Study of the effect of hydro alcoholic extract of walnut leaves on blood factor changes of IDI, HDI, trigly ceride and full cholesterol in female hypercholesterolemic rabbits.

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ABSTRACT

Introduction: walnut leaves contain phenol composition which reduce cardiovascular diseases, prevent oxidation of LDL in cells, prevent different types of cancers and stop creation of mutation as well as oxidation activity of lipids, therefore. The most important goal of the present paper is the study of the effect of the walnut leaf extract on blood factors and atherosclerosis in mature rabbits. Procedure: 30 mature female rabbits were prepared, and were divided into 6 groups of 5 rabbits. These groups were fed as follows: group 1 with normal diet, group 2 with corn oil (cholesterol solvent), group 3 with high cholesterol diet, group 4 with high cholesterol along with minimum dose of walnut leaf extract (100 milligram per kilogram), group 5 with high cholesterol diet along with a mean dose walnut leaf extract (200 milligram per kilogram) and group 6 with a high cholesterol diet along with a maximum dose of walnut leaf extract (400 milligram per kilogram). These diets were given during a period of 60 days through gavage. Then after anesthesia, blood samples were taken directly from their hearts, and the studies regarding the changes of blood factors were performed. Statistical data was then determined through ANOVA test (one-way analysis of variance) at the significance level (p<0.05) by SPSS software, version 18. Results: results obtained from the current study show that in group 3 (high cholesterol diet), the value of full cholesterol, triglyceride and LDL have a significant increase compared to those group 1 (normal diet). The thickness of HDL serum in experimental groups of 4, 5 and 6 showed a significant increase compared to those in control group (p<0.05). Also, the thickness of HDL in the experimental group 6 show a significant increase compared to that of the experimental groups of 4 and 5 while other measured factors did not show an important change compared to the control group. Based on the obtained results, the extract of walnut leaf can be considered as an effective factor in curing atherosclerosis illnesses in hypercholesterolemic rabbits and this effect is dependent on doses.

Key words: Walnut leaf, blood factor, atherosclerosis, rabbit

Introduction

Walnut is a very valuable and important tree, which is found in many parts of the world. In Iran, this plant grows on a height of 26 meters below sealevel in Mazandaran, to the geight of more than 2500 meters above sealevel in ChaharMahal Bakhtiari, and par from the coastal provinces of person gulf and th sea of oman, it is grown in other provinces of the country. Walnut belongs to the group of hidden-seeds, under the closs of cotyledons, the group of Amentales juglandacea family and the juglans material and its fruit is drupes. All parts of walnut, including the leaves and green skin of its fruits contain lots of medicinal compositions and have a useful effect on the health of humans [1]. The total of 13 phenol compositions including hydroxycinamic of acids which includes chlorogenic acid, kapheik, phrolic acid and synaptic acid, Hydroxi Benzoik of acids, the group which includes galic acid, protcateik acid, syringic acid and vanilyk, phlavanoids which includes katecline, epikatekin, mirsten and goglune; are identified in walnuts [2]. The herbal chemical materials such as phenol compositions, due to their useful properties, like antiradical property, preventability ability from oxidation of LDL and the hardness of other sclerosis, have the anti-cancer property, anti-oxidation and anti-microbe, and are useful for humans health [3,4]. Arthrosclerosis, is a vascular
disease and can involve aorta veins up to coronary veins. Arteriosclerosis is a progressive and slow process which starts from childhood and in adult hood it heads to clinical appearances. Interfering elements of this illness include genetic, diet, metabolic, homodynamic, inflammatory infectious and also other elements that are being identified. This illness is one of the main causes of fatality and disability in developed countries and most of the developing couontres [5,6]. Also, arteriosclerosis is a complicated process that we cannot determine one main element of danger for it because different elements and factors are involved with each other [7]. The most important cause of it is high consumption of fats and reduction of movement, and the increase of blood cholesterol is the most important danger factor in this illness [5,6]. Also, lipids in side blood are trans ferred in the form of a group of solved molecules in water which is called lipoprotein. In center of lipoprotein, strocholesterol and triglyceride and on their surface, a layer of phospholipids, free cholesterol and protein is located. Lipoproteins are divided to shilomicron, LDL,HDL and lipoprotein with very low density. LDL has 70 percent of the blood cholesterol and has the most important role in formation of arteriosclerosis, after oxidation [8]. Since arteriosclerosis is considered as an inflammatory disease, inflammation and the elements that affect it, are potential dangers [9,10]. Control of these danger elements are important in prevention of arteriosclerosis and today the use of medicinal herbs for controlling danger elements are considered [11]. About 50 percent of deaths are caused by arteriosclerosis [12]. There fore, with due regard to the importance and effects of walnuts and also the importance of prevention and control of having the cardio vascular disease and also, since so for no resurch has been carried out on the effects of walnuts on the changes of blood factors of HDL, LDL and triglyceride and cholesterol, the important goal of the current research is to investigate about the effect of different doses of walnut on the mentioned factors and reduction of being affected by cardiovascular diseases in hypercholesterolemic rabbits.

Procedure:

30 mature white rabbits of Newzealandi type with the approximate weight of 2000±150 grams were purchased from the center of lab animals of Shiraz medical college, and were exposed to the surrounding lab condition for a week to adapt to the atmosphere. These conditions include: temperature of 22±2 degrees centigrade and the cycle of 12 hours of light and 12 hours of dark. After specifying walnut leaf extract with the following procedure, and specifying LD50 which was the equivalent of 8

mg/kg/b.wt, there doses of minimum, medium and maximum were specified. Procedure of taking walnut leaf hydro alcoholic extract: 100 grams of the obtained powder was put inside a one-litter erlen and etlic alcohol of 96 rface of the powder and after 24 hours the solution clarified. In the next stage alcohol of 75 percent was added to the remaining walnut leaf grounds and after 24 hours it became clear. The smooth solutions were mixed and were thickened to one third of their original weights through the distillation machine in vacuum in 50 degrees centigrade and the rotation speed of 70 turns per minute. For separation of protein, fat and chloroplyll, the thickened mixture was decanted three times by 50 milliliter chloroform, the obtained solution from the last stage, became dry in autoclave and a temperature of 50 degrees centigrade at a sterile condition. The dry powder of extract was prepared and kept in temperature of 4 degrees centigrade. During the test, specific a mounts were solved in distilled water to different thicknesses to be fed to the test animals as their diet [13]. Then the rabbits were divided randomly into 6 groups of 5 rabbit each. Each group was given the following diet for a time duration of 60 days: group 1 with abnormal diet (control), group 2 with corn oil (cholesterol solvent) (evidence), group 3 with a high cholesterol diet prepared from Germany merk company which was fed on the base of 1% of daily food of rabbits in the form of gavaj, group 4 with high cholesterol digh along with the minimum dose of walnut leaf extract (100 mg/kg/b.wt), group 5 with a highcholesterol diet along with the minimum dose of walnut leaf extract (200 mg/kg / b.wt) and group 6 with a high cholesterol diet a long with maximum walnut leaf extract (400 mg/kg / b.wt). After care period; all robbits were weighed and after anesthesia, blood samples were taken directly from their hearts and their blood serum was taken. then the changes of LDL, HDL triglyceride and full cholesterol were measural. For comparison of the effects of extract different doses, the ANOVA statistical analysis and after that the Tukeys post hoc test with the use of spss software were utilized, and a meaningful level (p<0/01) was chosen. the results of statistical review of results are shown schematically by Excel software. Results: In the current study which has been carried out on Investigating about the effects of walnut leaf extract on the cure of arteriosclerosis, the amount of LDL according to diagram [1], In the experimental group 3 has an important increase compared to the group having normal diet (p< 0/05). The amount of LDL in other experimental groups has not shown important changes compared to group 1.

Also, the amount of HDL with regard to diagram [2] in the experiment groups 4, 5 and 6 compared to the group having a normal diet showed an important increase (p < 0.01). Also experimental group 6 has an important increase compared to groups 4 and 5 and groups 4, 5 and 6 have an important increase compared to the high cholesterol group and the receiving corn oil.
Diagram 1: Comparison of LDL thickness, in groups receiving different amounts of hydro alcoholic extract of walnut leaves.

Diagram 2: HDL thickness comparison in groups receiving different amount of hydro alcoholic extract of walnut leaves.

The amount of triglyceride serum thickness (TG) in the experimental group 3 has an important increase compared to groups having a normal diet (p<0.01).

Diagram 3: Comparison of triglyceride thickness in groups receiving different amount of hydro alcoholic extract of walnut leaves.
Also, the amount of full cholesterol with regard to diagram 4 in experimental group 3 which is high cholesterol has an important increase compared to the experimental group with normal diet (0.01).

In other experimental groups, compared to group 1 an important change has not been observed. Also, the experimental group 3 has an important increase compared to group 1 and experimental groups 4, 5 and 6 also compared to the experimental group 3 has an important decrease.

Diagram 4: Comparison of cholesterol thickness in groups receiving different of hydro alcoholic extract of walnut leaves.

Discussion:

In researches it has been mentioned that one of the stages of in caption of atherosclerosis is the entering of lipoprotein with low density in the wall if veins, gathering and oxidation of lipoprotein with low density prevents the process of stickiness, movement and distinction of monosyt and causes inflammation, also, in many researches it has been shown that there is a reverse relation between cardiovascular illnesses and HDL thickness factor [14], because this factor has the antioxidant, antithrombotic and anti inflammation properties and causes the control of atrogenic [15,16,17]. Also the past researches has shown that using diets containing %0.5 cholesterol caused the increase of HDL and using diets containing %1 cholesterol caused HDL thickness. Cholesterol percentage in the study was %1 and caused the decrease of HDL thickness [18,19]. In the current study, HDL in the experimental groups 4, 5 and 6 which were groups that received different doses of extract, and show an important increase compared to the group that has a normal diet. In the past studies it has been realized that the extract of walnut leaves contain some antioxidant like phenol compositions that exist in walnut leaves. The most important phenol acids of walnut leaves are kafeoilkueink acid, cholerogenic acid and its most important flavenoid is koerestin [2,13,20]. It has latery been realized that koerestin which is a plant flavenoid, has anti inflammation activity [21]. Flavenoids, and hydroxi dynamic acid products which is found abundantly in walnut leaves has an important role in decreasing the risk of cardiovascular factors through decreasing the gathering of plackets, atherosclerosis, blood pressure, and having antioxidant and into-inflammation activity, affects the activity of endothelial and gathers free radicals and prevents the death of endothelial cells [22,23,24]. Also, antosianins affect the cholesterol distribution and protect endothelial cells against CD40(25). Therefore the reason that in current study in experimental groups receiving walnut leaf extract, changes related to cholesterol and triglyceride and LDL not being important con be related to antioxidant properties and effective antioxidant materials which exist in walnut leaves. Also with regard to diagram 3 cholesterol thickness in the group receiving maximum extract has shown the least increase, although these changes have not been important and with regard to diagram 4 the thickness of LDL also in the experimental group 6 which has received maximum doses, has the minimum increase which shows the positive effect of extract in a way which is related to the doses. In past studies it has been mentioned that lipoprotein with a high density has good effects on the decreasing of fat deposit in people with hyperlipidemic and as a result of decrease of its level of serum, the problems of lipid in patients and people having vascular-coronary illnesses will appear [26]. As it was mentioned, in the current study the level of lipoprotein with high density in the experimental groups 4, 5 and 6 has had an important increase.
consumption of walnut kernel [30] which the current people having normal increased fat after amount of cholesterol, LDL-C and triglyceride in also have reported the decrease related to dose in the cholesterol (and non-diabetic) [29]. Koohsoltani et al. cholesterol and LDL-C in rats having high to dose, has caused the decrease of glyceride, of trans fatty walnut oil extract in a way which is related in the past, it has been mentioned that consumption in the current study shows the positive effects of walnut leaf extract. Also in another research which has been carried out in the past, it has been mentioned that consumption of Iranian walnut oil extract in a way which is related to dose, has caused the decrease of glyceride, cholesterol and LDL-C in rats having high cholesterol (and non-diabetic) [29]. Koohsoltani et al. Also have reported the decrease related to dose in the amount of cholesterol, LDL-C and triglyceride in people having normal increased fat after consumption of walnut kernel [30] which the current study the changes related to these blood factors compared to the group with a normal diet is not important. The study carried out by Jafar Nejat et al. showed that daily consumption of 35 grams of walnuts in ones diet for four weeks caused an important do crease in the level of glyceride, LDL-C and an increase in the level of HDL-C [31]. As it is shown in the current study, the level of lipoprotein with high density has increased, which is agreement with the past studies.

Conclusion :

With due regard to the obtained results from this study, we can say that hydro alcoholic extract of walnut leaves causes an increase in the level of HDL which it shows its positive effects on decreasing the chances of vein congestion, and this effect is related to the doses. Therefore through consumption of walnut leaf extract we can decrease the risk of atherosclerosis.

References


