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Antiatherosclerotic Effect of Licorice Extract on Hypercholestrolemic Male Rabbit by Electron Microscopy

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ABSTRACT

Coronary artery disease develops as a result of risk factors such as increased plasma low- density lipoprotein (LDL) level and hypertension. In the present study, we extended our investigation to analyze the antiatherogenic effects of licorice- root extract consumption in hypercholesterolemic rabbits. Fifty male rabbits in five separate groups of ten have gone under normal and high cholesterol (HC) diet for six weeks. One group of ten received HC and another had normal diet. Each of the other three groups of HC diet were fed with 1gr/kg, 0.1gr/kg and 0.01 gr/kg. Dietary consumption of licorice - root extract by hypercholesterolemic may act as a moderate hypocholesterolemic nutrient and potent antioxidant agent and, hence against cardiovascular disease.Electron and light microscopy study of the tissue samples showed the hypercholestrolemic group with 1gr/kg licorice per day had a significant difference with other groups. Licorice root supplementation reduced atherogeniclessions in the arteries.

KEYWORDS: atherosclerosis, coronary artery, ultra structure, licoric root.

1. INTRODUCTION

Today coronary artery disease is one of the most important problem in the developed countries that leads to high mortality and morbidity. Atherosclerosis, defined as thickening of the arterial wall through the accumulation of lipids, macrophages, T-Lymphocytes, smooth muscle cells, extra cellular matrix, calcium, and necrotic debrides, can affect any of the arteries in a condition known as cardiovascular disease (pathology Goodman).Atherosclerosis is a multifactorial disease, and other factors besides lipid peroxidation can accelerate thermogenesis independently or in association with lipid peroxidation ().Coronary artery disease develops as a result of risk factors such as increased plasma low density lipoprotein (LDL) level and hypertension or LDL atherogenic modifications such as retention, oxidation and aggregation (1-5).Coronary arteries carry oxygen to the myocardium. When these arteries become narrowed or blocked, the areas of the heart muscle supplied by that artery become ischemic and injured and infarction may result (Pathology Goodman).Despite improved clinical care, heightened public awareness, and widespread use of health innovations, atherosclerotic diseases and their thrombotic complications remain the number one cause of mortality and morbidity in the united states, (Pathology Good man).Consumption of flavonoids in the diet has been shown to be inversely associated with morbidity and mortality from coronary heart disease. (24), 6,7

Different classes of flavonoids are present in different fluits and vegetables and in beverages such us tea or wine. Phenolic flavonoids possess antioxidavtive properties toward LDL lipid, peroxidation (4-6) and they have been reported to exert free radical- scavenging capabilities. (7,8).Dietary supplementation in nutrients rich in polyphenols such as black or green tea, olive oil, (12, 13) Licorice root extract, (14, 15) or red wine (16) has been shown to be associated with increased resistance of their plasma LDL to oxidation and an increase in plasma antioxidant capacity.The antioxidant activity of the flavonoids is related to their chemical structures (9, 15).

Since oxidative modification of LDL is thought to play a key role in the pathogenesis of early atherosclerosis (18, 19, 20) the benefical effect of red wine consumption against the development of this disease was attributed to the antioxidant activity of the polyphenols in the wine (Hayek).Licorice acid (Glycyrrhiza glabra) a member of legum family, is an ancient herb that has been used for medical purposes for centuries. It contains flavonoids from the flavan and chalcone subclasses, which have lipophilic characteristics and antioxidative properties (19). In Chinese medicine it is one of the oldest and most frequently employed botanical and in Asia it is used as a sweetener or a spice.

consumption of red wine or its flavonol quercetin or of licorice extract by atherosclerotic Apo lipoprotein E- deficient (E˚) mice resulted in a significant reduction in the development of atherosclerotic lesions, along with a reduction in their LDL oxidation (12 , 13).

Dietary supplementation of healthy human volunteers with flavonoid - rich nutrients such as olive oil, red wine, or licorice root, resulted in a reduction in LDL oxidizability (10-12).Oxidized LDL is taken up by machrophages at an enhanced rate via their scavenger receptors (a), Leading to the formation of lipid - laden foam cells, the halmark of the early a atherosclerosis ().In the present study we investigaed the ultra structural effects of licorice root extract in normal, and hypercholestrolemic male rabbits

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MATERIALS AND METHODS

Licorice ethanolic extract was provided by shirin daroo company (shiraz, Iran).
Fifty male Dutch rabbits with normal weight and age from Razi Institule were used for this study. The animals were randomly divided to five groups of ten. They were fed for 6 weeks as below:
Groups 1: Normal diet.
Group 2: High cholestrol diet.
Group 3 : High cholestrol diet plus 1g/kg / day licorice root extract.
Group 4 : High cholestrol diet plus 0.1g/kg/day licorice root extract.
Group 5 : High cholestrol diet plus 0.01g/kg/day licorice root extract.
The rabbits received their regular licorice root diet via their drinking water.
After six weeks of consumption of above diet all animals were deeply anesthetized by 50 mg/kg pantobarbital intraperitoneal injection.

The heart and entire aorta were rapidly removed and washed in 0.9% normal saline and then fixed by immersion in 2.5% glutaraldehyde in 0.1mM phosphate buffer, pH 7.2-7.4 at room temperature.

After 2 hours, under a binocular steriomicroscope, the aortic arch was dissected and the fatty tissues surronding it were removed. When the origin of coronary arteries were recognized, the ascending aorta was removed.

The tissues were fixed over night in a new glutaraldehyde at 4˚c. They were then insed in 0.l mM phosphate buffer 3 times for 30 minutes.

The tissues were treated with 1% osmium tetroxide solution for 2 hours. They were next rinsed in phosphate buffer 3 times for 30 minutes, dehydrated in ascending acetons, prior to infiltration in Aceton - resin mixture and finally in pure resin and embedding in epoxyresin (TAAB-Microscopy).

The blocks were trimmed and semithin and then ultrathin transvers sections (50-70 nm) cut with diamond knif (Taab-Microstar) on a Leica ultramicrotome (Leica ultracut- R- Germany).

The sections were mounted on 200 mesh grids and stained with 5% uranyl acetat and 0.5% lead citrate.

The grids were observed by a transmission electron microscope. (zeiss EM 900, Germany).

For scanning electron microscpy the tissues after dehydration were kept in a freezer for 72 hours, in order to dry and then transferred to a Lyophilizer (JFD- 300 Jeol, Tokyo, Japan). They were dried under the vaccum for 6h. The samples were next mounted on metal stab by silver glue. After drying the glue, a layer of thin gold (12 nm) were coated on the samples by a sputter coater (SCD- 005 BAL- TEC Germany) and observed by a scanning electron microscope (zeiss - DSM - 940 A- Germany). After observation of the samples by light microscopy, the ones which had fatty streak, categoized according on lesion severity. Statistical analysis were done by spss (version 10).

Analysis of variance was used to compare between the groups. Duncan test was used to evaluate the differences between each group values were considered significant when ($P \leq 0.05$).

Ultrastructural of the intima layer of the coronary arteries was compared together and signs such as pinocytotic vesicles (include free LDL radicals), increased intima thickness, endothelial cell enlargment, phagocytotic fatty vesicules in macrophage cell and smooth muscle and endothelial layer interruption were evaluated.

Endothelial cells and fatty streak in internal surfaces of the coronary arteries were also evaluated by SEM in different groups.

RESULTS

TEM Observation: After 6 weeks of licorice - root extract consumption significant changes of coronary arteries were observed in the rabbits fed with high cholestrol diet (group 2) , and the rabbits fed with high cholestrol diet plus licorice root extract (groups 3, 4 , 5). No ultrastructural phathologic lesions were observed in normal diet groups. (Figures . 5 , 6).

Figure.6 (X-34000)

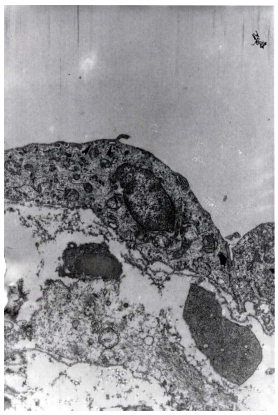
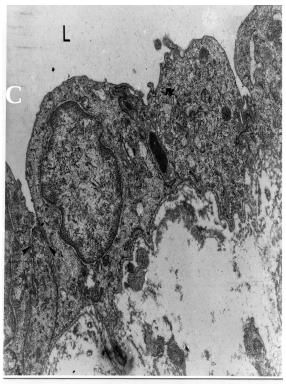


Figure.5 (X-15400)

A:spindle endothelial cell, B:tight junction(normal junction) ,c:some pinocytic vesicles with small size

Ultrastructural changes in high cholestrol group showed an increase in the volume of the endothelial cells toward the lumen of artery, endotheial junctions interruption and widdening of the inter cellular spaces (Figures 7, 8). Pinocytotic vesicules containing phagocytic free LDL radicals were observed (Figure 8).



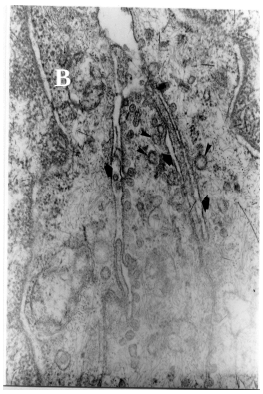
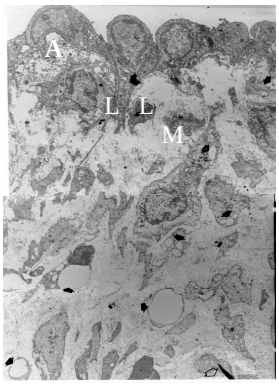


Figure.7 (X-15400) Figure.8(X-51000)

A:Endotelial cell(increased volume), B: wide inter cellular space, C:many pinocytic vesicles
Intima layer thickness and monocyte infiltration in to the subendothelial layer and macrophage accumulation had considerably increased in group (2). Many Phagocytotic fat vacuoles were observed (Figures 9 and 11).



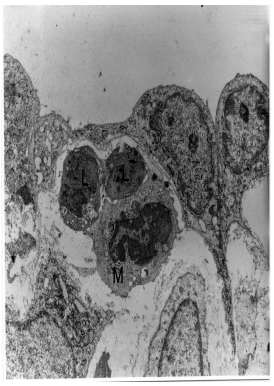


Figure.9(X-51000) Figure.11(X-7480)

A:Endotelial cell(increased volume),B:thikened intima layer, C:phagocytotic fat vacuole, L:lymphocyte,
M:monocyte

Endothelial layer had interrupted in some areas and basal membrane thickness had continuously increased (Figure 10).

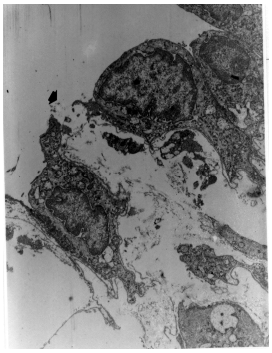


Figure.10(X-7480)
A:ruptured intima

There were no significant differences in phathological and ultrastructural changes between groups fed with high cholestrol diet plus licorice root extract in 0.1g/kg/day (group 4) and 0.01/kg/day (group 5) with high cholestrol diet wihout licorice root extract (group 2).

In the group fed with high cholestrol diet plus licorice root extrat 1g/kg/day (group 3) , ultrastructural phathologic lesion showed mild changes. Intima layer thickness was less than last groups and endothelial cells had a few fatty streaks (Figures 12, 14).

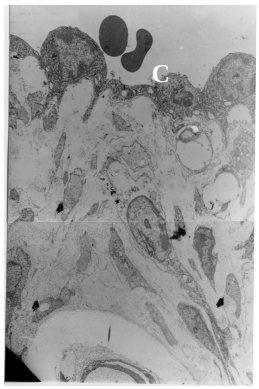


Figure12(X-5100)

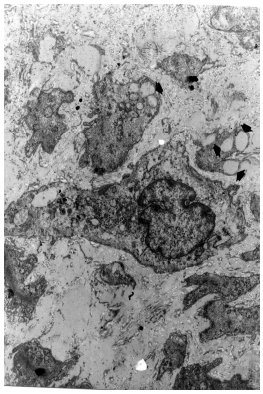


Figure14(X-9680)

A:Endotelial cell with less increased volume, B:less thickened intima lyer, C:less phagocytotic fat vacuole

Also endothelial cells outburging were not notable toward the artery lumen, and inter cellular junctions were completely continuous (Figures 12).

SEM Observation: Endothelial cells had regularly arranged in the direction of artery axis when internal surface of coronary arteries fed with normal diet were evaluated by SEM (Figure 15).

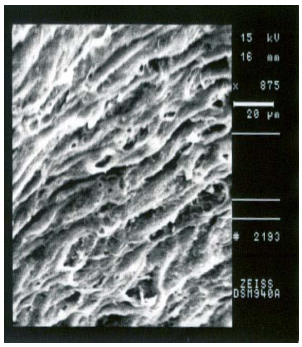


Figure.15(X-875)
A:Endotelial cells

In high cholestrol feeding group a lot of monocytes were attached to the endothelium and were doing diapedesis by pseudopodium (Figure 16).

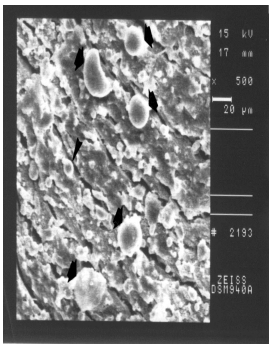


Figure.16(X-500)
A:monocytes doing diapedesis

In some area endothellium was also interrupted by the accumulation of coagolation factors (such as platelets, red and white cells) and clots (Figure 17).

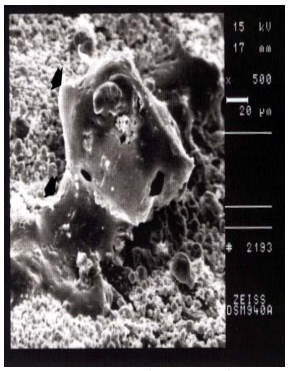


Figure17(X-3000)
A:an Endotelial cell that be detached from other cells and is embossed to the loman.

DISCUSSION

Atherosclerosis is a multi factorial disease associated with different risk factors (16), where more than one mechanism, along more than one step, contriobutes to macrogphage cholestrol accumulation and foam cell formation, the hallmark of early atherosclerosis.

Researchers attempts on studying the effect of licorice root extract show that this extract acts as an antidxidant (13 , 14), and a reducing factor for coronary artery diseases. The present study was designed to get exact and more certainly findings on ultrastructural phathologic lesions of artherosclerosis. There is a strong relationship between LDL and intensity of atherosclerosis in the wide epidemiologic analysis (15).

"Filtration theory" that was explained by a scientist named "Ancheskiu" expresses that nutrition with high cholestrol diet causes "Atheroma" (15).High LDL level that seems to be because of high cholestrol and esterification of it by cells, causes the accumulation of cholestrol linoleat in the smooth muscle cells. Many of th cells may be necrotic and send lipids to the extracellular space. In the presence of high plasma LDL level which is in cholestrol linoleat the remainders can be the mixture of two kinds of cholestrol esters.

In some studies has been suggested that in hyperlipidemic animals there are some factors in LDL that spontaneously lead to smooth muscle cells prolipferation and production of connective tissues in the cells (16).Therefore, there is a squences of tenses including the chronic endothelial damage, because of high LDL level , progress of the atherosclerotic lesions due to contact with high LDL level and finally low HDL level, causes the atherosclerotic lesions progress (17).It has been shown that when serum cholestrol level reduces by nutrition diet, some of atherosclerotic plaques begin to regress or stop progress (15).We showed that high cholestrol diet acts as an important factor in formation or intensification of the atherosclerotic plaques in the coronary arteries of rabbits in this study and licorice root extract can modify the progress of the disease. In high cholestrol diet group which received 1g/kg/day licorice root extract, Histopathologic evaluation about the presence of fatty streak in coronary arteries showed significant differences compared to the other groups.These differences was due to the indirect effect of licorice root extract on serum lipid metabolism (18).Fuhrman et al have also studied the antioxidant effect of licorice root extract on LDL oxidation and confirmed the antioxidant effect of licorice root and thereby inhibitory effect of licorice root extract on LDL oxidation in animal model (13).In the ultrastructural study of the endothelial and intima of the coronary arteries there were a significant differences between the goups fed with high cholestrol diet without receiving licorice root extract and the groups fed with high cholestrol diet plus 1g/kg/day licorice root. These differences include the reduction in fatty vacuoles, thickening of endothelial cell basal membran, macrophage and monocyte accumulation in group (3).Christophere et al suggested that there were significant differences in intima lesions between two group of normal and high cholestrol diet. Endothium layer interruption in highcholestrol diet and accumulation and lipid vacuoles, macrophages and monocytes in subendothelial layer were significantly increased (19).In conclusion, structural and ultrastruct studying showed that there was a significant reduction in intima lesions in highcholestrol diet associate with licorice root extract 0.1g and 0.01g/kg/day. Intima lesion had a regression but it is not statistically significant. Also in TEM & SEM evaluations have been shown that the inima layer lesions in high cholestrol diet had modified in high cholestrol diet with licorice root extract of 1g/kg/day.

This study showed that the licorice root extract resulted in a significant reduction in the development of atherosclerosis lesions.

REFERENCES

1.Ross R. the pathogenesis of atherosclerosis:A perspective for the 1990 .nature 1993;362:801-809.

2.Starg HC.Chandler AB,Glagous, et al.A definition of initial,fatty streak, and intermediate lesions of atherosclerosis.Circulation 1994;89:262-2478.

3.Foggiotto A,Ross R,Harker L.Studies of hypercholesterolemia in the nonhuman primate: I,changes that lead to fatty streak formation .Arteriosclerosis 1984;4:323-340.

4.Mann J,Davis M. Vulnerable plaqe.Velation of characteristies to degree of stenosis in human coronary arteries.Circulation. 1996;94;928-931.

5.Brown B,Bolson E,Dodge H.Dynamic mechanisms in human coronary stenosis.Circulation 1994;70:977-922.

6.Supperku HR, Krauss RM. Coronary artery disease regression: conuinclrg for the benefit of aggressive lipoprotein management.Circulation 1994;90:1059-1069.

7.Witzum JL, Steinbery D.Role of oxidized low density lipoprotein in atherogenesis.J Clin Invest 188:1785-92,1991.

8.Aviram M. modified forms of low density lipoprotein and atherosclerosis,98:19,1993.

9.Estebauer H, Gebicki J, Publ H, Jurgens G.The role of lipid peroxidation and antioxidants in oxidative modification of LDL.Free Radic Bio Med. 13:341-90.

10.Levine GN,Keaney JF,Vita JA.Cholesterol reduction in cardiovascular disease: clinical benefits and possible mechanisms.Natengle Med.332:512-21.1995.

11.Craig WJ.health-promoting properties of common herbs.Am J clin Nutr,70:4912-4995,1999.

12.Wang ZY,Nixon DW Licorice and cancer.Nutr Cancer,39:1-11,2001.

13.Fuhrman B,Volkova N,Rosenbelt M.Lycopene syhergistically inhibits LDS oxidation in combination with Vitamin E, glbridin,rosmarinic acid,Carnosic acid or garlic.Antioxid Redox Signal Z:491-506,2001.

14.Bellinky PA,Avirami M,Mehmood S,Vaya J.Structural aspects of the inhibitory effect of glabridin on LDL oxidation .24:1419-29,1998.

15.Frederiek J. Blood Vessles: In cotran,Kumar ,Robbins,Pathologic Basis of Disease :from W.B. Saunders Philadelphia USA,1999;473-484.

16.Kita T,Nagano Y ,Yokde M,et al.Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit,an animal model for familial hypercholesterolemia- induced atherosclerosis in the nonhuman primate by probucol: I.Is the extent of atherosclerosis related to resistance of LDL to oxidation? J Clin Invest,1994:94:155-164.

18.Robert A.Estrogen:Effects on the cardiovascular Tree. obstetrics &Gynecology,1996:87:27-35.

19.Cristofori P,Lanzoni A, Gauiarghi G,Turton J, Sbarbati A.Anti-atherosclerotic activity of the calcium antagonist lacidipine in cholesterol-fed hamsters: Biomed pharmacother.2000 Mar;54(2):93-9.