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Original Article

Evaluation of the effect of *Medicago sativa* on Lipid Profile and Iron Indices in Healthy Volunteers

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Abstract

Medicago sativa (alfalfa) has a long history of traditional use. Several studies indicate that the ingestion of *M. sativa* reduces cholesterol absorption and atherosclerotic plaque formation in animals and has been reported to be beneficial in the treatment of hemorrhage, as a tonic after blood loss and during anemia. Because hypercholesterolemia is an important risk factor for heart diseases and anemia is a common problem in developing countries, this double-blind, placebo-controlled, randomized clinical trial was designed to evaluate the effect of alfalfa extract on lipid profile and iron indices in healthy volunteers. 19 volunteers (all male) with an average age of 35 were elected for a randomized double-blind study. These volunteers were divided into two groups for drug (9 males) and placebo (10 persons). The capsules of 250 mg were taken three times a day for two months. Blood samples were collected at baseline, one month, and two months after taking the capsules. Ferritin, TIBC (Total Iron Binding Capacity), Iron, CBC (Complete Blood Count), cholesterol, triglyceride, LDL, and HDL were determined by laboratory testing standards. After two months in the treated group, serum iron showed more increase and TIBC factor decreased more in comparison with the control group and their LDL and triglyceride decreased and HDL increased but was not statistically significant. Alfalfa ethanolic extract, with 750 mg/day can help decrease LDL and triglyceride and increase HDL, and maybe increase serum iron as an adjunctive for hypercholesterolemia and anemia treatment.

Keywords: Alfalfa, *Medicago sativa*, Iron, Anemia, Cholesterol, Triglycerides.

1. Introduction

Anemia is defined as a low total amount of red blood cells or hemoglobin concentration in the blood that decrease the ability of the blood to carry oxygen. Anemia can be caused by blood loss, decreased red blood cell production, and increased red blood cell destruction. Causes of blood loss include trauma, gastrointestinal disorders, and genitourinary. Causes of decreased production include iron deficiency, vitamin B_{12} deficiency, thalassemia, and a

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number of neoplasms of the bone marrow. Causes of increased breakdown include genetic conditions such as sickle cell anemia, infections such as malaria, and certain autoimmune diseases. Anemia can also be classified based on the size of the red blood cells and the amount of hemoglobin in each cell. If the cells are small, it is called microcytic anemia; if they are large, it is called macrocytic anemia; and if they are normal-sized, it is called normocytic anemia. The diagnosis of anemia in men is based on hemoglobin of less than 130 to 140 g/L (13 to 14 g/dL); in women, it is less than 120 to 130 g/L (12 to 13 g/dL). Anemia is the most common blood disorder, affecting about a third of the global population [1]. The number of visits to emergency departments with anemia as the primary diagnosis: 890,000 [2]. In 2019, anemia due to iron deficiency resulted in about 5254 deaths in the US, 1.6 deaths per 100,000 populations [3]. It is more common in women than men during pregnancy, and in children and the elderly [1, 2]. Anemia increases the costs of medical care and lowers a person's productivity through a decreased ability to work [3]. Alfalfa is used in Iranian traditional medicine as a treatment for anemia [4].

Medicago sativa Linn. (Leguminosae), commonly known as the “father of all foods” (al-fal-fa), is a perennial herbaceous leguminous plant species that originated in Asia [5]. It has trifoliate dentate leaves with an underground stem that is often woody [6]. This is the most ancient plant, cultivated throughout the world as a fodder plant. In America, *M. sativa* has been extensively cultivated since the arrival of Europeans. *M. sativa* has been grown for a variety of purposes such as soil improvement, animal feed, and medicinal uses [7].

It is considered beneficial in bladder disorders, blood clotting disorders, boils, cough, diuresis, gastrointestinal tract disorders, breast cancer, cervical cancer, kidney disorders, prostate disorders, appetite stimulation, inflammation, increasing breast milk, asthma, indigestion, insect bites, jaundice, menopausal symptoms, allergies, increasing nutritional support, stomach ulcers, increasing peristaltic action of the stomach and bowels, thrombocytopenic purpura, uterine stimulant, rheumatoid arthritis, scurvy, vitamin supplementation (vitamins A, C, E, K) and wound healing [6, 7].

M. sativa has been reported to contain a variety of phytochemicals. It has the following different classes of phytoconstituents: alkaloids, digestive enzymes, carotenoids, coumarins, flavonoids, minerals, organic acids, non-protein amino acids, polyamines, phytoestrogens, phytosterols, phenolic compounds, proteins, saponins, vitamins, volatile components, etc. [4].

Several studies indicate that the ingestion of *M. sativa* reduces cholesterol absorption and atherosclerotic plaque formation in animals [9]. Saponins of *M. sativa* top (stem and leaves) have been reported to decrease plasma cholesterol concentrations, decrease intestinal absorption of cholesterol, increase excretion of neutral steroids and bile acids, prevent atherosclerosis, and induce the regression of atherosclerosis [10]. Hypocholesterolemic activity has been reported for root saponins when given to monkeys receiving a high-cholesterol diet [11]. In a study, the ability of

the *M. sativa* plant to reduce liver cholesterol accumulation in cholesterol-fed rats was enhanced by the removal of saponins. Therefore, *M. sativa* saponins appear to play an important role in neutral steroid excretion but are not essential for increasing bile acid excretion [12]. In an experiment with prairie dogs, the lowest incidence of cholesterol gallstones was served with a diet of a higher fiber content (85% alfalfa) [9]. This plant is used mixed with rice as one of the local foods in some regions of Iran.

Fifteen patients with hyperlipoproteinemia (HLP), types IIA (n = 8), IIB (n = 3), and IV (n = 4) were given 40 g of heat-prepared alfalfa seeds 3 times daily at mealtimes for 8 weeks with otherwise unchanged diet. In patients with type II HLP alfalfa treatment caused after 8 weeks a maximal lowering of pretreatment median values of total plasma cholesterol from 9.58 to 8.00 mmol/1 and low-density lipoprotein (LDL) cholesterol from 7.69 to 6.33 mmol/1, which corresponds to decreases of 17% and 18%, respectively. The maximal decrease was 26% in total cholesterol and 30% in LDL cholesterol. In two patients with hypercholesterolemia, the LDL cholesterol decreased by less than 5%. Apolipoprotein B decreased in the same period from 2.17 to 1.43 g/1 in type II HLP, corresponding to a 34% decrease, whereas apolipoprotein A-I did not change [13].

The effect of *M. sativa* leaves on blood parameters related to anemia and also on increasing blood cholesterol levels (traditional medicine claims), has not been done so far. Thus, the present study attempted to examine the effect of oral consumption of *Medicago sativa* leaves on blood lipids and blood factors associated with anemia in healthy volunteers in order to investigate the claims of traditional medicine.

2. Materials and Methods

2.1. Plant Identification and Extraction

The leaves of alfalfa (*Medicago sativa*) after preparation in the fall of 2013 were approved in the Medicinal Plants Laboratory of Shahid Beheshti School of Pharmacy (Herbarium Code-104-HSP by M. Kamilinejad). Maceration by 96° ethanol (for three consecutive nights) was used in extraction. After evaporation of the solvent, at a temperature of less than 50° C, 350 g of extract was obtained from 7 kg of alfalfa leaves, which has an efficiency of 5%. Alfalfa extract was mixed and pulverized with corn starch. The resulting powder was filled in capsules by the manual device. Finally, 250 mg capsules were prepared as a medicine and corn starch as a placebo using a manual capsule-making machine (in GLP conditions). Volunteers had to take three capsules a day, which is an average of 750 mg of plant extract daily. The dose of the extract was determined based on the dose of the dry aerial part of the plant [14].

2.2. Inclusion Criteria

Inclusion criteria included: completion of the project consent form, age range 25-60 years, normal liver function (ALT, AST <3 ULN), and normal kidney function (GFR> 60 ml/min); exclusion criteria included: medications containing iron, vitamin B_{12}, folic acid, and blood lipid-lowering drugs (during 1 month before participation), diseases such as intestinal

disorders and chronic diseases such as cancer and a history of allergies to alfalfa. 19 healthy men (college workers) were randomly divided into two groups of drugs (9 people) and placebo (10 people). The placebo was corn starch. The capsules were taken daily for two months at a dose of three per day. Blood samples were taken at intervals before, one month, and two months after taking the capsules. Patients were instructed to notify in the event of an allergy or any other unbearable complication (excluding any case). Laboratory tests to measure complete blood count (CBC), Iron, Ferritin, total iron-binding capacity (TIBC), Cholesterol, Triglyceride, LDL, and HDL were performed in the Central Laboratory of Taleghani Hospital (Shahid Beheshti University of Medical Sciences, Tehran). This clinical study has been registered with the number 2014083178032N in Iran Clinical Trial Center (IRCT).

2.3. Sample Size Calculation

In clinical trials, the sample size is calculated based on previous articles, and since a similar work sample was not performed on this plant or on these blood factors and the present clinical study was designed and performed for the first time, the sample size includes the maximum number of volunteers.

2.4. Data Analysis

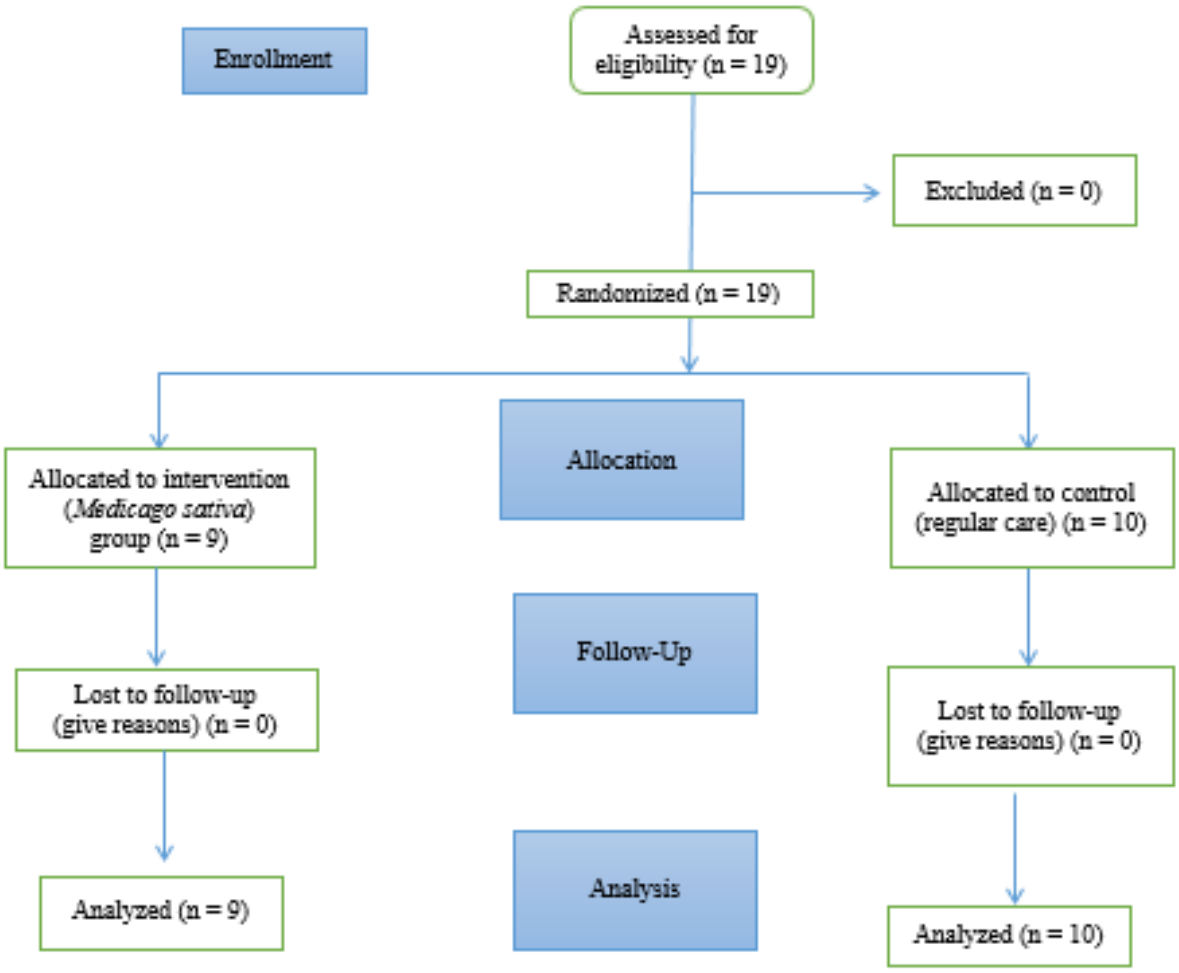
An unpaired T-test was used to compare the age of the control group with the experimental group. In other results, the data were expressed in Mean and SD. To compare the means and homogeneity of the data, D'Agostino & Pearson test was used to determine the type of distribution. Because the data distribution was normal, the 2-Way ANOVA method and then Bonferroni's auxiliary test was used to determine the significance of the differences between the groups. Values of $P < 0.05$ were considered as the quorum for significant differences.

3. Results and Discussion

Based on the main conditions for inclusion and exclusion criteria in the study, a total of 19 volunteers with an average age of 35 years, consumed alfalfa extract capsules (9 people) (SD=32.2±13.64) and placebo group (10 people) (SD=39.22 ±7.52). None of the volunteers were excluded from the study. To compare the age of patients between the two groups, which was a small variable with a distance scale, and due to the normal age of the volunteers in the two groups, a t-test was used and there was no significant difference between the two groups (**Scheme 1**).

To evaluate the effect of capsules on iron indices, changes in ferritin factor during two months of capsule use were investigated. In the control group, ferritin increased in the first month, unchanged in the second month, and increased in the whole two months. It also decreased in group therapy in the first and second months and a total of two months (P Value> 0.99) (**Figure 1**). Regarding the effect of capsules on changes in iron factor during two months of use, in the control group, serum iron decreased in the first month, increased in the second month, and increased for a total of two months.

Also in the treatment group, serum iron decreased in the first month, increased in the second month, and increased more in two months than in the control group (P Value> 0.99) (**Figure 2**). Total Iron Binding Capacity (TIBC) in the control group increased in the first month



Scheme 1. CONSORT flow diagram.

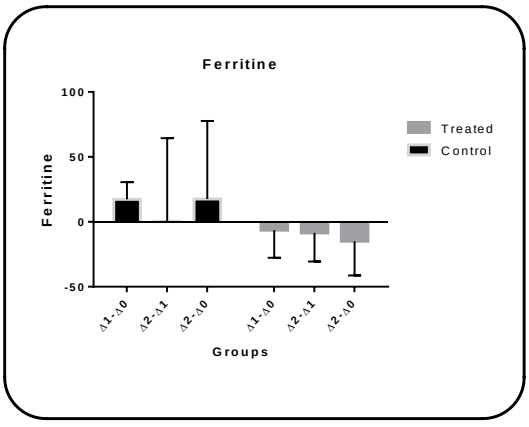


Figure 1. The effect of drug and placebo capsules on ferritin.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

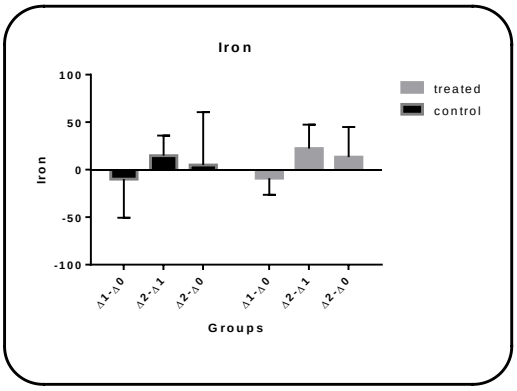


Figure 2. The effect of drug and placebo capsules on serum Iron.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

and decreased in the second month and decreased in the whole two months. TIBC in group therapy also increased in the first month and decreased in the second month and decreased for a total of two months (P Value> 0.99) (**Figure 3**). The effect of

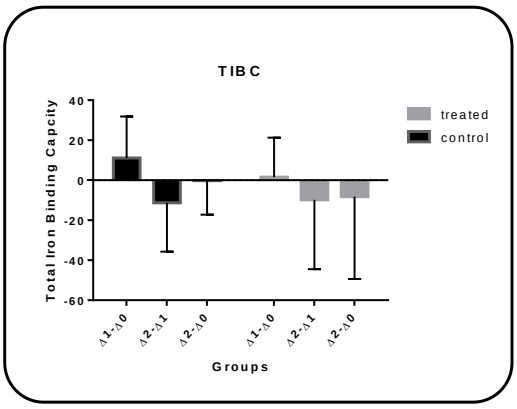


Figure 3. The effect of drug and placebo capsules on blood TIBC.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

capsules on hemoglobin changes for two months was investigated, too. In the control group, hemoglobin decreased in the first and second months and a total of two months. In case group, it decreased in the first month, increased in the second month, and decreased in total for two months (P Value> 0.99) (**Figure 4**). Triglyceride

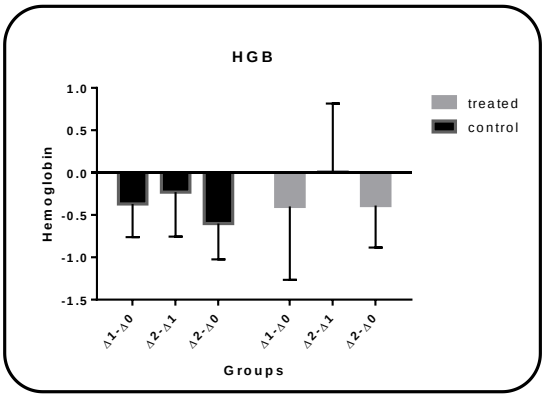


Figure 4. The effect of drug and placebo capsules on Hemoglobin.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

(TG) decreased in both control and treatment groups but decreased more in the treatment group (P Value> 0.99), too (**Figure 5**).

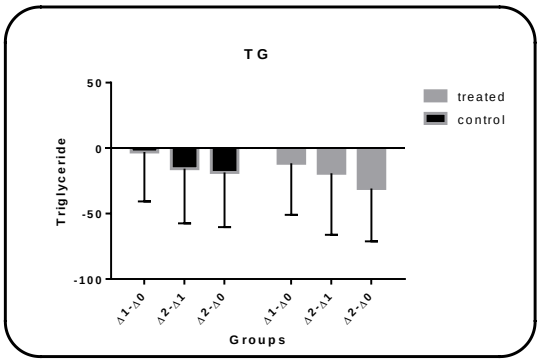


Figure 5. The effect of drug and placebo capsules on total blood TG.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

Total cholesterol also decreased in both groups during two months of drug use, but was further reduced in the treatment group (P Value> 0.99) (**Figure 6**). Also, LDL decreased during

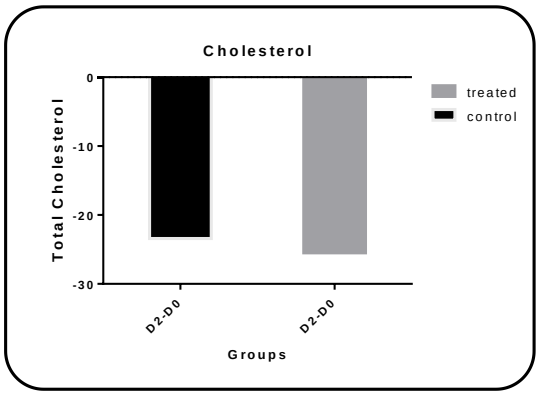


Figure 6. The effect of drug and placebo capsules on total blood cholesterol.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

two months of capsule use in both groups (**Figure 7**). HDL increased in the control group in the first month and decreased in the second month and increased for a total of two months, too. In the treatment group, it increased in the first month, decreased in the second month, and increased

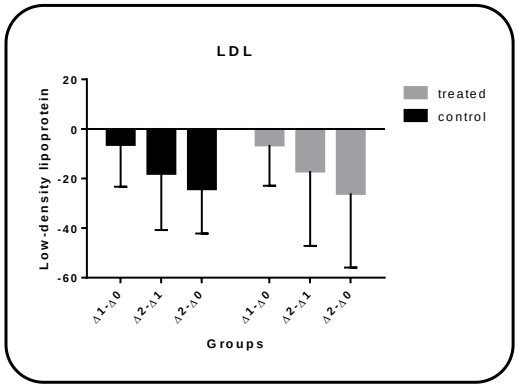


Figure 7. The effect of drug and placebo capsules on LDL factor.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

more in total for two months (P Value> 0.99) (**Figure 8**). SD seems too high, which may be due to the inaccurate use of drugs by volunteers.

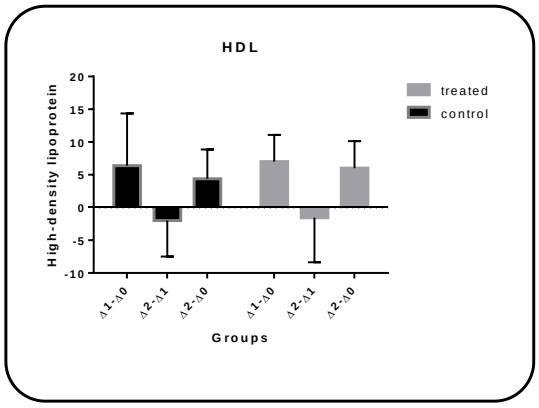


Figure 8. The effect of drug and placebo capsules on HDL factor.
The period of the 1st month of taking the capsules Δ0-Δ1, The period of the 2nd month of taking the capsules Δ1-Δ2, The period of the 2 months of taking the capsules Δ0-Δ2

The results of this study demonstrated that after two months of using alfalfa capsules, serum iron in the drug group increased more than in the control group, although it was not statistically significant. In taking iron supplements, first serum iron increases, and more time is needed to observe an increase in hemoglobin and ferritin. It can be concluded that the consumption of alfalfa extract was done in two months, and this period was not enough to increase ferritin and hemoglobin, we only saw an increase in serum iron levels of volunteers. One of the important reasons that the difference between the two groups is not significant can be the low number of patients and the limitation in admitting patients to enter the study.

TIBC shows the Total Iron Binding Capacity to the transferrin protein. TIBC decreased in the drug-treated group over two months, indicating greater iron uptake by transferring from the gut. Alfalfa is said to have twice as much iron as spinach (5.5 mg %). In the treatment group, we saw an increase in transferrin iron intake compared to the control group. It can be said that consuming alfalfa extract at a dose of 750 mg per day may help treat iron deficiency anemia. More effects can probably be seen by increasing the dose and duration of use and taking the capsules of the plant extract on an empty stomach.

Hemoglobin decreased in the control and treatment groups but decreased less in the treatment group. Because hemoglobin shows its changes with delay, the decrease in hemoglobin is related to the nutrition and condition of the volunteers before taking the capsules.

Ferritin increased in the control group and decreased in the treatment group. Because the nutritional content of the two groups may be different, despite the necessary precautions and warnings, these changes can be justified. Triglycerides were significantly lower in the herbal group than in the control. Alfalfa may help lower triglycerides and could be used as adjunctive therapy in hypertriglyceridemia.

LDL was further reduced in the herbal capsule group than in the control. Alfalfa may

also help lower blood LDL and may be used as adjunctive therapy in hyperlipidemia. In the treatment group, we also saw an increase in blood HDL levels during two months of taking the drug. One of the limitations of the study was the age and sex and the number of participants in the study, and the other was that the subjects were not sick and all were healthy. It is recommended that this be done on elderly patients with hyperlipidemia (both men and women).

In the treatment group, we saw a significant reduction in total cholesterol levels of participants taking herbal extract capsules. It is possible to reduce the risk of stroke and atherosclerosis by consuming these capsules that reduce harmful fats. The capsules should be taken three times a day with food to prevent the absorption of harmful fats.

The results of this study are consistent with the study of other researchers on the effect of alfalfa on lowering blood lipids. In a study in which heat-treated alfalfa seeds (40 g, three times a day for 8 weeks) were consumed by eight patients with type 2a hyperlipidemia and three patients with type 2b, a significant reduction in total serum cholesterol concentration, LDL and apolipoprotein B were observed. In two of the 11 patients' concentrations of LDL less than 5% were reduced [15]. In another study, alfalfa with the highest amount of saponin content just before fruiting showed high anti-atherosclerosis and hypocholesterolemic activity. This study showed that alfalfa lowers natural cholesterol. The extracts had the most significant effect in reducing total cholesterol and LDL by 85.1% and 88% respectively, in rabbits with high cholesterol. This reduction is much greater than the reduction made by gemfibrozil in the same animal at the same dose [13]. Recent studies have also mentioned the antihyperlipidemic effect of alfalfa, and they also confirm the effect observed in the present article [16, 17]. Also, alfalfa ethanolic extract, with 750 mg/day can help to increase serum iron, in animal studies; alfalfa extract consumption has an anti-anemic effect based on increasing hemoglobin levels [18].

4. Conclusion

The conclusion is that alfalfa extract can be used to help treat hyperlipidemia, but it seems that more research is needed to help treat iron deficiency anemia, and the decision is a bit early. The exact mechanism of these effects is unknown, and obviously goes back to the effective compounds of alfalfa such as saponins, phenolics, etc.

According to this study, 750 mg of *Medicago sativa* extract for two months can increase serum iron, and lower cholesterol, and triglycerides, but its use in the treatment of anemia and hypercholesterolemia requires further clinical studies.

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Conflict of interest

The authors declare to have no conflict of interest.

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