Effect of licorice extract on lipid profile in hypercholestermic male rabbits

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الخلاصة

التصلب العصيدي هو ضمور وتثخن وفقدان المطاطية ,تشمل تراكم الدهون والخلايا الالتهابية كذلك الانسجة الليفية في بطانة الشرايين مما يسبب تثخن بطانة الشرايين الكبيرة والمتوسطة .الاجهاد التاكسدي يلعب دورا كبيرا في بتداء وتطور وظهور لويحة عصيدية.عرق السوس من النباتات التي تحوي مواد لها تاثير خافض للدهون وفلافونويدات لها تاثير مضاد للاكسدة.

اجريت هذه الدراسة لتقييم تاثيرالمستخلص المائي والكحولي لعرق السوس على مستوى الدهون في مصل الدم والتصلب العصيدي في ذكور الارانب التي اعطيت غذاء عالي الكولسترول واظهرت النتائج انخفاضا معنويا بمستوى الكولسترول الكلي والكولسترول واطئ الكثافة والكليسيرول الثلاثي وارتفاعا معنويا بتركيز عالي الكثافة

Abstract

Atherosclerosis is degeneration, hardening and loss of elasticity ,it includes accumulation of lipid, inflammatory cells, and fibrous tissue in the intima, which causes intimal thickening of large and mid-sized arteries. Oxidative stress plays a major role in initiation, propagation and rupture of atherosclerotic plaque. *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.Results show that *Glycyrrhiza glabra* significantly decrease total cholesterol (TC), low- density lipoprotein cholesterol (LDL) and triglyceride (TG) levels with increase in high- density lipoprotein cholesterol (HDL), also it significantly decreases MDA level and increases serum GSH level. Hence *Glycyrrhiza glabra* extract has hypolipidemic and antioxidant effect.

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Introduction

Hyperlipidemia is identified as dyslipidemia , to describe the manifestations of different disorders of lipoprotein metabolism . dyslipidemia is a major risk factor for atherosclerosis and cardiovascular events in middle - aged and older adults, also associated with subclinical atherosclerosis in children and young adults **(** 1**)**.

Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk , dyslipidemia can also describe elevated TC or TG , or low levels of HDL **(2)**. Oxidized LDL is particularly atherogenic and is chemotactic for monocyte-macrophages. Macrophages bind intra-intimal LDL via a family of novel receptors known as scavenger receptors, which recognize LDL only after it has been oxidized , uptake of oxidized LDL renders the macrophages less mobile, thereby promoting the accumulation of these lipid-laden cells in the intima . The foam cells retain their metabolic activity and secrete a variety of cytokines and inflammatory mediators ,Outcomes of their activation include recruitment and proliferation of smooth muscle cells (which in turn elaborate additional locally active cytokines), further LDL oxidation, recruitment of additional monocyte/foam cells and additional impairment of endothelial function (3 ; 4 **).**

Licorice (liquorice) is a plant of ancient origin and steeped in history. It has a long and storied history of use in both Eastern and Western cultures predating the Babylonian and Egyptian empires (5), it contain saponins, flavonoids , stilbenoids and miscellaneous compounds (6). Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities (7).

This study was done to investigate the hypolipidemic effect of alcoholic and watery extract of licorice root .

Materials and methods

Twenty four local domestic male rabbits were used in this study. Their weight was between (2-2.5 kg). The rabbits were housed in the animal house in Collage of Medicine / Babylon University in cages (6 rabbits in each cage) and kept at room temperature of 25$\pm$2°c, relative humidity of 75$\pm$5% and 12 hours light-dark cycles with 12.00 AM being

the mid dark period, rabbits had free access to drink water *ad libitum* and standard chow diet.

After two weeks of adaptation the rabbits were randomly divided into four groups (six rabbits in each group) as follows :

Group 1 : Normal control group, this group was maintained on standard chow diet throughout the duration of the experiment .

Group 2 : Atherosclerosis induced group**,** this group was maintained on atherogenic diet (1% cholesterol mixed with standard chow diet(7) .

Group 3 : Alcoholic extract treated group , this group was maintained on atherogenic diet and alcoholic extract of glycyrrhiza glabra (50 mg/kg) (7) .

Group 4 : Watery extract treated group , this group was maintained on atherogenic diet and watery extract of glycyrrhiza glabra (170 mg/kg) .

The experiment lasted 60 days and blood samples were taken directly 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 prepared by centrifugation at 3000 RPM for 10 minutes. the serum was used to measure the selected parameters .

Preparation of glycyrrhiza glabra extract :

The plant was purchased from local market, after verification of the plant by botanist Nida'a Adnan, glycyrrhiza glabra roots was crushed by a blender, plant extraction was done according to (8), extracts of plant were prepared as follows :

- **Hot watery extraction of glycyrrhiza glabra :**

Watery extract was prepared by mixing 1 gm of powdered plant with 5 ml of distilled water (DW) and boiled at 100°c in a flask with continuous mixing for 10 minutes, then it was mixed for 15-20 minutes away from heat . Then the mixture was filtered through a piece of soft cloth and filter paper to remove all the residual materials. Further separation was done by centrifugation at 3000 RPM for 10 minutes to obtain clear solution of the extract . Then , it was dried at 45 °c by using hot air oven , and kept at 4 °c to be used.

170mg/kg of watery extract (equivalent of 1 g dry powder of dried herb per kg body weight) was given orally every other day throughout the duration of the experiment to watery extract treated group.

- **Ethanolic extraction of glycyrrhiza glabra :**

Ethanolic extract was prepared by mixing 1gm of powdered plant with 10 ml of absolute ethanol and the mixture was left for 3 days in dark place at room temperature, after that it was filtered through a clean piece of cloth and filter paper, the filtrate was centrifuged at 3000 RPM for 10 minutes and the filtrate was dried at 45 °c by using hot air oven , and kept at 4 °c to be used.50 mg/kg of alcoholic extract (equivalent of 1 g dry powder of dried herb per kg body weight) was given orally every other day throughout the duration of the experiment to alcoholic extract treated group (7).

According to (9) , LDL and VLDL concentration was measured as follows :

LDL = total cholesterol – (HDL + VLDL)

VLDL = serum TG /5

According to (10), atherogenic index plasma (AIP) was calculated as follows :

AIP = log(TG/HDL).

Results

Serum TC ,TG ,HDL,LDL and VLDL concentrations was changed as shown in figure (1,2,3,4,5) , at 30 day and 60 day, there was a significant increase in TC ,TG ,LDL and VLDL concentrations and significant decrease in HDL concentration in atherosclerosis induced group as compared with normal control group ($P < 0.01$) . At these times, there is a significant reduction in the serum concentration of TC ,TG ,LDL and VLDL and significant elevation in HDL concentration in alcoholic extract and watery extract treated groups as compared with atherosclerosis induced group ($P < 0.01$) , with greater effect for watery extract than alcoholic extract ($P < 0.01$).

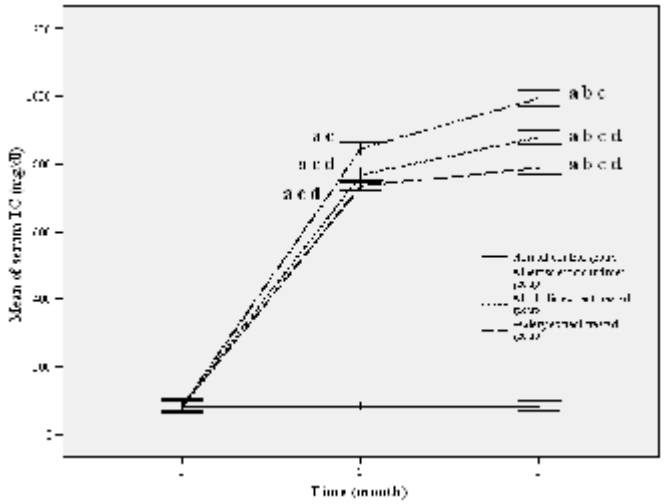
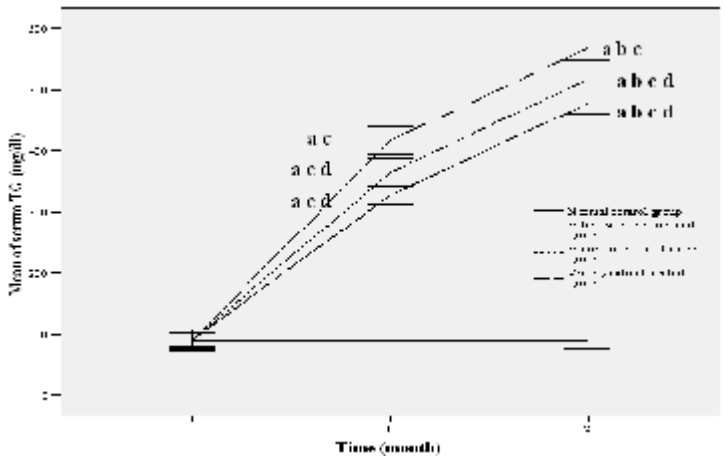


Figure (1) : Changes in the concentration of serum TC expressed as mean ± SD .

a : significant difference from baseline value. c :significant difference from normal control group.

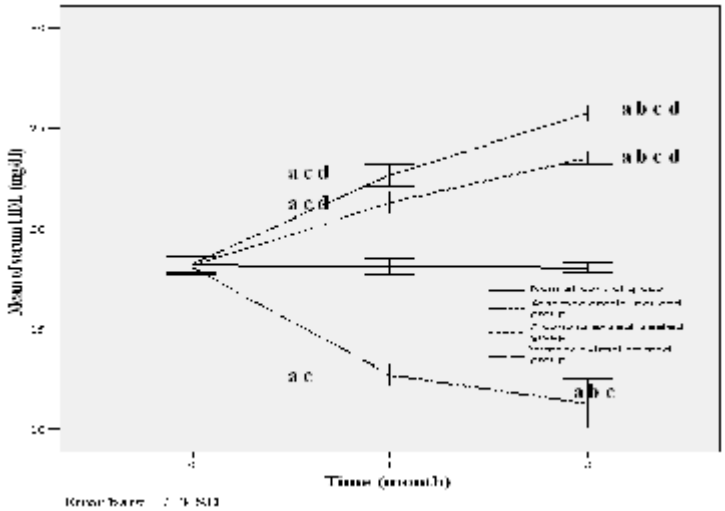
b: significant difference from time1. d :significant difference from atherosclerosis induced group.



a : significant difference from baseline value. c :significant difference from normal control group.

b: significant difference from time1. d :significant difference from atherosclerosis induced group.

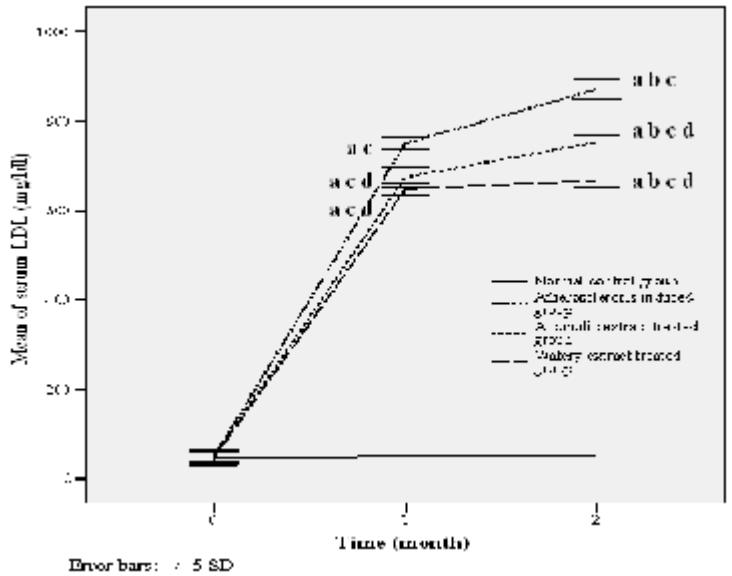
Figure (2) : Changes in the concentration of serum TG expressed as mean ± SD .



a : significant difference from baseline value. c :significant difference from normal control group.

b: significant difference from time1. d :significant difference from atherosclerosis induced group.

Figure (3) : Changes in the concentration of serum HDL expressed as mean ± SD .



Figure(4) :Changes in the concentration of serum LDL expressed as mean± SD

a : significant difference from baseline value. c :significant difference from normal control group.

b: significant difference from time1. d :significant difference from atherosclerosis induced group.

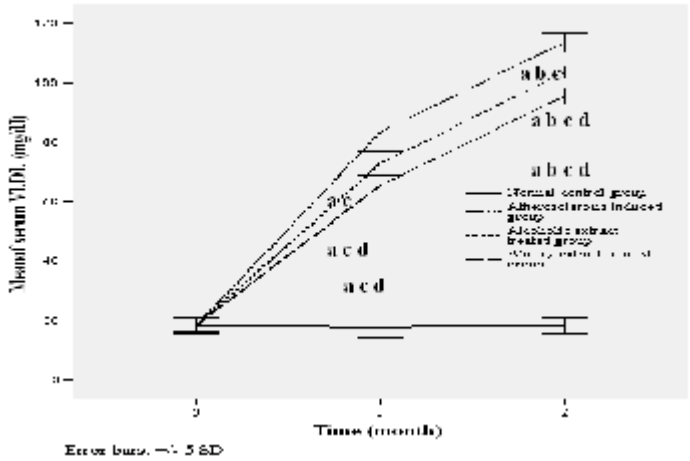


Figure (5) : Changes in the concentration of serum VLDL expressed as mean ± SD.

a : significant difference from baseline value.
b: significant difference from time1.
c :significant difference from normal control group.
d :significant difference from atherosclerosis induced group

Changes in the AIP is shown in figure (6) , within time there was significant increase in the AIP in the atherosclerosis induced group as compared with the normal control group ($p < 0.001$), licorice extract treated groups (alcoholic and watery extract) showed significant reduction in the AIP as compared with the atherosclerosis induced group ($p < 0.001$), with significant difference from normal control group ($p < 0.001$).

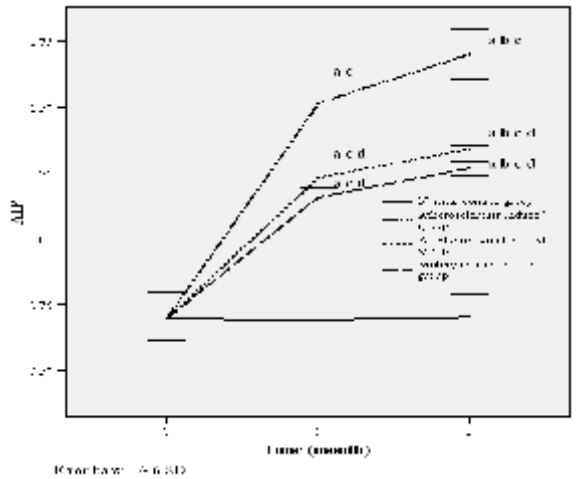


Figure (6) : Changes in the AIP expressed as mean ± SD .

a : significant difference from baseline value.
b: significant difference from time1.
c :significant difference from normal control group.
d :significant difference from atherosclerosis induced group.

Discussion

Within the time , alcoholic extract treated group has shown a significant decline in serum concentration of TC, TG, LDL and VLDL, with a significant increase in the serum concentration of HDL as compared with atherosclerosis induced group , these data is agreed with (7), (11). also there was a significant reduction in the AIP in alcoholic extract treated group as compared with atherosclerosis induced group , these results is agreed with (7).

Watery extract treated group has also shown a significant decrease in serum concentration of TC, TG, LDL and VLDL with significant increase in serum HDL and significant reduction in AIP as compared with atherosclerosis induced group , also we have found that watery extract has greater effect than alcoholic extract , no data yet available to compare our result with.

These results may be due to the presence of phytosterols and saponins in the root of the plant could play an important rule in affecting serum lipid profile parameters (11) .

Phytosterols are well known for their ability to inhibit absorption of cholesterol and lowering of serum cholesterol by two main processes, preferential uptake in the gut for plant sterols versus cholesterol, and improving elimination of cholesterol (12), fat solubility of phytosterol esters followed by their effective intestinal hydrolysis, preferentially of unsaturated fatty acid esters, allows sufficient micellar solubilization of unesterified plant sterols for prevention of cholesterol absorption, and subsequent lowering of their serum concentrations (13).

Saponins form strong insoluble complexes with cholesterol and bile making them unavailable for absorption , this mixture is then removed from the body through the normal elimination process, increased bile acid excretion may cause compensatory increase in bile acid synthesis from cholesterol in the liver. As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the blood stream through increase the hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aid its catabolism to bile acids, thus lowers serum cholesterol (14).

Saponins are known to inhibit pancreatic lipase, leading to greater fat excretion due to reduced intestinal absorption of dietary fats (15), by

this mechanism saponins can lower TG level (16), furthermore, the decline in VLDL cholesterol levels in treated groups could be directly correlated to a decline in TG levels of these groups, as it is well established that VLDL particles are the main transporters of TG in plasma (17).

Saponins could also potentially act to activate the peroxisome proliferators activated receptor (PPAR),a class of nuclear receptors that act on the isoforms PPAR-α and PPAR-γ respectively to induce an increase in LPL activity, and may, therefore, be proposed to be a candidate for raising LPL , LPL acts to hydrolyze the core triacylglycerol (TAG) of circulating TAG-rich lipoproteins to regulate the entry of fatty acids into the underlying tissues, leading to increase lipolytic rate of the chylomicrons and the very-low-density lipoproteins (VLDL) (18).

Plasma HDL-c concentrations correlate positively with plasma LPL activity, In the process of hydrolyzing (TG)-rich lipoproteins (TRL: chylomicrons and VLDL) , redundant surface lipid (free cholesterol and phospholipid) and apolipoproteins are transferred from TRL to HDL particles, contributing significantly to plasma HDL-c and HDL associated apoA-I concentrations .

The roots of the plant contain both ascorbic acid and flavonoids that could have contributed to an increase in HDL cholesterol concentrations in treated dyslipidaemic animals (11).

Ascorbic acid seems to affect the post transcriptional step of the expression of Apolipoprotein A-I (major constituent protein of HDL) (19),and seems to increase the activity of lipoprotein lipase, which in turn appears to participate in the regulation of HDL metabolism (20).

The mechanisms by which flavonoids elevate plasma HDL concentrations remains unclear. One hypothesis is that increase the expression and production of apolipoproteinA1, the major protein component of HDL, has a role in increasing HDL (21).

Flavonoids may augment the activity of lecithin acyl transferase (LCAT) which plays an important role in the incorporation of free cholesterol into HDL, causing an increase in the serum HDL concentration (22).

Conclusions

According to the results of the present study , the following can be concluded out of this study :

1- Watery extract had greater effect on serum lipid profile and atherogenic index than alcoholic extract .
2- Alcoholic extract had greater effect on serum GSH and MDA than watery extract .
3- Both extracts had atherolytic effect .

Recommendations

The following can be recommended for the next studies :

1- Measurement of another antioxidants parameters like SOD and catalase activity.
2- Studying of licorice anti-inflammatory effects .
3- Fractionation of both alcoholic and watery extract and determination of the active constituents that have the effects on serum lipid profile and atherosclerosis .

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