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Original Research Article

Effects of Hydroalcoholic Extract of Dill (Anethum graveolens) on the serum levels of blood lipids (cholesterol, Triglycerides, LDL and HDL) in male NMRI mice

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ABSTRACT

Increasing blood lipids, particularly cholesterol and triglyceride levels are dangerous factors causing cardiovascular disease and myocardial infarction in humans. The aim of this study was to investigate the effect of drinkable hydroalcoholic extract of dill on the serum cholesterol, Triglycerides, LDL and HDL levels. This study was conducted on 18 male NMRI mice. The mice were divided into 3 groups (n=6). The first group was given tap water as the control group. The second group, the extract at a dose of 250 mg / lit and the third group extract at a dose of 500 mg / lit were given in drinking water for 30 days. After the period of treatment under complete anesthesia, blood samples were taken from animals and serum cholesterol, triglycerides, LDL and HDL levels were measured. The result showed that consumption of hydroalcoholic extract of dill results in significant reducing of cholesterol, triglycerides and LDL in both extract - receiving groups compared to control group($P<0.001$). Also, the extract caused a significant increase in HDL levels in both treatment groups compared to the control group ($P<0.001$); meanwhile, these changes were dependent on the dill extract concentration. Results obtained in this study suggesting that administration of hydroalcoholic extract of dill via drinking water has effective concentration-dependent effect in decreasing cholesterol, triglycerides and LDL and increasing HDL.

Keyword: Dill; LDL; HDL; Cholesterol; Triglycerides

INTRODUCTION

Heart disease is often caused by blockage of the coronary arteries due to atherosclerosis [1]. Increasing oxidized LDL and the formation of foam cells rich in fats are two important and decisive factors in the formation and development of atherosclerotic lesions. In fact, Foam cells form, while macrophages in the artery wall remove a large amount of oxidized

LDL which is active in terms of antigenic and cholesterol esters accumulate in large amounts in the their cytoplasm. Formation and accumulation of foam cells is a major factor in the development of fatty streaks, complex lesions and atherosclerotic plaques [2,3,4].One of the most important sources of medicine are plants from a long time ago which are used for prevention, health, treatment and relief from work. Currently, about 50 thousand plant species are used in the preparation of medicines due to combination of secondary compounds such as essential oils, aromatic, resin, sap and gum in various organs such as roots, stems, leaves, flowers, fruits and the seeds [5]. Dill with the scientific name of *Anethum graveolens* is a plant from the Apiaceae family. It is read Dill in English. Dill fruit have 1/2 to 7/7 percent oil, dillanoside, coumarin, kaempferol, vicenin, myristicin and other fiavonoids, phenol acids, proteins, fats and others [6]. In traditional medicine, dill is used as an antiseptic and anti-seizure and tonic, stomach tonic, carminative and relieve bloating, indigestion, anti-vomiting and spasms, making menstruation, soothing pain, laxative and reducing blood lipids [7] . Aqueous extract of dill seed is effective on plasma lipids and have more effect in reducing cholesterol and LDL compared to Lovastatin [1]. Findings from studying on New Zealand male rabbits suggested positive effects to the reduction of inflammatory cytokines and lipid and liver enzymes [8]. Kazemi et al stated that, dill can be used as one of the most effective drugs in the treatment of hyperlipidemia in human [9]. But a study conducted on 150 patients with hyperlipidemia was demonstrated that the dill extract could not modify lipids and plasma lipoprotein in Hyperlipidemic patients [10]. The study was performed on 88 normal and diabetic mice showed that, the combined dill extract have strong anti-lipidemic on normal rats fed with high fat diet, but it was not effective drug in reducing plasma lipids in streptozotocin-induced diabetic rats fed with high fat diet [11]. Using dill aqueous leaf extract for 14 days reduced levels of triglycerides and cholesterol in rats [12]. Considering this background it becomes clear that there are still contradictions in some studies, whilst different methods have been used. This study also aimed to evaluate the effect of dill hydroalcoholic leaf extract with two different concentrations and different method (via drinkable water) on cholesterol, triglycerides, LDL and HDL in small laboratory mice that this study had not been done yet on them.

MATERIALS AND METHODS

This experimental study was conducted on 18 male NMRI mice. Animals were taken from Razi Institute and they were transferred to the laboratory Animal House of Razi University. Animals kept in conditions of temperature, humidity, light, nutrition and other environmental factors were under control. A week before the start of the experiment, the mice were allowed to adapt to the conditions of the animal house. Then, the animals were divided into 3 groups of 6 as follows: Group1 the recipient of normal food and drinking water in drinkable water network lacking dill extract, Group 2 the recipient normal food and dill extract with concentration 250 mg / lit in drinking water, group 3 the recipient normal food and dill extract with concentration 500 mg /lit in drinking water (table 1). This dose of hydroalcoholic extract of *Anethum graveolens* is not toxic [13]. Proposal and experimental actions used in this investigation were reviewed by the Animal Ethics Committee of Razi University (1225/SF/AECRU).

Extract preparation method

Dill was used after preparing from farms around Islamabad and approving by an expert in plant systematic in order to prepare the hydroalcoholic extract. The leaves and stems of this plant were dried in the shade after

washing, and then dried leaf and stem were powdered in crusher. Amount of 200 grams of powder was poured into a liter flask and then 70% ethyl alcohol was added in a way that completely cover the surface of the powder and powdered plant is immersed in it ,then we completely blocked the door of flask with Nescofilm. The mixture was left for 72 hours in this condition. During this time, we shook thoroughly the container every 12 hours; next we were filtrated resultant compound with whatman filter paper 1. Then, we condensed the filtrate of the first and second process with rotary machine at a temperature of 60 ° C and rotation speed of 110 rpm up to one-third of the original volume. Obtained soluble poured into Petri dish and was dried on an electric heater with indirect heat with temperatures below 50 ° C and under sterile conditions. The resulting powder was kept in a freezer until testing [14,15].

Blood Sample collection

After 30-day treatment period, the animals were anesthetized by inhalation anesthesia with chloroform. Then put it into the supine position for surgery and during opening chest, blood samples were taken from the right atrium of the animal heart with a syringe 2 ml. After blood collection from the animal, blood samples for serum preparation was transferred to a sterile tube. After 1 hour, remaining constant in the Laboratory temperature for blood clotting, the samples were centrifuged with 3000 revolutions per minute (RPM) and for 15 min. Then clear serums had been removed by clean tip samplers from remaining sediment and were kept in clean vial until analysis. Unclear and dark colored serum samples were overlooked. The serum samples were taken for measuring to the Zagros lab.

Statistical analysis

In this study, the results obtained from the data were studied using Graphpad Prism statistical software which is an appropriate program for analysis of biological parameters. For comparing several groups, One– way analyze variance (ANOVA) and subsequent Turkey's test were used. For comparison between two groups, if necessary, statistical test in Student-t- test [2] was used. The data were expressed as Mean ± Standard Error Mean and for all values of difference P <0/05 was considered statistically significant.

RESULTS

Results of statistical analysis of serum LDL levels that were obtained in terms of milligrams per decilitre showed a significant reduction (P <0/001) between both experimental groups treated with the dill extract compared to control group. Also comparison of treatment group by the dill extract concentration of 500 mg / lit with the treatment group by the dill extract concentration of 250 mg / lit showed that LDL levels in the group treated with a dose 500 mg / lit compared to the group treated with a dose 250 mg / lit is reduced (P <0.05.) (Fig. 1A). The results of the measurement of serum cholesterol levels in milligrams per deciliter showed that cholesterol levels in both treatment group compared to the control group was significantly decreases (at p <0/001). Comparison of two treatment group showed that cholesterol level in the treatment group received concentration of 500 mg / lit compared to the group received concentration of 250 mg / lit is decreased (P = <0.05) (Fig.1B). The results of the assessment serum triglycerides in terms of milligrams per decilitre showed a significant reduction in both treatment group compared to the control group (P <0/001), as well as a significant decrease (P <0/01) in the amount TG levels in received 500 mg / lit dill extract compared to the treatment group received 250 mg / lit dill extract. (P <0.01). (Fig.1C). HDL level in the treatment group receiving dill extract with Concentration of 500 mg / lit has a significant increase

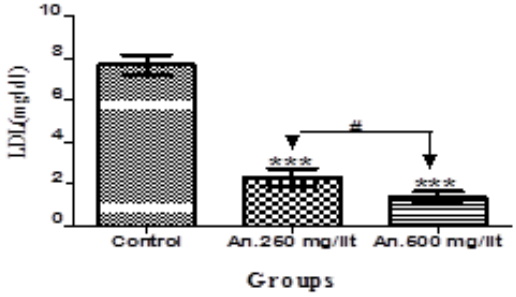

Fig. 1A

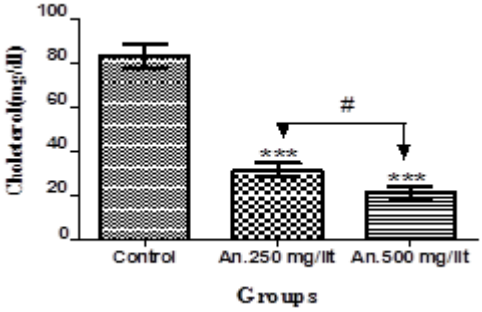

Fig. 1 B

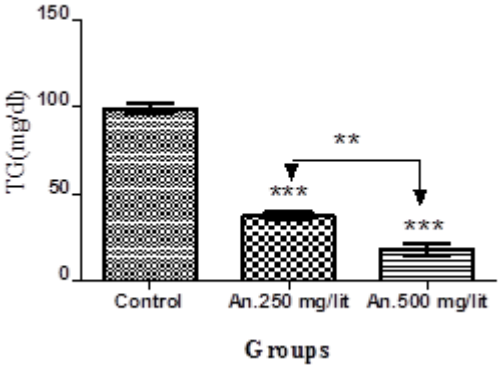

Fig. 1C

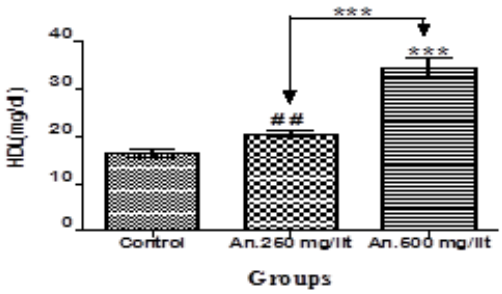

Fig. 1D

Fig.1: Effect of hydroalcoholic extract of dill on the serum cholesterol, Triglycerides, LDL and HDL level.

(A) The serum level of LDL significantly decreased both experimental groups treated with the dill extract compared to control group. (B) The serum level of cholesterol significantly decreased in both treatment group compared to the control group .(C) The serum triglycerides showed a significant reduction in both treatment group compared to the control group.(D) HDL level in the treatment group receiving dill extract with concentration of 500 mg / lit has a significant increase compared to control group. **=*P*<0.01, ***=*P*<0.001 and *t*-test. (#) = *P*<0.05, ##=P<0.01

Table 1: Estimate the amount of drinking water consumption and the amount of dill extract consumption per day and total period

Group	Extract Consentration mg/lit	Water consumption (ml/24 hour) For each Mouse	Water Intake (ml/30 day) For each Mouse	Extract Intake mg/ 24hour	Extract Intake mg/30day
G1: Control	-	6.143± 0.26	184.30±7.8	-	-
G2: 250 mg/lit	250	6.120 ± 0.54	183.60±16.2	1.53±0.14	45.90±4.05
G3: 500 mg/lit	500	5.957 ± 0.34	169±10.2	2.98±0.17	89.40±5.1

compared to control group (p <0/001), as well as the data analysis showed that serum HDL levels in treatments group receiving dill extract with concentration 500 mg / lit had have a significant increase compared to the treatments group receiving dill extract with concentration 250 mg / lit (P <0.001).

DISCUSSION

Increasing LDL-c causes the cholesterol from the blood goes to the vessels wall and deposit there**,** then the person affected with atherosclerosis, if these deposits is done in the arteries that supply the heart wall, the person exposed to myocardial infarction [16]. The findings of the our study showed that consumption of dill extract caused a significant decrease in serum and LDL cholesterol levels in both treatment groups compared to the control group. HDL levels have increased significantly in both treatment groups compared to controls. In a study on hyperlipidemic patients, it have been reported that, dill extract causes significant decrease in the triglyceride levels while lowering LDL cholesterol was not significant [17]. In another study which was done on the tendency of LDL to the related receptor, it had been shown that limonene (one of the combinations available in dill), had antioxidative effect and had increased LDL tendency (normal and oxidized) to the receiver LDL on adrenal cells levels [18]. Antioxidants are compounds that effectively and in different ways prevent free radical reactions in forms of active oxygen and nitrogen with bimolecular such as proteins, amino acids, lipids, DNA leading to a decrease in damage or cell death, cardiovascular disease and cancers [19]. The mechanism of action of antioxidant compounds in reducing lipids and lipoproteins are via inhibition of cholesterol biosynthesis and increased conversions cholesterol to bile acids, as well as increasing lipoprotein lipase activity, thus cholesterol concentration is reduced which is a component of lipoproteins and subsequently lipoprotein synthesis is reduced [20] This report strongly confirmed our findings. Dill by having flavonoids and quercetin, is known as a rich source of antioxidants [6]. Therefore in our study, it is likely, antioxidant activity may be serum lipids-lowering agent. It has been reported that, dill hydroalcoholic extract prescription via intraperitoneal injection has caused significant reducing in the level of total cholesterol, triglycerides, LDL, VLDL and increasing HDL [20]. Another report obtained from effects of dill on lipid profiles in mice suggesting that Serum triglyceride and LDL in hyperlipidemic mice decreases after using dill [21]. It has been reported that quercetin decreased cholesterol [22] and hence it is likely reducing cholesterol in the groups treated with dill extract due to the presence of quercetin. Presence of flavonoids and phenolic compounds in dill have been reported [23, 24,25]. According to a report in People, flavonoids in Hypercholesterolemic People cause reducing LDL-C and increasing HDL-C **[**26]**.** On the other hand, Flavonoids can increase the number of these receptors on the surface of liver cells while affecting on LDL receptor gene**.** With identification of available apoprotein, the receptor LDL attached to it and LDL is pulled into hepatocytes and is removed from the blood stream [27]. In a study to investigate the effects of dill leaf powder in hyperlipidemic patients conducted in the year 2012, was reported that a significant decrease in lipid profile and atherosclerosis index was observed [28]. It has been reported that dill in hyperlipidemic rats caused a significant decrease in the levels of cholesterol, triglycerides and a significant increase in LDL-c and HDL-c levels [29,30]. Results obtained of these experiments confirm the results of our experiments that with another method were performed on small laboratory mice. Kojuri et al examined the effect of dill and garlic on lipid

profile in hyperlipidemic patients, reported that prescription of dill tablet twice a day for 6 weeks had no significant effect on the lipid profile [10] so, results from this study not consistent with our survey. According to the studies, Carvone will effect on cholesterol biosynthesis [31]**.** Tannins is also effective in reducing appetite in animals and reduction of serum lipids [32] .Yugarani, et al who examined the effect of Tannic acid on serum lipid levels in hypercholesterolemic mice and normo cholesterolemic mice expressed that serum cholesterol levels in hyper cholesterolemic and normocholesterolemic mice fed with high-fat diet with Tannic Acid is lower than mice fed with high-fat diet without Tannic Acid [33] Therefore, because the tannin and Carvone is available in dill , so it is probably effective on reducing cholesterol and its biosynthesis. Phytosterols or plant sterols are three terpenes that are part of structural components of plant cell membranes and their function is similar to cholesterol in animal cell membranes [34]**.** It is also expressed that, Phytosterols through the reduction of harmful blood lipid reducing the risk of cardiovascular disease and mortality from these diseases is lowered [35,36]. Most plant sterols have two important medicinal properties, such as inhibition of tumor growth and helping regulate blood cholesterol [5].Significant results obtained in our study is that: firstly, prescription of dill hydroalcoholic extract in drinking water confirm most of the relating reports on the lowering effect of risk blood factors and secondly, the effect of dill hydroalcoholic extract is dependent on consumed concentration over a period of a month oral administration.

CONCLUSION

Results obtained in this study suggesting that administration of hydroalcoholic extract of dill via drinking water has effective concentration-dependent effect in decreasing cholesterol, triglycerides and LDL and increasing HDL. Dill hydroalcoholic extract in drinking water confirm most of the relating reports on the lowering effect of risk blood factors, therefore, may there are effective agents of Dill in its hydroalcoholic extract that may thought is useful for syrup production for reducing of blood fat from its extract .

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