Allium hirtifolium Boiss: Radical scavenging property and the lowering effects on blood fibrinogen and factor VII

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Abstract: Enhancement of blood fibrinogen or factor VII increases cardiovascular diseases. *Allium hirtifolium* Boiss (Mosir) has been shown to have cardioprotective effect. This study, therefore, aimed to evaluate the effects of *Allium hirtifolium* Boiss on factor VII and fibrinogen blood levels. Its radical scavenging property was also measured. Twenty four NewZealand male rabbits were randomly designated into 3 groups of 8 and were fed for 60 days with normal diet, hypercholestrol (1%) diet or hypercholestrol (1%) diet+ Mosir. At the beginning and 60 days after the start of the study, the blood fibrinogen and factor 7 were measured and compared in different groups. The Mosir radical scavenging property was measured using the beta-carotene linoleate method. The blood fibrinogen and factor 7 were higher in hypercholesterolemic group (26.7 ± 329.22 and 17.1 ± 277.7mg/dl) compared to normal diet group (13.7 ± 287.25 and 18.2 ± 230.0 mg / dl, respectively) ($P<0.05$), at the end of the experiment. The amount of blood fibrinogen and factor 7 were decreased in hypercholesterol+Mosir group (23.9 ± 180.00 and 53.3 ± 237.0 mg / dl) compared to hypercholesterol diet group ($P<0.05$). radical scavenging activity of Mosir extract was 52.1 ± 3.3%. Mosir may have beneficial effect on heart by decreasing blood fibrinogen and factor 7 as cardiovascular risk factors. These effects of Mosir should be considered carefully in patients with hemostatic disorders.

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Introduction:

Cardiovascular diseases, especially atherosclerosis, are growing rapidly and are considered the first cause of death in industrialized societies (1, 2). Studies done in America and Europe have estimated occurrence range of cardiovascular diseases at 3 to 8 percent. According to surveys conducted by the Ministry of Health and Medical Education, 5.38 percent of deaths in Iran occur due to coronary artery disease (1). Studies have shown that regulation of hemostatic and Thrombogenic factors potential by simple changes in lifestyle can be considered as primary or secondary prevention (3).

Today, the factor VII and fibrinogen activity (Hemostatic factors) have been recognized as risk factors involved in cardiovascular diseases. Now, different procedures and medications including supplements of vitamins E, folic acid, B6 and B12 vitamins, and if necessary, anticoagulant drugs are used for the control of these risk factors and prevention of vascular disease (4, 5). However despite the use of these supplements, it seems that outbreak of atherosclerosis, progression of acute coronary syndromes and brain ischemia or ischemia in other tissues can be still prevented by inhibiting the coagulation and inflammation activity.

Factor VII is a vitamin K dependent factor that is produced by hepatocytes and is released in the blood stream. High plasma level of factor VII is associated with a risk increase in development of arterial thrombosis. 25 percent increase in factor VII activity increases risk of fatal coronary artery disease by 55 percent (6). It also has been identified that factor VII levels is associated with serum triglyceride levels. Nutrition with a high fat diet caused increase in the lipoproteins and triglycerides concentration and lipolysis of large lipoprotein particles causes increase in activated factor VII (7). Factor VII activity levels in people who have a high fat diet is 16 percent more than those who consume low-fat diet (8).

Fibrinogen is a protein with a high molecular weight produced by the liver and its normal average level is 250mg/dl. Plasma fibrinogen levels increase in inflammatory, malignant and liver diseases (9). Plasma

fibrinogen increase could be prelude to clot formation increase (10). Although the increase in plasma fibrinogen associated with other risk factors for coronary artery disease such as age, smoking, high blood pressure, elevated blood fats, diabetes and obesity can be seen, but fibrinogen as an independent risk factor also plays a role in the development of atherosclerosis disease (11, 12). Fibrinogen by affecting on plasma viscosity fibrinogen, platelet aggregation and the fibrin level which it forms provides the risk of developing coronary artery disease (14, 15).
Several studies have shown the relationship between plasma fibrinogen level and severity of coronary artery disease in the angiography. Most of these studies consider this matter mainly due to the obstruction of the vessel lumen which is a sign of this subject that plasma fibrinogen increase is a thrombogen factor (16). Reports show correlation between components of the clotting system (fibrinogen and factor VII) and fibrinolytic factors such as tissue plasminogen activator (TPA) and plasminogen activator inhibitor (PAI) and atherosclerosis (17).
Studies show that flavonoids and phenolic compounds in plants have many biological effects including antioxidant properties, inhibition of free radicals and anti-inflammatory effects (18). According to increasing prevalence of heart disease and reduction in age of getting this disease, investigating for new drugs and herbs for control and prevention of atherosclerosis plaque and reduction in cardiovascular disease risk factors is important. Shallot (*Allium hirtifolium* Boiss) is a species of tulips large family consisting of about 500 known different species. The family in addition to shallot includes other important species known as garlic, onions and leeks which have food and drug application throughout the world (20, 19). Allium plants use has been common as local traditional drug since ancient times and such plants have been used for centuries because of its medicinal value and properties. These plants are rich in flavonols and organosulfur compounds and have shown the anticancer properties in *in vitro* studies (21). Shallot is a traditional plant like garlic, but its chive is darker than garlic and is often used as a food flavoring in the diet. Heretofore, numerous studies have been conducted on the properties and characteristics of shallot which among them we can mention Hypocholesterolemia effects (22), having activity of inhibiting hemolysis and depletion of glutathione due to stress in human erythrocyte (23), hypoglycemia and hypercholesterolemia effects (24, 25), antibacterial effects (26), high antioxidant potential (27) and the Hematological effects (28). Moreover, some of effective compounds in this plant such as antifungal peptide askaline (29) and mannose-specific lectin (30) have been identified and isolated. The results showed that edible prescription of alcoholic extract of shallot for 20 days cause decrease in total cholesterol level and LDL and increase in HDL and prescription of this plant has no effect on serum triglycerides level (31). Also it has been proven that prescription of shallot extract in male rats could reduce lipid peroxidation due to having antioxidant (31). As for the existing compounds in shallots and its hypocholesterolemic effects and effect of blood hemostatic potential on atherosclerosis and cardiovascular diseases, if this plant causes decrease in hemostatic factors, it could be very helpful in cardiovascular diseases. Since the plant was proposed as a powerful antioxidant compound and some reports suggest its protective effect on cardiology system, therefore the aim of this study was to evaluate the shallot effect on fibrinogen and Factor VII in rabbits consuming high cholesterol. The Mosir radical scavenging property was also measured using the beta-carotene linoleate method.

Methodology

Extraction

In this experiment, the collected shallots from Koohrang area in Chaharmahal & Bakhtiari province was approved by Medicinal Plants Research Center of Shahrekord University of Medical Sciences and the extract obtained from it were used intraperitoneally in rabbits. During the period, body weight and food intake were measured.
After drying shallot in the shade and crushing, act of the extraction was performed at temperature of 20 -15 with 80 percent ethanol. For this purpose, the powder was soaked in solvent. 100 g of plant powder was poured in 500 ml of 80% ethanol. 48 hours later, after filtering the extract, extraction repeated twice and the collected plant extract was transferred to the vacuum distillation unit and concentrated. Then it was dried at a temperature of 40 ° C (32).

Grouping and rabbits treatment

In an interventional study, 24 New Zealand male rabbits weighing 9/12 ± 2010 g were purchased from Razi Institute, Karaj and were kept in animal kennel of Medicinal Plants Research Center, Shahrekord University of Medical Sciences for two weeks at an appropriate temperature and humidity, hours of natural darkness and light and standard basal diet and then were treated. Rabbit nourishment was done by standard prepared grain food purchased from Pars feed company (Tehran) which contains 15% protein, 40-50% carbohydrates, 2% vegetable fat and 15 to 25% fiber. In order to prepare food after scientific confirmation, the obtained powder after grinding cholesterol with weight ratio of 0.01 was mixed with the powdered and standard food and animal feed was produced again. During the experiment period the animals had access to

enough food and water. Rabbits were randomly divided into 3 groups of 8 as described below and were under the various regimes for 60 days as follows: Basal diet, high cholesterol diet (1%), high cholesterol diet (1%) + shallot extract (1g/kg BW) (32). Shallot extract was injected intraperitoneally once a day (about 11 am) for 60 days.

Biochemical factors measurement:

Before the beginning and end of the study, the animals were fasted for 12 hours, and blood samples of rabbits were taken from the central ear artery. Blood taken from rabbits was poured in two separate tubes to prepare serum and plasma. Tubes with specific number and date were centrifuged for 20 minutes at 3500 rpm in order to prepare serum and plasma. Fibrinogen level was measured by Mahsayaran kit and factor VII was measured by STA-Deficient VII kit (15).

Measurement of the antioxidant activity by beta-carotene-linoleate method

Beta-carotene-linoleate method was used for measuring antioxidant capacity (33). Two milligrams of beta-carotene was solved in 2/0 ml of chloroform. 20 mg linoleic acid and 200 mg Tween 40 were added to the emulsion. 40 ml of water saturated with oxygen was added to the above materials. Shallot extract was prepared at a concentration of 2/0 milligrams per liter in pure ethanol. 4 ml of the prepared solution was added to 2 ml of the prepared extract and control (ethanol). Antioxidant activity of extract based on Beta-Carotene Bleaching rate at a wavelength of 470 nm and at 180 minutes (15 minute intervals), and by using the following formula was calculated and the mean of obtained values was considered as the percent of antioxidant activity of the extract.

Formula 1:

$$AA = 100\ [1 - (A_o - A_t) / (A^o_o - A^o_t)]$$

A_o and A^o_o represent the absorbance of light at time zero, A_t A^o_t represent the absorbance of light at different times during 180 minutes for the sample and control (33).

Measurement of plasma antioxidant capacity:

Plasma antioxidant capacity was measured by using Tripyridyl Triazine (*TPTZ*) (Sigma Co, USA) and based on dr Heydarian et al method. Reduction of ferric to ferrous ions in the presence of antioxidants creates colored ferrous tri-pyridyl-triazine complex which has maximum absorbance of light at 593 nm. $FeSO_4$ in the range of 100 to 1000 mM was used in order to draw the standard curve. In this method, first a working solution was prepared with a ratio of 10:1:1 from 10 mM triazine in 40mM chloridric acid, 20 mM solution of FeCl3 (Merck, Germany) and 300 mM acetate buffer (buffer solution 10 volume, each of triazine and FeCl3 1 volume). Then 25 µl of each sample's plasma was poured in clean tubes and 1/5 ml of working solution was added to each tube (One test tube was prepared for blank that instead of sample, distilled water was added to it). The tubes were incubated for 10 minutes in water bath at 37°C and subsequently each sample's absorbance of light relative to blank at a wavelength of 593 nm was read (34).

Statistical Analysis:

Results were analyzed using Instat 3 software. To investigate the biochemical results and comparison of the experimental groups, Kruskal - Wallis and Dunn tests were used and $P<0.05$ was considered statistically significant.

Results:

The results of this study showed that at the beginning of the period, biochemical factors values among the study groups were not significantly different ($P> 0.05$). Comparison of blood's fibrinogen level in the basal diet (normal) and high-cholesterol diet (1%) groups indicated that fibrinogen level in high-cholesterol group compared to basal diet group had significant increase ($P<0.05$). Also significant reduction was observed in high-cholesterol+shallot group compared to high-cholesterol diet ($P<0.05$) (Table 1). Factor VII level in high-cholesterol group had significant increase compared to basal diet ($P<0.05$) and significant decrease in high-cholesterol+shallot group compared to high-cholesterol diet was observed ($P<0.05$) (Table 1).

Table 1: Mean (mg/dl) of fibrinogen and factor VII levels in the diet groups under study

	diet	Fibrinogen ± SD	Factor VII ± SD
1	Basal (normal)	287/25±13/7	230/0±18/2
2	High-cholesterol (1%)	329/22±26/7a	277/7±17/1a
3	High cholesterol (1%) + shallot	180/00±23/9b	237/0±53/3b

a:$P<0.05$,compared with the basal diet group

b:$P<0.05$,compared with high-cholesterol diet group

In this study, the antioxidant capacity of shallot extract with concentration of 0.2 g / L, equivalent to 52.1 ± 3.3%, was obtained. The antioxidant capacity of plasma in shallots + high-cholesterol group was obtained 943.907 ± 249.51μM that was far more than the basal (normal) group, 629.675 ± 130.73μM. The results showed that there is a significant correlation between the antioxidant capacity of shallots + high-cholesterol group (943.907 ± 249.51μM) and antioxidant capacity of samples before the intervention (440.089 ± 99.99μM) (P <0.001).

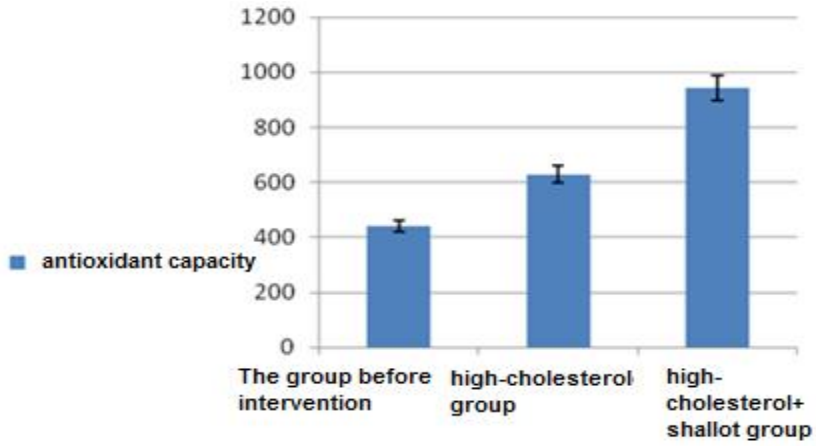

Figure 1: Comparison of the antioxidant capacity of plasma samples in tested groups (μM)

Discussion:

The results of this study showed that groups treated with shallots had significant decrease in fibrinogen and factor VII levels compared to the high-cholesterol group. Disorders of fibrinogen and coagulation are related to the development of cardiovascular diseases such as coronary artery disease, hypertension and ischemic shock (34).

Some vegetarian diets are effective in reducing concentration of clotting factors or increasing fibrinolysis and are involved in this process by reducing blood clotting with reducing fibrinogen, inhibition of platelet aggregation and increasing prothrombin time (35). The results have shown that edible prescription of alcoholic extract of shallot for 20 days cause decrease in total cholesterol level and LDL and increase in HDL (25). HALL studies in 1996 showed that there is a relationship between HDL and fibrinogen. People who have high HDL levels have low fibrinogen levels. The inhibitory effect of HDL on factor X activates proteins C and S (these proteins may inhibit coagulation factor V) that these factors reduce production of fibrinogen. HDL also inhibits platelet aggregation. Therefore, decrease in fibrinogen could also be due to an increase in HDL. Effect on reducing blood cholesterol is done by inhibiting production of HMG-CoA reductase. This enzyme causes conversion of hydroxymethyl glutaryl-CoA to mevalonic acid and its revival which is a one-way reaction and occurs in endoplasmic reticulum of cells (36). Studies have shown that garlic powder has anti-thrombotic effects due to having allicin, level of thrombin-antithrombin(III) complex have been decreased to normal level (37). Allicin is the major factor for its beneficial effects against blood lipids, blood pressure and blood clotting (36). It's recognized that long-term and edible use of shallot causes reduction of free radicals and protecting cells against chemical damage, reduction of lipid peroxidation and protecting liver against a variety of stresses that its main reason is its high level of antioxidants (38). Therefore, the use of the plant applies protective effects on body tissues and works toward reduction of oxidative stress (39). Flavonoids such as coarsetin, compferrol, myristin are inhibitors of platelet aggregation. Given that Shallot has several medicinal effects such as antioxidant property (39) make polyphenols rapidly absorbed and cause increase in the blood concentration to induce expression increase of mRNA and fibrinolytic proteins such as t-PA and setup reduction of PAI (40), which could partly explain the anti-inflammatory effects and improve blood lipid profiles and reduce lipid peroxidation in hypercholesterolemic individuals (8). Treatment with antioxidant factors such as Allium ampeloprasum with high doses of cysteine sulfoxide compounds can be effective in this regard (41). Furthermore it has been recognized that the antioxidant effect of this group of materials is applied by

increasing enzymes level related to dismutase and catalase antioxidant systems. On the other hand, this material is able to reduce the production of end products of lipid peroxidation such as malondialdehyde and hydroproxside (42). On the other hand, part of observed effects of the plant use in the present study should be attributed to the high percentage of anthocyanins with protective properties (41). Thus, by inhibition of coagulation and inflammatory activities of platelets, atherosclerosis and acute coronary syndromes and brain ischemia or ischemia in other tissues can be likely prevented.

Conclusion:

By considering the use of shallot in modern societies and according to its antioxidant effect and its certain role in the occurrence and development of various chronic diseases including cardiovascular disease, its preventive role in the incidence of these diseases is so important. In addition, according to researcher's interest for the discovery and use of drugs with natural origin because of fewer side effects to treat these diseases, shallot plant can be more considered in this field. Of course, finding probable effective compound or compounds and their affecting mechanism in cardiovascular disease is an issue that its clarification requires further and more quantitative research.

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