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## Effect of *Glycyrrhiza glabra* Extract on Aorta Wall Atherosclerotic Lesion in Hypercholesterolemic Rabbits

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**Abstract:** Atherosclerosis which results from gradual deposition of lipids in medium and large arteries is a leading cause of mortality worldwide. *Glycyrrhiza glabra* is an herb of Fabacea family which contain hypolipidemic compounds and flavonoids with high antioxidative properties. This study was conducted to determine the effect of *Glycyrrhiza glabra* extract on blood lipids and atherosclerosis in rabbits fed with high cholesterol diet. Fifteen male rabbits were randomly divided into three groups (normal diet group, high-cholesterol diet (1% cholesterol) and a group which received high-cholesterol diet supplemented with *Glycyrrhiza glabra* extract (50 mg/kg body weight every other day). The concentration of Total cholesterol (TC), LDL cholesterol, triglycerides (TG) and HDL cholesterol was determined in rabbits at the start of the experiment, and at the end of the first and second month of the study. At the end of the experimental period the aorta was removed for assessment of atherosclerotic plaques. Results show that *Glycyrrhiza glabra* significantly decreases TC, LDL and TG levels and increase HDL and lessens atherosclerotic lesion in aorta. Hence *Glycyrrhiza glabra* extract can effectively prevent the progress of atherosclerosis. This is likely due to the effect of *Glycyrrhiza glabra* on plasma lipoproteins and its antioxidant and anti-inflammatory properties.

**Key words:** *Glycyrrhiza glabra*, high-cholesterol, rabbit

### Introduction

Atherosclerosis the principal cause of death in industrialized countries, is an inflammatory disease that generally begins from childhood. Clinical manifestations is plaque formation in wall artery. In many cases, plaque protrude into the lumen of the artery and if sufficiently large, compromise the flow of blood (Braunwald, 1997). Epidemiologic studies have demonstrated a positive significant relationship between plasma cholesterol concentration with coronary artery disease (Moarrea, 2004). Cholesterol-lowering drugs (statins) and synthetic antioxidant (probuco) are widely used to treat atherosclerosis. Unfortunately, these drugs have different side-effects (Lankin *et al.*, 2003). Hence for treatment of atherosclerosis much attention has been focused on the use of herbal drugs as substances with the fewest side-effects (Cooke, 1998). Licorice, the root of the *Glycyrrhiza* species, is one of the most frequently employed botanicals in traditional medicine. The history of licorice, as a medicinal plant, is very old and has been used in many societies throughout the millennia (Wang, 2001). There are many useful compound in licorice root such as, glycyrrhizin (saponin-like glycoside -50 time sweeter than sugar) and its aglycone, glycyrrhetic acid which are clinically used for hyperlipidemia (Tamir *et al.*, 2001). Licorice flavonoid constituents mainly include flavones, flavonols, isoflavones, chalcones, bihydroflavones and

bihydrochalcones. Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities (Vaya *et al.*, 1997). This study was performed to determine the effects of *Glycyrrhiza glabra* extract on serum lipoproteins and progression of atherosclerosis.

### Materials and Methods

**Extract preparation:** *Glycyrrhiza glabra* was supplied and its genus and species were verified by Mr. Asgarzadeh from the Research Center of Isfahan Province Natural Resources. The required parts of drug including its root were removed and dried in shade and ground into powder using a grinding machine. Extract was prepared by concentrating the solution made by dissolving herb powder in 96% and 70% ethanol, decanting the concentrated solution with chloroform, and drying the solution resulting from decantation under sterile conditions and suitable temperature.

**Treatment of rabbits:** Fifteen male white New zealand rabbits weighing 2-2/5 kg were purchased from Razi serum production and vaccine Research Institute in Karaj. They were acclimatized in an air conditioned room for two weeks and provided with free access for food (Super Fosskorn Standard Rabbit Chow) and water. The rabbits were then randomly divided into three groups: normal diet group, high-cholesterol diet group (1%

cholesterol) and *Glycyrrhiza glabra* group which along with high-cholesterol diet by gavage received *Glycyrrhiza glabra* with a dose of 50 mg/kg body weight (equivalent of 1 g dry powder of dried herb per kg body weight) every other day. The experiment lasted 60 days and the animals had unlimited access to food and water.

**Measurement of serum lipids:** Blood samples were taken from the rabbits' hearts on day 0 (start of study), day 30 (middle of study), and day 60 (end of study) and the serum was used to determine levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL - C) and high-density lipoprotein cholesterol (HDL - C). The concentration of the aforesaid parameters was measured using biochemical test kits with the colorimetric method (Rifai *et al.*, 1999). The atherogenic index plasma (AIP) which is a measure of the extent of atherosclerotic lesions based on plasma lipids was determined in all three groups. The atherogenic index was calculated using the formula  $AIP = \log(TG/HDL)$  (Dobiasova, 2004).

**Assessment of the severity of atherosclerotic lesions:** At the conclusion of the study, blood samples were taken from the rabbits and chloroform was used to anesthetize the animals. Following chest incision, the animals' aortas were excised to study fatty streaks. After slicing and staining with hematoxylin, atherosclerotic thickness was assessed in hematoxylin stained sections on an arbitrary scale 1-4.

**Trace:** Minimal thickness of subintimal with little injury to aorta endothelium.

**Grade 1:** Atherosclerotic thickness less than half as thick as the media with some form of endothelial dysfunction, macrophages and isolated foam cell inside the endothelium.

**Grade 2:** Atherosclerotic thickness half as thick as the media with accumulation of intracellular lipid, macrophage and smooth muscle cells.

**Grade 3:** Atherosclerotic thickness as thick as the with an abundance of macrophages, smooth muscle cells and connective tissue.

**Grade 4:** Atherosclerotic thickness more than as thick as the media with a large extracellular intimal lipid core that appears as a large nucleus from the endothelial surface (Chekanov, 2003).

**Statistical analysis:** Serological and histological results have been expressed as mean  $\pm$  standard deviation. Serological data were analyzed using a bifactorial method and repeated measurements of one factor.

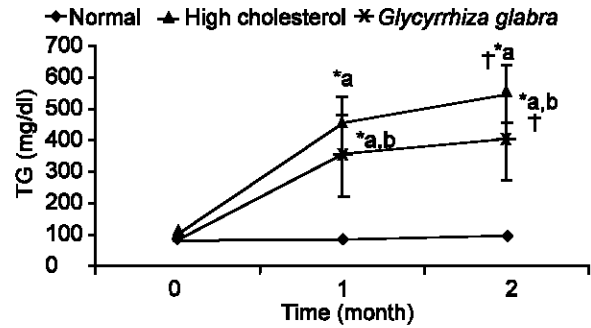


Fig. 1: Changes in serum triglyceride (TG) concentration in the studied groups. Results are expressed as mean  $\pm$ SD.

- \*: Comparison of value at different time with respect to time 0 in respective groups.
- †: Comparison of value at time 2 with respect to time 1 in respective groups.
- a: Comparison between studied groups with respect to Normal group.
- b: Comparison between *Glycyrrhiza glabra* group with respect to Highcholesterol group.

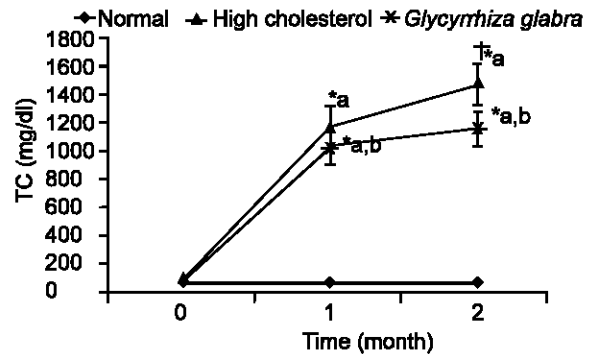


Fig. 2: Changes in serum total cholesterol (TC) concentration in the studied groups. Results are expressed as mean  $\pm$ SD.

- \*: Comparison of value at different time with respect to time 0 in respective groups.
- †: Comparison of value at time 2 with respect to time 1 in respective groups.
- a: Comparison between studied groups with respect to Normal group.
- b: Comparison between *Glycyrrhiza glabra* group with respect to Highcholesterol group.

Histological data were analyzed according to a random method. SPSS software was used to compare mean values within and between the groups. One-way ANOVA and Duncan tests were used. P values less than 0.05 were considered as significant.

## Results

Alterations in TG, TC, LDL cholesterol and HDL cholesterol concentrations in all three groups were

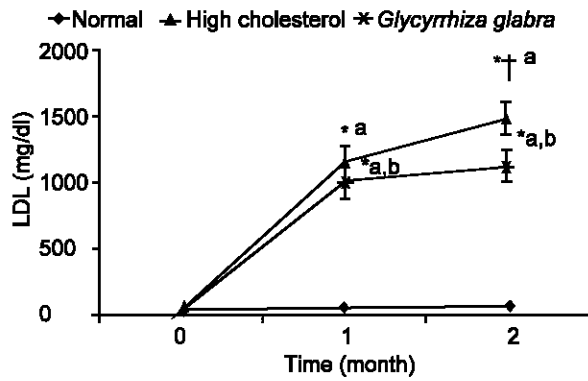


Fig. 3: Changes in serum LDL concentration in the studied groups. Results are expressed as mean±SD.

- \*: Comparison of value at different time with respect to time 0 in respective groups.
- †: Comparison of value at time 2 with respect to time 1 in respective groups.
- a: Comparison between studied groups with respect to Normal group.
- b: Comparison between *Glycyrrhiza glabra* group with respect to Highcholesterol group.

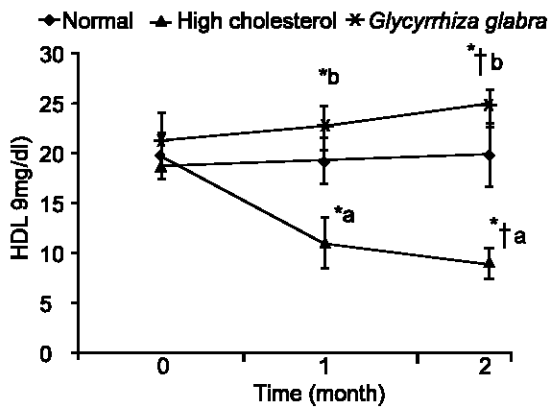


Fig. 4: Changes in serum HDL concentration in the studied groups. Results are expressed as mean ±SD.

- \*: Comparison of value at different time with respect to time 0 in respective groups.
- †: Comparison of value at time 2 with respect to time 1 in respective groups.
- a: Comparison between studied groups with respect to Normal group.
- b: Comparison between *Glycyrrhiza glabra* group with respect to Highcholesterol group.

shown in Fig. 1, 2, 3 and 4 respectively. At the end of the first and second months of experiment, TG, TC and LDL concentrations in the high-cholesterol group were increase significantly compared to the normal diet group whereas HDL concentration in this group were

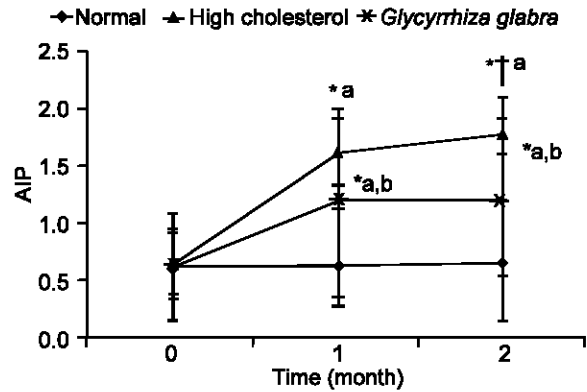


Fig. 5: Changes of AIP level in the studied groups. Results are expressed as mean ±SD.

- \*: Comparison of value at different time with respect to time 0 in respective groups.
- †: Comparison of value at time 2 with respect to time 1 in respective groups.
- a: Comparison between studied groups with respect to Normal group.
- b: Comparison between *Glycyrrhiza glabra* group with respect to Highcholesterol group

decreased significantly compared to the normal diet group ( $p < 0.05$ ). In this times TG, TC and LDL concentrations in the *Glycyrrhiza glabra* were decrease significantly compared to the high-cholesterol group and HDL concentration in this group was increased significantly compared to the high-cholesterol group ( $p < 0.05$ ). Fig. 5 represents atherogenic index in the studied groups. This index were decreased significantly in the *Glycyrrhiza glabra* group compared to the high-cholesterol group ( $p < 0.05$ ). Histological sections of aorta stained from the 3 groups were shown in Fig. 6 and the results of atherosclerotic thickness grading in these groups were summarized in Fig. 7. Atherosclerotic changes were absent in normal diet group (Fig. 6a), whereas in the intimal surface of the aortas from high-cholesterol diet group was seen many fat-laden macrophages. The cytoplasm of the macrophages filled with lipid droplets (foam cell) as the result of lipid digestion by the macrophage. In addition to lipid accumulation, there were smooth muscle cells in the intima. (Fig. 6b). In the *Glycyrrhiza glabra* group some endothelial dysfunction along a few foam cell and macrophages were seen in the intimal surface of the aorta (Fig. 6c). Atherosclerotic thickness grade in the *Glycyrrhiza glabra* group decreased significantly compared to the high-cholesterol group ( $p < 0.05$ ).

### Discussion

The significant decrease in TC, TG and LDL cholesterol and the significant increase in HDL cholesterol in rabbits receiving *Glycyrrhiza glabra* as compared to the high-cholesterol group represent that this extract is

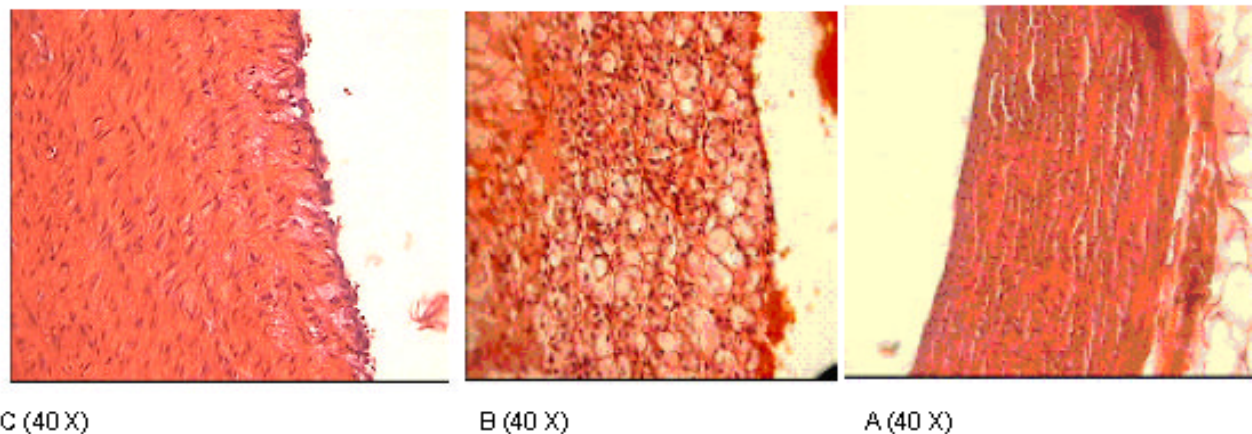


Fig. 6 Aortic intima cross-section in the studied groups.

- Grade 1: Plaque less than half as thick as the media with some form of endothelial dysfunction  
 Grade 2: Plaque at least half as thick as media with accumulation of intracellular lipid, macrophages, and smooth muscle cells.  
 Grade 3: Plaque as thick as the media with an abundance of macrophages, smooth muscle cell, and connective tissue.  
 Grade 4: Plaque thicker than the media with a large extracellular intimal lipid core and inflammatory cell infiltration (Chekanov, 2003).

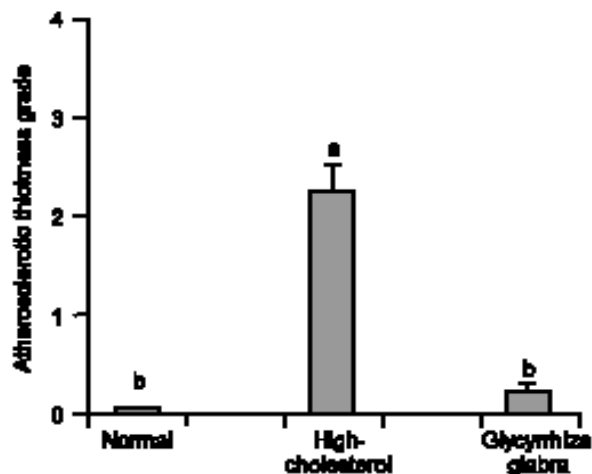


Fig. 7: Mean of atherosclerotic thickness grade in the studied groups.

- a: Comparison between studied groups with respect to Normal group  
 b: Comparison between groups with respect to Highcholesterol group

effective in moderating the dyslipidemic condition arising from a high-cholesterol diet ( $p < 0.05$ ). Fuhrman *et al.* (2002) reported that *Glycyrrhiza glabra* extract decrease TC, TG and LDL cholesterol and increase HDL cholesterol in hypercholesterolemic patients (Fuhrman *et al.*, 2002). Histological results indicate that *Glycyrrhiza glabra* extract significantly reduced arterial wall atherosclerotic lesions, when compared to the high-cholesterol groups ( $p < 0.05$ ). Results from

atherosclerotic thickness grading and atherogenic index plasma were present that these factors in the *Glycyrrhiza glabra* group were decreased significantly compared to the high-cholesterol diet group. On the other hand atherogenic index plasma difference between the *Glycyrrhiza glabra* group and the normal diet group is significant, whereas atherosclerotic thickness grading difference between these two group is not significant. This suggests that decrease in lesions in the group receiving *Glycyrrhiza glabra* has likely been due to antioxidative and anti-inflammatory properties other than the effect of the extract on plasma lipoproteins.

*In vivo* studies have shown that supplementation of licorice extract to atherosclerotic mice deficient in apolipoprotein E reduces the susceptibility of their LDL to oxidation and significantly reduces the development of aortic atherosclerotic lesions (Fuhrman *et al.*, 2002). Studies have shown that licorice-derived glabridin binds to the LDL particle and protects it from oxidation because of its capacity to scavenger free radicals and decrease activation of the NADPH-oxidase system (Belinky *et al.*, 1998; Rosenbla *et al.*, 1999). LDL Aggregation can induce macrophage cholesterol accumulation and foam cell formation. It is considered as a risk factor for atherosclerosis (Braunwald, 1997). Licorice root extract can inhibit LDL aggregation. The inhibitory effect of licorice on LDL aggregation can be attributed to possible binding of licorice extract constituents such as its polyphenols to the LDL particle,

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and such interactions between the lipoprotein hydrophobic domains and the licorice polyphenols can affect interactions between lipoproteins and their subsequent aggregation (Belinky *et al.*, 1998; Fuhrman *et al.*, 1997; Fuhrman *et al.*, 2002). High doses of licorice when used daily over a prolonged period can cause a fluid imbalance in the body, involving salt potassium, and water metabolism. Licorice associated hypertension is thought to be due to increased renal sodium retention (Uum, 2005). Persistence of some licorice-derived components in plasma was caused by this hypertension sustained during the placebo consumption (Fuhrman *et al.*, 2002). Therefore it is suggested that blood pressure is measured during the experiment and also sometimes after this period which determined the effects of extract on this factor.

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