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Effects of Alcoholic Extract of Stem and Aerial Parts of the *Alcea angulate* Plant on Aorta Atherosclerotic Plaque Formation in Rabbit

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Abstract

Background: Atherosclerosis is the main cause of mortality in heart disease patients that its dependence on cholesterol levels has been shown. The side effects of chemical drugs have created a tendency toward medicinal plants for treatment of diseases.

Objective: The aim of this study is to evaluate the effect of alcoholic extract of the *Alcea angulate* on the formation of atherosclerotic plaque due to hypercholesterolemia in rabbit aorta.

Methods: 25 male New Zealand white rabbits were divided randomly into 5 groups. Two months respectively treated with basic diet [group 1], basic diet plus 1% cholesterol [control group, group 2], 1% cholesterol with diet intervention *Alcea angular* at doses of 200, 400 and 800 mg/kg of body weight [groups 3, 4 and 5]. Blood lipid profile levels were measured after 60 days. At the end of the study, the animals were scarified and lipid profile and atherosclerotic plaque formation in the aorta arteries was measured. Data using SPSS ver. 16 were analyzed. Tukey HSD test was used to compare paired groups.

Results: Serum levels of total cholesterol and LDL.C of the groups which treated with the *Alcea angulate* was significantly lower than the control group ($p < 0.01$). Serum levels of HDL in treated groups compared to the control group significantly increased ($p < 0.01$). Pathology studies showed the desired effects and mitigation of extract on the progression of aorta atherosclerosis in hypercholesterolemic rabbits.

Conclusion: The overall result demonstrated that use of the *Alcea angulate* plant reduced total cholesterol and LDL in the blood and inhibited the atherosclerotic plaque formation.

Introduction

Heart disease, especially artery disease, is a leading cause of disability and death in industrialized societies and about one million people lose their lives due to ischemic heart disease annually in America [1]. Several factors are significant in the development of atherosclerosis in aorta artery [1]. Ischemic heart disease (IHD) is the most important markers of atherosclerosis today. Virtually all patients suffer a heart attack, Myocardial Infarction (MI) and most of the victims with sudden death have had coronary atherosclerosis [2]. Medicinal plants usage to treat diseases have centuries of history and in many developed countries is considered as a primary way of treatment [3]. Many medicinal plants have been identified as an adjunctive therapy in reducing cardiovascular risk factors [4]. *Alcea angulata* is a plant of the mallow family (Malvaceae), that knows its origin to different parts of Asia and Europe [5]. Hibiscus has been used as a therapeutic plant commonly since ancient times. Mallow has a soothing and calming effects and relieve skin irritation and inflammation [6] and has been applied for treatment of severe coughs [7], angina, bronchitis [8], inflammatory diseases [9], kidney stones, constipation and tenesmus [10]. Important ingredient of this plant are known such as flavonoids, polyphenols groups [11,12], poly saccharides [9,13], and mucin [14] and anthocyanins and fiber [10]. These compounds have antioxidant property and free-radical oxygen scavenger and also is consumed as food [15,16].

In clinical studies the effects of antioxidants in improving cardiovascular disease have been reported [17,18]. Malvaceae family consumption is suggested for the treatment of cardiovascular disorders [19]. Since most of the herbal combination, in controlled amounts, have less toxic side effects in comparison with the chemicals ones, in this study effects of Malvaceae family was assessed in preventing the formation of Atherosclerotic plaques in rabbit aorta.

Keywords: *Alcea Angulate*; Atherosclerosis; LDL; HDL; Cholesterol

Materials and Methods

Material

Pointy marshmallow plants (*Aleca angulate*) was collected in mid-September from different areas of Ilam province and was identified and confirmed by the Research Center for Agriculture and Natural Resources of Ilam. After being dried in the shade away from direct sunlight and moisture and grinding into powder, was stored inside the bags until extraction. 150 grams of powder was inserted in ml 1000 ethanol (70%) and extraction operation was carried out by Soxhlet apparatus for 18-12 hours. Extract was concentrated by solvent removal device, transferred into clean glassy container and was placed in the oven at 40°C for 24 hours to dry extract obtained. The resulting powder was resolved in an appropriate solution (distilled water) and was used in a proper dosage.

Laboratory animals

25 adult male rabbits of New Zealand race with an average weight of 1.8-2.4 kg were used. The rabbits were purchased from Razi Institute of Karaj. In order to adapt to new circumstances, rabbits were kept for two weeks in appropriate temperature, humidity and lightening (12 hours of darkness and 12 hours of light) in randomly and then were treated. The Rabbit were feed with Standard grain ready (pellet) of the Dame Pars company, containing 15% protein, 50-40% carbohydrate, 2% fat and 15 to 25 percent plant fiber. All animals in the experiment had access to enough food and water. The protocol of all experiments was approved in advance by the Ilam University of Medical Sciences Ethics Committee and performed in accordance with NIH guidelines for care and use of laboratory animals (ir.medilam.rec.1396.93).

Grouping and treatment of animals

After habituation period for two weeks, the rabbits were randomly divided into 5 groups of 5 as follow: First group received a normal diet (non-hypercholesterolemic); Second group (hypercholesterolemic diet) as control group; Third group, hypercholesterolemic diet + stem hibiscus extract (200 mg/kg); Forth group, hypercholesterolemic diet + stem hibiscus extract (400 mg/kg); Fifth group, hypercholesterolemic diet + stem hibiscus extract (800 mg/kg).

To prepare a 1% cholesterol diet, one gram of cholesterol (Merck) was solved in some olive oil (2 cc) and rabbits were force feeded (gavage) with it as high cholesterol diet. The study was conducted over a 60-day period that continued until the animals reached the ideal weight. After that animal were anesthetized, rabbits heart and vascular were removed and fixed in formalin (10%). histological sections were prepared and examined under a microscope.

Biochemical tests and histological studies

Blood samples were collected from the middle ear artery of fasting rabbits (12 hours of fasting). Procaine and lidocaine 2% was used to numb ears. Blood samples in the test tube were

centrifuged at 3500 rpm for 20 minutes. Serum was separated carefully by sampler and were stored into micro-tubes of Eppendorf in a freezer at -70°C until doing biochemical tests

Measurement of serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) all are done with spectrophotometric method (enzymatic colorimetry). The low-density lipoprotein cholesterol (LDL.C) also was calculated using Friedewald formula: $[LDL.C (mg/dl) = TC - (HDL.C + TG/5)]$ [19]. At the end of the biochemical experiments (60 days), rabbit sacrificed under chloroform (overdose) and their aorta artery was isolated and fixed in formalin (10%) taken sections from the aorta were stained with Hematoxylin and eosin [20].

Data collection

Using an optical microscope aorta artery wall changes were evaluated. The results of microscopic observations based on the frequency and severity of lesions were graded from 0-4. Zero grade: no lesion, first grade: Plaque thickness less than the thickness of the media, mild forms of endothelial dysfunction, second grade: Media thickness half the thickness of plaque in the presence of macrophages and smooth muscle cells within the plaque, Third grade: thickness of plaque equal Media thickness and plenty of connective tissue within the plaque that represents the synthesis and proliferation of extracellular matrix by smooth muscle cells. Fourth grade: thickness of plaque greater than the media thickness, large lipid core plaque which fully raised the surface of endothelial [21].

Data analysis

Normal distribution of data was evaluated with Kolmogorov-Smirnov test in both groups. Statistical differences were determined using ANOVA. Tukey HSD test was used to compare paired groups. Statistical significance was defined at $P < 0.05$. Using SPSS ver. 16 Data were analyzed.

Results

Biochemical findings

The results showed that the rate of serum cholesterol in hypercholesterolemic rabbits treated with alcoholic extract of hibiscus stem (doses of 200, 400 and 800 mg/kg) significantly reduced compared to the control group ($p < 0.001$) (Figure 1A). Extract of hibiscus stem significantly decreased the serum LDL in hypercholesterolemic rabbits compared to the control group ($p < 0.001$) (Figure 1B). The results also demonstrated a significant increase in serum HDL in hypercholesterolemic rabbits treated with alcoholic extract of marshmallow compared to the control group ($p < 0.001$) (Figure 1C). Consumption of alcoholic extract stem *Aleca* didn't show any changes in the serum VLDL hypercholesterolemic rabbits treated with doses of 200 and 400 mg/kg compared to control group but at dose of 800 mg/kg this parameter decreased compared to the control group ($p < 0.001$) (Figure 1D). The consumption of alcoholic extract in doses of 400 and 800 mg resulted in a significant

decrease in serum TG levels compared to the control group ($p < 0.05$) (Figure 1E).

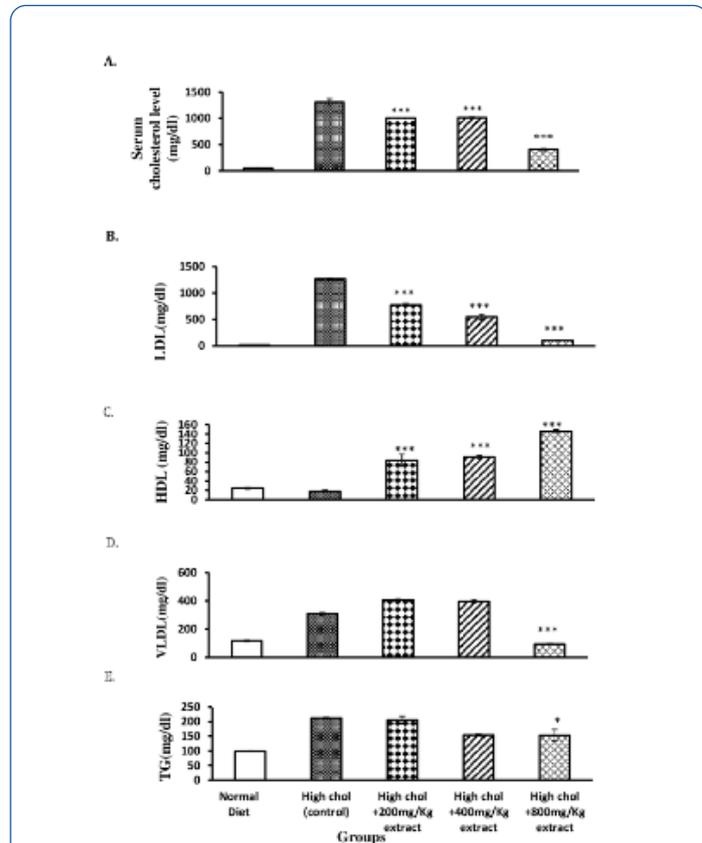


Figure 1: Comparison of serum cholesterol(A), HDL(B), LDL(C), VLDL(D), TG(E) levels in the control group (hyper cholesterol) versus groups treated with alcoholic extract of marshmallow extract at doses of 200, 400 and 800 mg/kg of body weight.

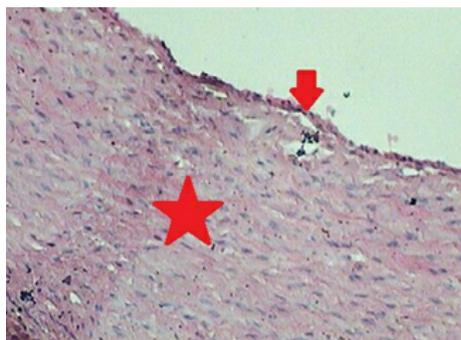


Figure 2: Intima (arrow) and media (star) layers on the normal aorta wall.

Histological results

The histological results showed that in the normal diet group vessels were completely normal and no lesion in the vessels intima and media observed (Figure 2). aortic plaque were detectable in the sections prepared from the group treated with cholesterol-1%, Lipid-laden macrophage shave produced Foamy

cells, smooth muscle cells were seen in these plaques and Plaque thicknesses had been increased (Figures 3A, 3B and 5). Consumption of hibiscus extract with hyper cholesterol diet decreased the severity of lesions compared to hyper cholesterol group significantly so that at dose of 800 mg/kg of hibiscus extract complication was limited just to formation of very few macrophages in intimal layer (Figures 4 and 5).

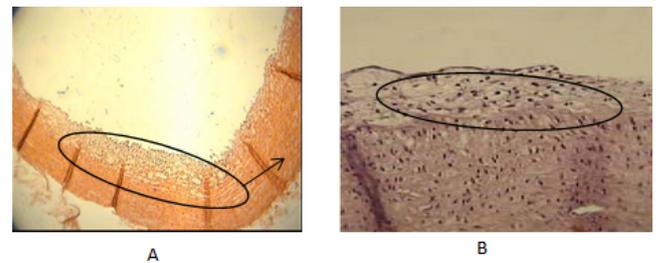


Figure 3: Formation of an atherosclerotic plaque under intima layer, H&E 40X (A), including macrophage foam cells and lymphoplasmocyte, H&E 100X (B) in high-cholesterol diet group.

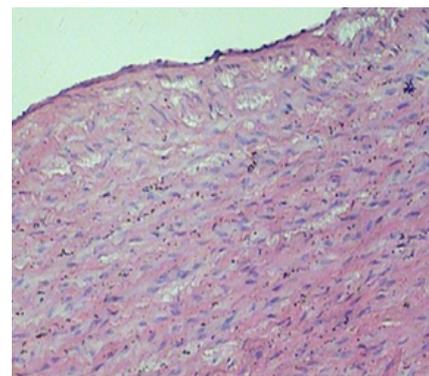


Figure 4: Fairly normal aorta artery (High-cholesterol diet + extracts of 800 mg/kg group, H&E 100X).

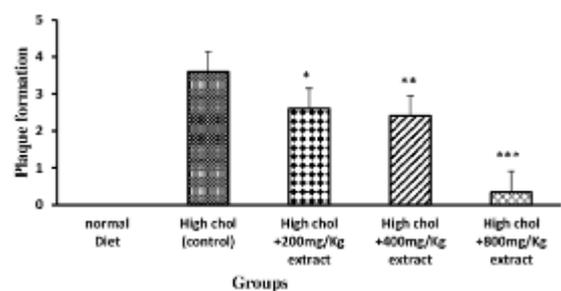


Figure 5: Comparison of plaque formation in the control group (hyper cholesterol) versus groups treated with alcoholic extract of marshmallow extract at doses of 200, 400 and 800 mg/kg of body weight.

Discussion and conclusion

This study showed that cholesterol-rich diet increased total cholesterol, triglycerides, LDL and VLDL respectively. Similar changes in these parameters have already been reported by other studies [22]. This diet created also a significant decrease in HDL concentration. Ramirez et al. (1999) reported that using of cholesterol-1% for 7 weeks caused creation of fatty streak in rabbit aorta and ultimately atherosclerosis [23]. In this study, consumption of a diet with 1% cholesterol for 60 days has been able to establish plaques. In present study, three doses of alcoholic extract of aerial parts of the stems and angular hollyhock *Aleca angulate* (200, 400 and 800 mg/kg) in the form of gavage for 60 days in hyperlipidemic rabbit, in addition to reducing cholesterol and LDL levels ($P < 0.01$) also increased HDL level compared to the control group significantly ($P < 0.01$) and these changes were in a dose dependent manner.

This study demonstrated a reduction in the aorta artery wall lesion in the rabbits which treated with alcoholic extract of marshmallow to greatly was compared to high cholesterol diet ones. Extent of the impairment in the group that had consumed the hibiscus extract at a dose of 800 mg/kg was limited to establishment of foamy cells in a very small amount into the vessel intima, while in the rabbits with rich cholesterol diet fully raised thromatous plaques in the vessel were seen.

Studies suggest that consumption of a diet rich in fruits and vegetables caused a significant decrease in cardiovascular disease [24]. These diets contain compounds such as antioxidants [25]. Positive effects of consumption of foods containing antioxidants in improving cardiovascular disease have been reported in clinical researches [17]. Antioxidants are able to prevent free radical damage resulting from the oxidation of LDL cholesterol, so can blockage atherosclerosis and platelet adhesion which leads to thrombosis and inhibit their risk of heart disease or stroke [26]. Yamakoshi et al. reported that the use of plant extracts Malvaceae family for 8 weeks in hypercholesterolemic rabbits decreased Serum cholesterol and LDL-C respectively by 14% and 17%, but did not have much effect on HDL-C and triglycerides [27] that partly consistent with our research results. In another study Chen and colleagues observed that administration of the extract of *Hibiscus sabdariffa* (Plant of the family Malvaceae) in hypercholesterolemia rabbits decreased cholesterol by 28%, LDL-C and triglycerides by 53 and 55% respectively [28]. *Hibiscus sabdariffa* extract inhibits LDL oxidation in vitro [29] and this extract has the property of lowering cholesterol level. Researchers also showed that this plant extract prevents the progression of atherosclerosis [30,31]. Studies have shown that *Hibiscus sabdariffa* extract prevented migration of smooth muscle cells resulting in thickening of the arteries in hypercholesterolemic rabbits. The results of this study are consistent with our research findings. Direct role of free radicals in the development and progression of atherosclerosis, suggests antioxidants as influential factors in preventing of progression of the disease. There are numerous reports about antioxidant property of the species of hibiscus extract [12]. Flavonoids by enhancing the LDL receptors and increase catabolism of LDL in the liver have reduced serum cholesterol levels [32]. Hibiscus extract mechanism in reducing

atherosclerosis can be due to presence of flavonoids. Hibiscus root extract like other herbal extracts contains compounds with abundant biological effects. Sabu et al. reported Green tea polyphenols inhibit lipid peroxidation and superoxide radicals in diabetic rats [33]. Hibiscus plants like green tea plant is belong to the Malvaceae family. These plants as well as plants that contain flavanols and isoflavonols have vascular protective effects [33]. Studies have shown that hibiscus is useful in the treatment of inflammatory diseases [34]. In other hand it has been shown that atherosclerosis is an inflammatory disease [34]. Regard to the effect of inflammation in the progression of atherosclerosis, anti-sclerotic property of marshmallow may be due to their anti-inflammatory effect.

Taken together the results obtained in this study have shown that alcoholic extracts of the stem and aerial parts of hibiscus reduced serum cholesterol level and LDL-C and inhibits atherosclerosis plaques appropriately. The results of this study and previous researches show that hibiscus extract could be considered as complement drug for the treatment and prevention of complications of atherosclerotic plaque in human.

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