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Original Article

Effect of Nettle (*Urtica dioica* L.) Leaf Hydroalcoholic Extract on Atherosclerosis Plaque Formation of Cardiovascular Diseases

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ABSTRACT

High levels of circulating fat are considered an important factor in atherosclerosis. So the most important object of the present study is to investigate the effect of nettle leaf extract mixture on atherosclerosis plaque formation and biochemical factors of cardiovascular diseases. Twenty-eight rabbits were randomly divided into four groups, normal diet as the control group, sunflower oil as a sham group, high cholesterolemic group (1% cholesterol), and high-cholesterol + nettle leaf extract (500 mg/kg of BW) group. Components were given to the sham and all 2 experimental groups as gavage. Obtained results of the present study indicated that body weight was reduced significantly in group III compared with the control groups and group II. In the slices prepared from the aorta of the 1% cholesterol-treated group, athreptic plaques were detectable. The plaques were formed of lipid-laden macrophages, and foam cells, with smooth muscle cells within the plaques. The thickness of the plaque increased so that it was more than half of the thickness of the environment. Pathology results showed that the nettle extract reduced significantly the damage in the arterial wall compared with the high-cholesterol group. The atheroma was fully raised and observable in the inner surface of the vessel (P<0/01). The results indicated that Grande ortie extract is efficient and tended to improve atherosclerosis complications in hypercholesterolemia rabbits.

INTRODUCTION

Cardiovascular diseases and atherosclerosis arising from the gradual deposition of fat in the endolethium of vessels are the leading causes of death worldwide [1]. Hyperlipidemia [2], diabetes [3], high blood pressure, age, sex, and smoking are important risk factors for cardiovascular disease and cause an abnormal increase in serum total cholesterol and LDL-c and a decrease in HDL-c [4].

The high amount of fat in the blood circulation is one of the important factors that cause atherosclerosis. Studies show that in countries where the average amount of fat consumption is increased, the probability of death due to coronary diseases is also higher [5, 6]. Hypercholesterolemia is common and follows a polygenetic pattern. It is usually arisen from nutritional factors, such as obesity and an unsaturated fat-rich diet, along with its polygenic favorable background. The disorder is associated with the overproduction of LDL which genetic factor that does not seem to be monogenic unless it is very severe. Hypercholesterolemia can also have a complete genetic reason. A common example is monogenic familial hypercholesterolemia, an autosomal dominant disorder in which cholesterol is increased from birth; it has a dominant genetic pattern and is recognized with early onset coronary disease [7].

First-line intervention for the treatment of hyperlipidemia includes a change in lifestyle, weight control, and decreasing smoking. Synthetic lipid-lowering drugs (statins) and synthetic antioxidants are clinically used to treat hyperlipidemia and atherosclerosis [8, 9]. When they are ineffective in high-risk patients, regulating medicines such as statins, fibric acids, cholesterol absorption inhibitors, bile acid sequestrants, and nicotinic acids, are used and their mode of consumption depends on the type of lipid disorder [10]. But due to the many side effects of these drugs, the use of medicinal plants has been considered. Referring again to the use of herbal medicines and medicinal plants (as the main sources of medicines) has increased the need for accurate and scientific identification and description of plants [11].

Nowadays, the research and development of new drugs from natural sources a systematic way with strategic and economic value has gained special importance in the world. So that currently more than 30% of herbal medicines taken from natural sources are used in hospitals and clinics [12, 13]. The nettle (*Urtica dioica* L.) leaf is very effective as a traditional remedy in countries such as Morocco, Turkey, Brazil, Jordan, and parts of Europe and Iran due to its distribution [14]. Nettle compounds include flavonoids, hydrophilic compounds such as lectin, and polysaccharides, steroid compounds such as stigmasterol, as well as substances such as histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotriene, and 5-hydroxytryptamine [15, 16].

Golalipour *et al*. (2009) studied the protective impact of the hydro-alcoholic extract of nettle leaf on histological and morphological alterations in hyperglycemic rats' kidneys and found that the extract can prevent blood glucose increment as well as morphological and histopathological changes in hyperglycemic rats' kidneys [17].

This research aimed to determine the effect of the alcoholic extract of the aerial parts of the nettle plant (*U. dioica* L.) on the number of lipoproteins in the blood and the formation and progression of atherosclerosis plaques in the aorta of hypercholesterolemic rabbits.

MATERIAL AND METHODS

Animals and Experimental Conditions

This research was carried out as a laboratory work. The code of professional ethics regarding laboratory animals was fully observed. Twenty-eight male, mature rabbits of the New Zealand race with an approximate weight of 2.0-2.5 kg were purchased from the Razi Institute of Shiraz and transferred to the animal house. To adapt them to the environment, they received a baseline diet and were kept in standard conditions in terms of lighting and temperature (12 h darkness and 12 h light at 24±2 °C) for 2 weeks. The animals were kept in 360×450×710 mm^3 cages made of poly-carbonate. The cage floor was covered with sawdust and wood chips and emptied, cleaned, and disinfected once per day. Water was provided in special plastic containers which were cleaned and filled with water every day.

Extract Preparation

The climatic zone for sampling was located 15 km from Shiraz between 52.15 longitude 52.10 and latitude 29.45 to 29.40. First, one kilogram of nettle was collected and after confirmation by the botanist of the Shiraz University of Medical Sciences, its leaves were separated from the stem. The collected leaves were dried in the shade, then the fine powder was prepared in an airflow of 35 to 40 °C. For extraction, the maceration method was carried out for 72 hours with continuous stirring (solvent ratio using the hydroalcoholic solution of 75% of ethanol). The filtered solutions were mixed and concentrated with a vacuum distillation device at a temperature of 50 °C and a rotation speed of 70 revolutions per minute to one-third of the initial volume, and then it was decanted three times with 50 milliliters of chloroform. The solution obtained from the last step was dried in an autoclave at a temperature below 50 °C and under sterile conditions. The dry powder of the extract was prepared and stored at 4 °C.

Experiment Design

The LD_{50} method was used to determine the nettle leaf extracts doses. To investigate the effect of cholesterol, since it is provided as a powder and 1% of the total weight of the diet should contain cholesterol, it was dissolved in sunflower oil. Therefore, a solution was prepared with a cholesterol concentration of 0.5 g/mL, and the rabbits received it through a feeding tube according to their weights and diet relative amount.

The rabbits were randomly divided into four groups of seven animals each, as follows; Group I) as controls with a normal diet; Group II) as a sham with sunflower oil as cholesterol solvent (2 mL/Kg of BW); Group III) with high-cholesterol diet (1% of feed weight); Group IV) with nettle leaf extract (500 mg/kg of BW) and high-cholesterol diet (1% of feed weight). All groups received the diet for 60 days during which the animals had access to feed and water without limitation. To provide a high cholesterol diet, cholesterol prepared from Merck, Germany, was fed to rabbits by stomach tube based on 1% of the diet weight. The extracts of plants with the mentioned dose were also fed to the samples through a stomach tube at a specified time after ingestion of high cholesterol every day.

Measurement of Biochemical Factors

The rabbits were anesthetized by chloroform at the end of the experiment. After blood sampling, their chests were dissected, and the aortas were removed and washed with physiologic saline. The aortas were placed in 10% formalin for preparing tissue sections. The sections were stained with hematoxylin-eosin. Atherosclerotic plaques were graded according to the ratio of plaque thickness to media thickness on a 1-4 scale [18].

LDL-cholesterol and HDL-cholesterol were measured from the obtained serum in the laboratory using a biochemical enzyme kit (manufactured by Kavshiar Co., Ltd. of Iran) and by Hitachi Automatic Analyzer 902.

Data Analysis

For the statistical analysis of serological data, a one-way analysis of variance was used based on the following statistical model. The results in each time unit were compared between groups and in different time units in each group. SPSS-27 software was used for analysis and Duncan's post-test methods were used to compare means at the 5% level.

The statistical model of the design was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij}: The observed value, μ: mean, T_i: treatment effect, e_{ij}: Effect of experimental error

RESULTS

Figure 1 shows changes in the concentration of LDL, HDL, and HDL: LDL ratio in different experimental groups. HDL concentration in the hypercholesterol group II (3.5±0.56 mg/dl) showed a significant decrease compared to treatment III (27.33±1.76 mg/dl) as well as sham (I) and control ($P<0.05$). Also, the level of LDL in group III (29.83±1.49 mg/dl) showed a significant decrease compared to group II (45.66±1.60 mg/dl) ($P<0.05$). The results showed that in group III, due to the consumption of nettle extract, the HDL: LDL ratio improved by 0.92, so this increase compared to group II (0.08) was recorded as more than 1100% ($P<0.05$).

Body weight changes in the experimental groups receiving nettle hydroalcoholic extract compared to the control group are shown in Fig 2. Body weight in group II did not show a significant change compared to the control group ($P<0.05$). But this index demonstrated a significant decline in group III. The plaque scale also raised with cholesterol consumption, from 0 in the control group to 3.33±0.21 (II). By consuming nettle extract in group III, the amount of plaque scale decreased to 2.00±0.026 ($P<0.05$) (Fig. 3).

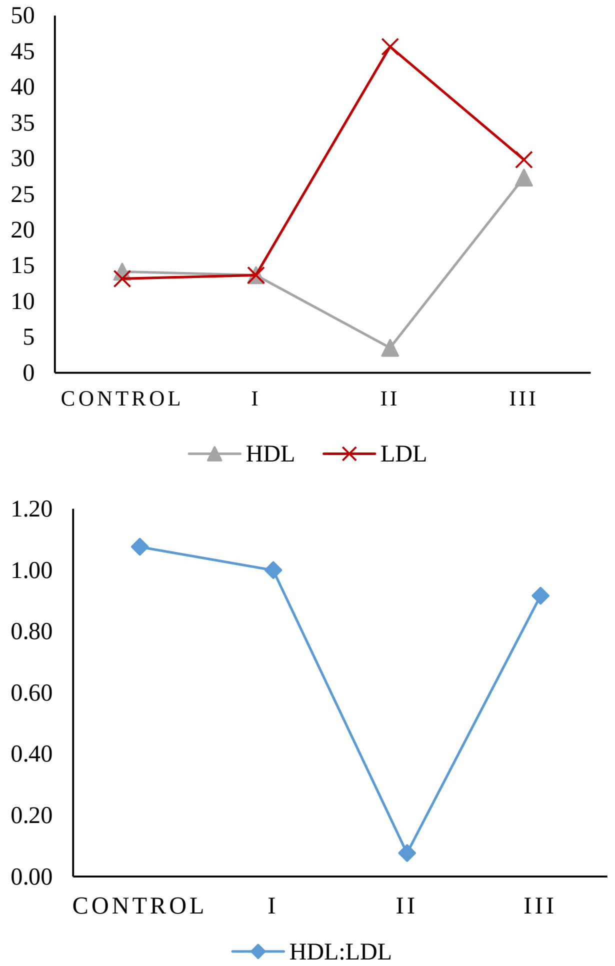

Fig. 1 Changes in the concentration of LDL, HDL and HDL:LDL ratio in different treatments compared to the control group and high cholesterol group.

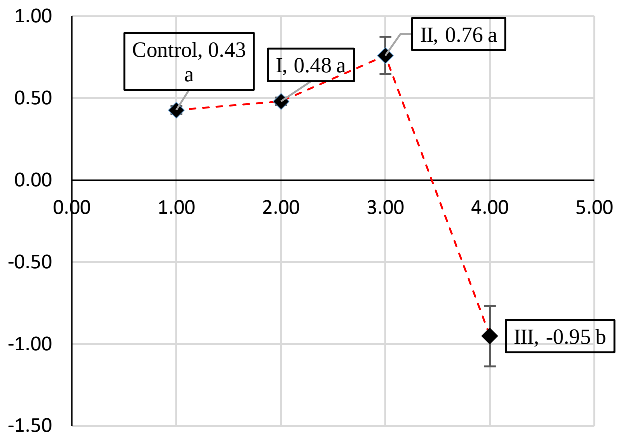

Fig. 2 Body weight changes in the experimental groups receiving nettle hydroalcoholic extract compared to the control group and high cholesterol group. The means having at least one common letter are not significantly different at the 5%.

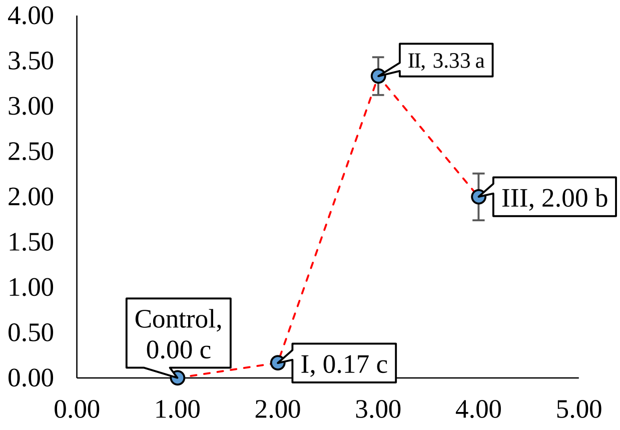

Fig. 3 Plaque scale changes in the experimental groups receiving nettle hydroalcoholic extract compared to the control group and high cholesterol group. The means having at least one common letter are not significantly different at the 5%.

Histological results showed that in the group treated with a normal diet (control), the vessel was completely normal and did not show any lesion in the intima and media (Fig. 4).

In the slices prepared from the aorta of the 1% cholesterol-treated group, atherotic plaques were detectable. The plaques were formed of lipid-laden macrophages, and foam cells, with smooth muscle cells within the plaques. The plaque thickness was increased and was more than half the thickness of the media (Fig. 5 and 6).

Pathology results revealed that the extract reduced significantly the damage in the arterial wall compared with the high-cholesterol group in whom atheroma was fully raised and observable in the inner surface of the vessel.

In the groups with high-cholesterol and nettle leaf extract, the severity of the lesion was decreased in comparison with the high-cholesterol group and the thickness of plaque was half the thickness of media (Fig. 7 and 8).

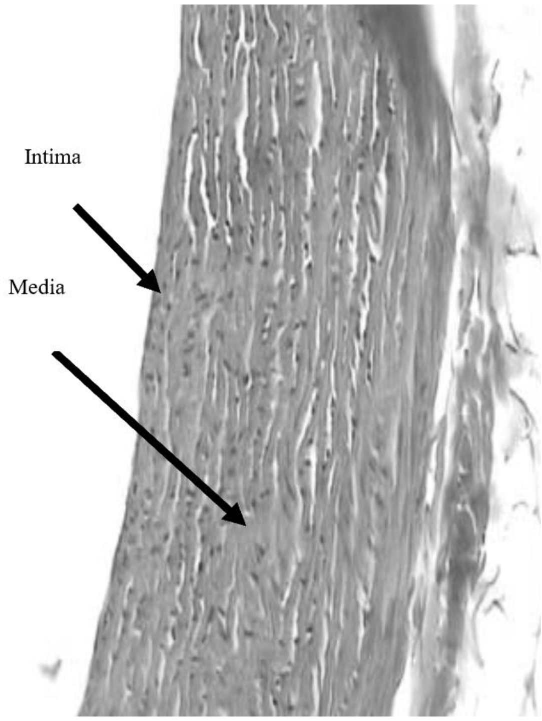

Fig. 4 Transverse section of the aorta of control group rabbits (normal diet) with x40 magnification.

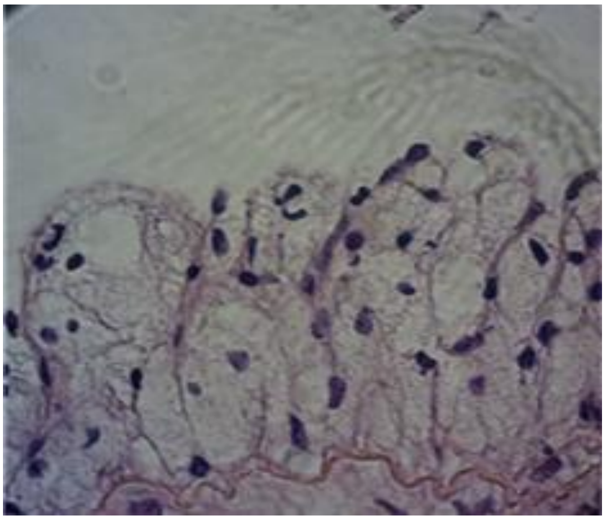

Fig. 5 Transverse section of aorta of hypercholesterol group rabbits with x100 magnification.

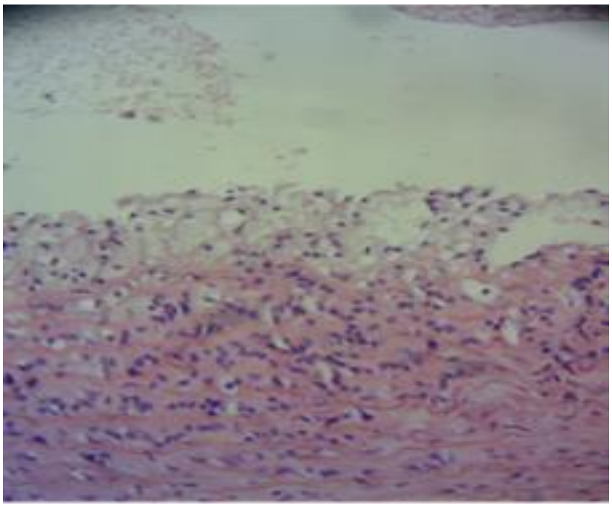

Fig. 6 Transverse section of the aorta of hypercholesterol group rabbits with x40 magnification.

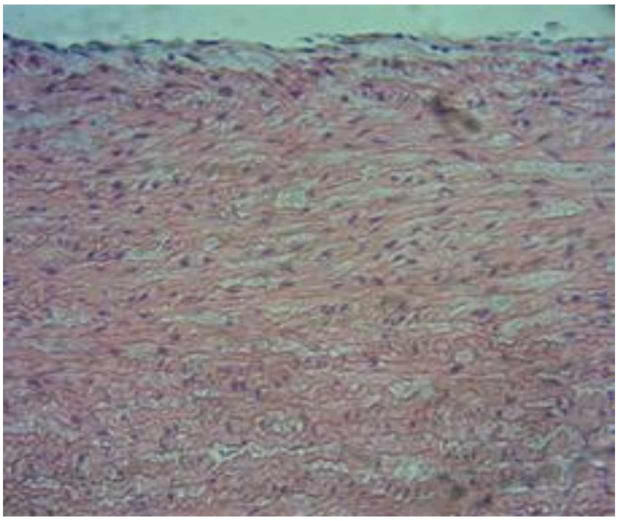

Fig. 7 Transverse section of aorta of hypercholesterol + nettle extract rabbits with x100 magnification.

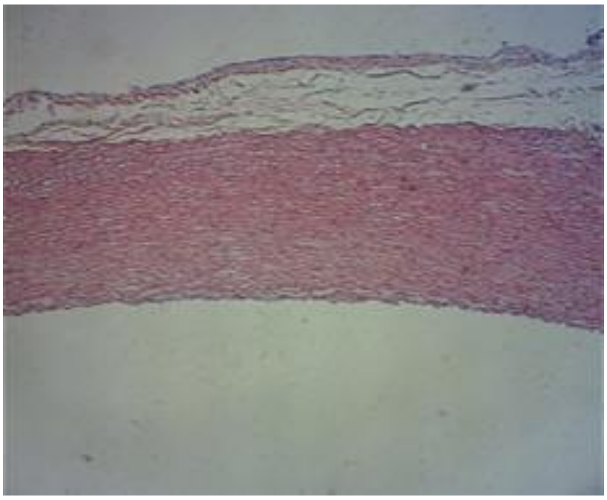

Fig. 8 Transverse section of the aorta of hypercholesterol + nettle extract group rabbits with x40 magnification.

Discussion

Deposition of cholesterol- and lipid-containing plaques along with fibrous tissue and deposits of calcium salts in the intima or inner walls of large and medium arteries of different organs result in atherosclerosis [19]. Atherosclerosis is a complex process for which a major risk factor cannot be identified since a variety of factors are involved [20]. Control of these risk factors is important in the prevention of atherosclerosis and today the use of herbs is taken into consideration to control the risk factors [1].

To improve a variety of diseases including hyperlipidemia, the use of alternative therapies, especially herbal therapy and dietary supplements, was increased in most countries among people in recent decades. One of the major problems facing practitioners and consumers of medicinal plants is the lack of sufficient information regarding their safety and impacts on diseases. Fortunately, extensive research was carried out on the efficacy of medicinal plants used in traditional medicine over the past 30 years to prove their performance [21].

Since in the current study, the level of LDL in the fourth group showed a significant increase and a positive relationship between leptin and blood fats, it can be said that leptin, by increasing blood fat, causes a decrease in appetite and As a result, weight has been reduced. On the other hand, factors such as nausea and keeping conditions of laboratory animals may be effective in the results related to body weight, which cannot be ignored. There is a contradiction regarding the effect of a hypercholesterol diet on HDL concentration. In some studies, this concentration shows an increase and in some studies, a decrease. The reason for this discrepancy is not clear, but according to Prasad and Lee (2003), it may be due to the effect of the percentage of cholesterol used in the diet and the duration of the diet [22].

According to Fig 2, a significant reduction in the final weight of laboratory animals was achieved in group III. Previously performed research stated that a positive relationship exists between leptin and plasma cholesterol and triglyceride [23]. Leptin acts as a satiety factor and hence affects appetite and satiety center in the brain. This hormone is released in the blood and circulates toward the hypothalamus where it binds to its specific receptors and reduces food intake and increases energy use in the body [24, 25]. Leptin conveys information about body fat storage and energy status to the hypothalamus and leads to the regulation of food intake and energy expenditure to stabilize body weight [26, 27]. The effects of this hormone are probably exerted through its actions on the hypothalamus and include most importantly reduction of body fat mass, reduction of hyperglycemia, and increase of fat metabolism and hence weight loss [28, 29].

The deposition of plaques including cholesterol and fatty substances along with fibrous tissue and deposits of calcium salts in the intima or the inner wall of the large and medium arteries of different organs causes atherosclerosis [19]. Atherosclerosis is a complex process that cannot be a risk factor. The main reason for this is that different agents and factors are involved with each other [20] control of these risk factors is important in the prevention of atherosclerosis and nowadays the use of medicinal plants to control risk factors has been considered. In addition to the oxidation of lipids, blood platelet activity is another important factor that could accelerate the formation of atherosclerotic plaque. Increased cholesterol is associated with increased coagulability and increased platelet count [5].

Since in the present study, total cholesterol, triglycerides, and LDL were significantly increased in groups II and III, and given the positive relationship between leptin and blood lipids, one can state that leptin reduced appetite and resulted in weight loss through increasing blood fat [21]. However, factors such as nausea and maintenance conditions of laboratory animals may affect body weight and should not be ignored.

Various biological activities such as anti-oxidative, antimicrobial, and anti-inflammatory are observed in plants which are mainly due to flavonoids and other phenolic components. It was stated in some research that plants similar to those examined in this study such as *Silybum marianum* contain flavonoids and exert a positive influence on the improvement of several diseases including hyperlipidemia. It also stated that the *Silybum marianum* can lower blood cholesterol in patients with hypercholesterolemia [30]. In a study, flavonoids-containing *Silybum marianum* reduced total cholesterol, LDL, and triglycerides [31]. Specific mechanism or mechanisms that determine the improvement of lipid profile or reduction of the risk of coronary artery disease is unknown. Hetrog *et al*. (1993) found in their studies that regular consumption of flavonoids reduces the risk of death from cardiovascular disease in elderly men [32]. Cook and Samman (1996) reported that flavonoids can reduce LDL oxidation through reducing lipid peroxidation, reduction of free radicals, supporting α-tocopherol-LDL or reduction of oxidized α-tocopherol-LDL, and separation of metal ions that participate in oxidation reactions [33]. The antioxidative effect of flavonoids on the affinity of LDL to its receptor was studied [34]. The results showed that flavonoids with good anti-oxidative properties increase the affinity of normal and oxidized LDL to its receptor and thus have beneficial effects in the treatment of atherosclerosis and reduction of plasma cholesterol. In addition to antioxidative properties, flavonoids exert also anti-platelet and anti-inflammatory properties. Lale (1992) showed that flavonoids have inhibitory effects on platelet and leukocyte functions and protective effects on endothelial cells and thus prevent the interaction between the vessel wall and blood which can initiate thrombosis. Flavonoids perform this action through effects on the tissue factor of human monocytes which is per se a blood clotting factor starter [35].

Borradaile *et al.* (2003) showed that flavonoids may reduce plasma lipids and atherosclerosis [36]. It was identified that the cholesterol-lowering effect of naringenin flavonoids is associated with a decrease in hepatic HMG-CoA reductase enzyme, as well as reduced secretion of apo-B from hepatocytes which is related to reduction in acyl-cholesterol acyl transferase (ACAT) activity and reduced ACAT expression. HMG-CoA reductase is the rate-limiting enzyme of cholesterol formation in the liver and other tissues and decreases the reductase gene expression through negative feedback regulation. Consumption of vitamins and herbal antioxidants reduces the risk of cardiovascular disease possibly through decreasing free radicals and improves vascular endothelial function in hyperlipidemic patients [37]. Free radicals can oxidize LDL in the vascular wall and hence reduces vascular endothelial-related vasodilatation [38].

In patients with hyperlipidemia, the need for antioxidants increases, and adding vitamins with antioxidant properties to the diet or drug regimen may reduce blood fat. As an antioxidant, vitamin C found in plants reduces lipid peroxidation and oxidative damage of vessels. The use of high doses of lipid-lowering drugs is associated with side effects; in addition, vitamin C and using a diet rich in these anti-oxidative vitamins improves health and reduces the risk of cardiac disease [39]. According to the results, it seems that vitamin C exerts its favorable changes in HDL and LDL levels through two main mechanisms: 1) decreasing LDL oxidation and increasing its recognition by its receptor; 2) competition with glucose (due to structural similarity) in the HDL and LDL glycation process which results in increased LDL catabolism and decreased HDL excretion.

CONCLUSION

Consumption of nettle aerial parts has been able to prevent fatty streaks in high-cholesterol groups. This effect has also been demonstrated by affecting other cardiovascular risk factors, including apolipoproteins. It is worth mentioning that apolipoprotein B is one of the new risk factors for cardiovascular diseases, which is more important than other risk factors. According to the results obtained in this study, it can be stated that nettle leaf extract was somewhat effective in the reduction of blood lipids in the experimental groups with a high-cholesterol diet. The results of this study can be partially extended to humans.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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