained soluble transferred into the Petri dish and was dried in an incubator (BD400-400L, Binder, Germany) at temperatures below 50 °C. The treatment groups received hydroalcoholic extract of dill processing by-product at a dose of 300 mg/kg body weight (dill extract by products were diluted (3 gr/10 ml) in saline solution), orally via gavage for 10 consecutive weeks (three days/week), and others groups received a saline solution via gavage for the same period time (14).

Laboratory measurements

At the end of 10 weeks' experiment (48 hours after the last intervention session), animals were anesthetized with 70-100 mg/kg ketamine and 7-10 mg/kg xylazine and sacrificed after 12h of fasting. Blood samples were collected into sterile tubes containing EDTA from the heart and plasma was separated immediately by centrifugation (3000 rpm/15 min) (BH-1200, Universal Centrifuge, Germany) for determination of the lipid profile. Plasma TC was determined by a colorimetric method (Pars Azmoon Co, Tehran, Iran) with a coefficient of variation and sensitivity of 1.1% and 5 mg/dl, respectively. Similarly, plasma TG was used through the same instrument with a coefficient of variation and sensitivity of 1.6% and 5 mg/dl, respectively. Plasma HDL-C was determined by the direct Immuno method (Pars Azmoon Co, Tehran, Iran) with a coefficient of variation and sensitivity of 1.8% and 1 mg/dl. Whereas, LDL-C was calculated by Freidwald's formula [LDL-C = TC- (HDL-C - TG/5)] and VLDL-C was calculated by the TG/5 formula (14). Optical absorption of TC, TG, and HDL-C plasma variables was measured using ELISA reader (Epoch, BioTek, USA).

Statistical analysis

All data are presented as mean ± standard deviation (SD). The results were analyzed using SPSS version 22 at the significance level of $P < 0.05$. The anatomical variables were analyzed using an ANCOVA with Bonferroni post hoc test. Lipid profile data was analyzed using a one-way ANOVA with Tukey's post hoc test.

Results

Changes of anatomical variables

Changes in the anatomical variables of rats at the beginning and end of the study are presented in Table 1. The baseline values of body weight and lee obese index of obese groups were significantly more than the NCt group ($P = 0.001$). The results revealed that there was a significant decrease in body weight in the ETr and ETr+DEx groups compared to the Ct group ($P = 0.03$ and $P = 0.007$; respectively) (Table 1). Also, the lee obese index was decreased significantly in the ETr and ETr+DEx groups compared to the Ct group ($P = 0.007$ and $P = 0.009$; respectively) (Table 1). There was no significant difference in other groups ($P > 0.05$).

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Table 1. Changes in anatomical variables before and after the intervention.

Groups	Body weight (g)		Length body (cm)		Lee obese index	
	before	after	before	after	before	after
ETr	373.45±11.97*	413.21±30.14†	21.43±0.67	23.50±0.37	336.17±10.77*	316.78±5.58†
DEx	393.02±36.49*	463.83±82.56	21.43±0.41	23.11±0.75	341.49±11.59*	333.96±21.56
ETr + DEx	378.50±20.36*	410.22±29.24†	21.81±0.45	23.28±0.64	331.61±7.14*	319.03±8.70†
Ct	391.88±22.87*	485.60±44.16	21.31±0.79	22.96±0.50	343.62±12.94*	342.10±10.15
NCt	261.78±21.56	380.95±29.35	22.06±0.41	23.27±0.65	289.81±8.68	311.48±11.17
ANCOVA results						
P value	0.001		0.53		0.004	

Data are presented as mean ± standard deviation; ETr, endurance training; DEx, dill extract; ETr + DEx, endurance training+dill extract; Ct, obese control; NCt, non-obese control.
*Significant difference compared with the NCt group, †significant difference compared with the Ct group ($P < 0.05$).

Changes of plasma lipid profile

The results showed that there was a significant increase in TC, LDL-C, VLDL-C, and TG levels and TC/HDL-C and LDL-C/HDL-C ratio in the Ct group compared to the NCt group (P = 0.01, P = 0.001, P = 0.001, P = 0.001, P = 0.0001 and P = 0.001; respectively) (Figure 1). Also, LDL-C level, TC/HDL-C, and LDL-C/HDL-C ratio was increased significantly in the DEx group compared to the NCt group (P = 0.001, 0.002, and P = 0.003; respectively (Figure 1C, E, and F).

The findings showed that there was a significant decrease in LDL-C, VLDL-C, and TG levels and TC/HDL-C and LDL-C/HDL-C ratio in the ETr+DEx group compared to the Ct group (P = 0.007, P = 0.04, P = 0.04, P = 0.005 and P = 0.02; respectively) (Figure 1). In addition, the TC/HDL-C ratio was decreased significantly in the ETr group compared to the Ct group (P = 0.03) (Figure 1E). There was no significant difference in other groups (P > 0.05). Based on the findings, there were no significant differences in the level of HDL-C after 10 weeks of endurance exercise training and oral administration of the dill extract (P = 0.13) (Figure 1G).

Discussion

The purpose of the present study was to determine the effect of endurance exercise training and dill extract on cardiovascular risk factors in obese male rats. The results showed that following 10 weeks of endurance training and endurance training + dill extract simultaneous combination led to reducing the body weight and lee obese index. Also, endurance training and dill extract simultaneous combination led to reducing LDL-C, VLDL-C, and TG levels and TC/HDL-C and LDL-C/HDL-C ratio. In addition, endurance training reduced the TC/HDL-C ratio. There was no difference between groups in the HDL-C level.

Obesity contributes substantially to increased CVD incidence, and more attention has been paid to lipid profile levels since studies have shown distinct relationships between obesity and CVD risk (24-26). Exercise training and medicinal herbs have been suggested to improve the plasma lipid profile and reduce cardiovascular complications related to obesity (14, 15, 18). We showed that 10 weeks of endurance exercise training combined with hydroalcoholic extract of dill decreased LDL-C, VLDL-C, and TG levels and TC/HDL-C and LDL-C/HDL-C ratio in obese rats. The exact mechanism of these changes is unclear. Dill seed is one of the important traditional medicinal herbs, having potent treatment abilities, and reduces the absorption of fat from the intestine and contains tannins, ketones, limonene, and carotene that affects cholesterol depletion and its biosynthesis (14, 15).

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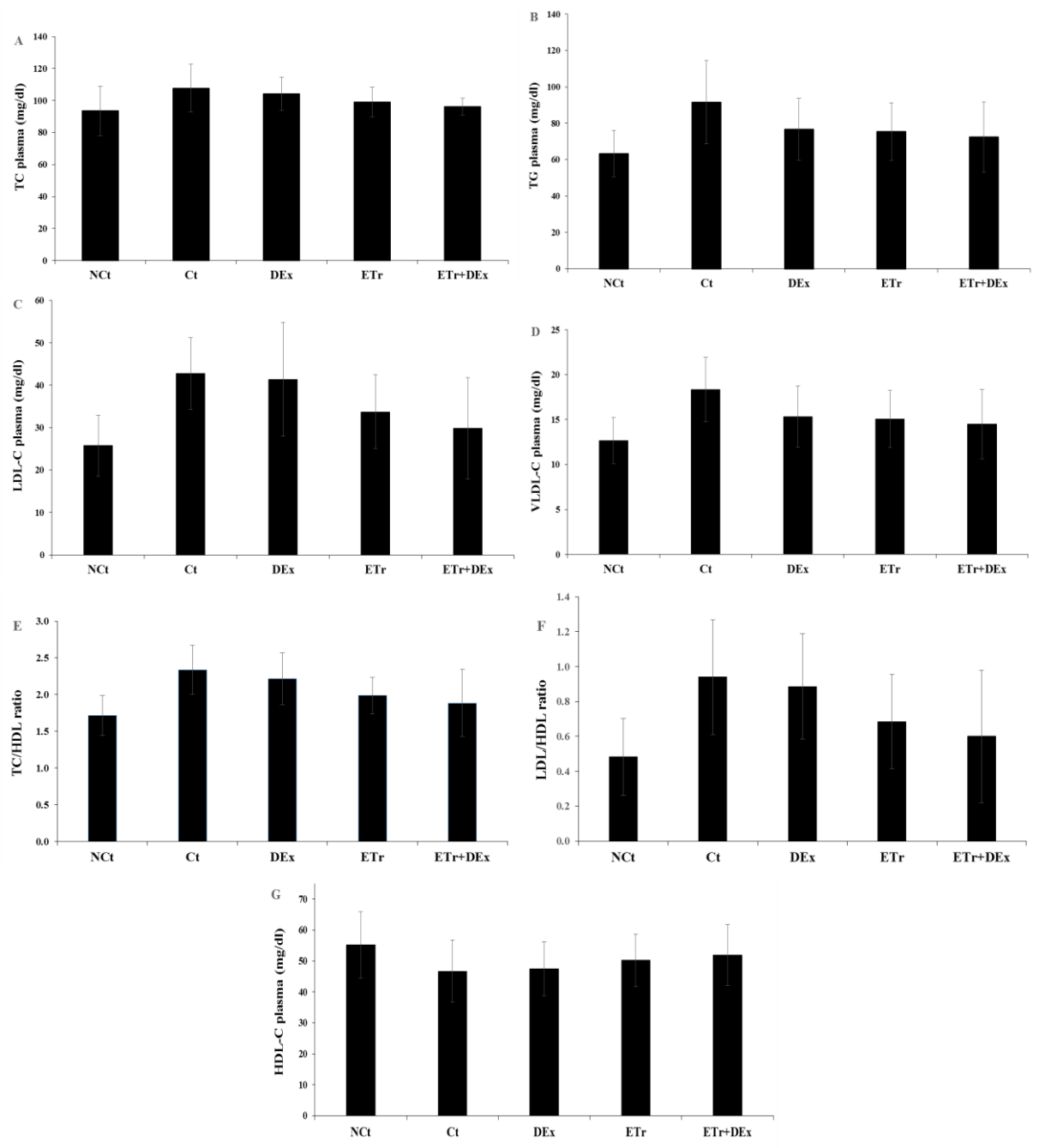


Figure 1. Effect of endurance training and dill extract on plasma lipid profile in obese rats. TC, total cholesterol (A); TG, triglyceride (B); LDL-C, low-density lipoprotein (C); VLDL-C, very low-density lipoprotein (D); TC/HDL ratio (E); LDL/HDL ratio (F); HDL-C, high-density lipoprotein (G); ETr, endurance training; DEx, dill extract; Ct, obese control; NCt, non-obese control; Data are presented as mean ± SD. * Significant difference compared with the NCt group, † significant difference compared with the Ct group ($P < 0.05$).

In line with this, a study showed that six weeks of hydroalcoholic extract of dill consumption, swimming training, and their combination, had a significant effect on lowering LDL-C, VLDL-C, TG, and TC levels in diabetic rats (18).The possible mechanism of responsible for the decrease in TC and LDL-C may be related to the involvement of pathways of cholesterol in the digestive system that by forming insoluble cholesterol, can excrete cholesterol through the stool and is a factor in reducing cholesterol absorption (15). Reduction in cholesterol uptake in the liver

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increase the expression and activity of LDL receptors and this increase in hepatocytes results in the removal of cholesterol from the bloodstream. Also, dill seed contains flavonoids such as quercetin, isorhamnetin, anethole, and dillapiole, and it has been reported that the hydroalcoholic extract of dill has the strong anti-lipolytic activity due to the presence of two phenolic compounds of anethole and dillapiole. Anethole and dillapiole have biological properties of reducing cholesterol, LDL-C, and TG and increasing HDL-C levels (15). Flavonoids in dill extract are effective for inhibiting LDL oxidation. On the other hand, the polyphenol in dill, reduce cardiovascular disease by inhibiting LDL-oxidation, increasing antioxidant activity and increasing HDL-C levels. Quercetin and isorhamnetin can reduce TG levels. Part of this quercetin lowering effect is due to a decrease in liver production of Apo B-100. The decrease in ApoB-100 production results in a decrease in VLDL-C and consequently a decrease in plasma TG (18). Lipoprotein lipase (LPL) is one of the lipoproteins regulating enzymes and decomposition of triglyceride found in triglyceride-rich lipoproteins. Exercise training increases LPL and decreases hepatic lipase (HL), and this enzyme plays a major role in converting VLDL-C to HDL-C. Considering increased LPL activity increases the catabolism of triglyceride-rich lipoproteins; therefore, LDL-C levels decrease with exercise training. Exercise training has also been shown to increase the lecithin cholesterol acyltransferase (LCAT) enzyme, which increases intramuscular cholesterol esterification to HDL-C. Increased HDL-C after exercise training results in lower triglycerides, and increased LPL activity. It may accelerate the degradation of glycerol in VLDL-C and eliminate the lipoprotein particles. This may end to resulting in the formation of free lipid cortex (free cholesterol and phospholipid) that transmit to HDL-C. In addition, increased LCAT activity due to exercise training results in feeding HDL-C particles (24, 25). According to the above studies, it seems that administration of dill extract through reduces the absorption of fat from the intestine, cholesterol depletion, and its biosynthesis, increase the expression and activity of LDL receptors, inhibiting LDL oxidation, decrease in liver production of Apo B-100 may enhance the good effects of endurance exercise training on lipid metabolism, including anti-lipidemic, anti-inflammatory, and anti-oxidant activity. Therefore, the combined effects of endurance exercise training and dill extract have a positive effect on the lipid profile of obese rats that can lead to preventing undesirable changes in cardiovascular risk indicators.

In this study, the obtained results showed that the hydroalcoholic extract of dill had no effect on biochemical cardiovascular risk factors. In line with results from this study, Piri et al. (2010) have shown that consumption of dill extract (50, 100 and 200 mg/kg for 21 days) in diabetic rats with the high-fat diet, had no significant effect on plasma lipid profile (19). Another study also reported that the administration of dill seeds for three months (600 mg/day) had no significant effect on the lipid profile of patients with metabolic syndrome (27). On the other hand, Abbasi et al. (2015) reported that consumption of dill extract (100 and 200 mg/kg of body weight) for 30 days in rats fed with a high-fat diet reduced lipid profile (14). In addition, another study has shown that hydroalcoholic extract of dill to be able to reduce levels of TG, TC, and LDL-C in rats, whose blood cholesterol levels were increased by high-fat diet (28). The therapeutic effect of the dill extract may be related to flavonoids and volatile oils in this plant. However, the present study did not show any improvement in the lipid profile due to the presence of the hydroalcoholic extract of dill. The exact mechanism of these changes is unclear, but it may be due to a high-fat diet. Adding cholesterol to the diet led to reduces endogenous cholesterol synthesis and

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expression of hepatic LDL receptors, and decreased LDL receptors also led to decrease hepatic uptake of LDL from the blood and therefore TC, LDL, and VLDL are increased in plasma (19).

In the present study, endurance exercise training reduced the body weight, lee obese index, and TC/HDL ratio, but no effect on other biochemical cardiovascular risk factors. Similar to our study, Badalzadeh et al. (2014) showed that aerobic exercise training (eight weeks and five sessions/week for 90 minutes) had no significant effect on the lipid profile of rats (29). Another study also has shown that eight weeks of moderate exercise training was able to reduce the body weight, but it had no beneficial effect on lipid profile in diabetic obese mice (30). On the other hand, Shorabi et al. (2017) showed that endurance training decreased the levels of TC, TG, LDL-C in obese rats (31). Considering the mechanisms involved in the process of promoting cardiovascular health resulting from exercise training, there is a consensus that long-term exercise training by restorative the body's antioxidant system and reducing LDL-C oxidation has beneficial effects on the lipid profile and prevention of obesity-related atherosclerosis (10). In line with this, it has been reported that 12 weeks of endurance exercise training decreased TG and LDL-C and increased HDL-C of obese rats (32). Therefore, perhaps the reason for a not significant change of the lipid profile by endurance exercise training in the present study is related to the duration and intensity of performed exercise training.

Our study had several limitations, including no overload of exercise training and also no measuring the calorie intake of obese rats during the investigation period. Also, taking different doses of the extract of dill can have different effects on the lipid profile. Therefore, future examinations should consider the effect of endurance exercise training with different overload protocols as well as administration of hydroalcoholic dill extract in different doses on lipid profile in obese rats. In summary, endurance exercise training or dill extract alone has no effect on lipid profile in obese male rats, but the results of this study showed endurance training combined with dill extract improved the plasma lipid profile. Therefore, it can be more effective in obesity complications reduction than exercise training or dill extract alone in obese rats.

Conclusion

The results of this study showed that endurance training combined with hydroalcoholic extract of dill for 10 weeks improved biochemical cardiovascular risk factors in obese male rats. Further studies are needed to clarify the mechanism of these effects.

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Conflict of interest

The authors declare that no conflict of interest exists.

Authors' contribution

Marziyeh Saghebjoo and Mahdi Aliakbari-Baydokhty designed of research; Mahdi Aliakbari-Baydokhty performed experiments; Mahdi Aliakbari-Baydokhty analyzed the data; Mehdi Hedayati and Hadi Sarir contributed to set up and lab experiments work; Mahdi Aliakbari-Baydokhty drafted the manuscript; Marziyeh Saghebjoo and Mehdi Hedayati edited and revised the manuscript; all the authors approved the final version of the manuscript.

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