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ORIGINAL ARTICLE

ANTIDYSLIPIDAEMIC ACTIVITY OF *GLYCYRRHIZA GLABRA* IN HIGH FRUCTOSE DIET INDUCED DSYSLIPIDAEMIC SYRIAN GOLDEN HAMSTERS

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ABSTRACT

The root of Glycyrrhiza glabra is a traditional medicine used mainly for the treatment of peptic ulcer, hepatitis C, pulmonary and skin diseases, although clinical and experimental studies suggest that it has several other useful pharmacological properties such as antiinflammatory, antiviral, antimicrobial, antioxidative, anticancer activities, immunomodulatory, hepatoprotective and cardioprotective effects. Glycyrrhizinic acid, a major component of licorice, has antiulcer effect by raising the local concentration of prostaglandins that promote mucous secretion and cell proliferation in the stomach. Glycyrrhizin shows hepatoprotective effect by preventing changes in cell membrane permeability, inhibiting phospholipase A_2 (PLA_2) and increasing survival rate of hepatocytes. Glabridin has effect in melanogenesis and inflammation by inhibiting the tyrosinase activity of melanocytes. α-glycyhrritinic acid exhibits anti-inflammatory activity by inhibiting glucocorticoid metabolism. In present study ethanolic (95%) extract of root of Glycyrrhiza glabra and its fractions were investigated for its antidyslipidaemic activity on HFD induced dyslipidaemic hamsters. Ethanolic extract and its ethyl acetate soluble, water soluble and hexane soluble fractions decreased serum level of total cholesterol by 25.9, 38.0, 39.0 and 26.3%, respectively. On the other hand ethanolic extract, ethyl acetate soluble, water soluble and hexane soluble fraction increased the serum HDL-cholesterol level by 14.8, 34.3, 27.3 and 17.2%, respectively. Ethanolic extract, ethyl acetate fraction, aqueous fraction and hexane fraction decreased triglyceride level by 31.3, 37.2, 41.2 and 28.9%, respectively. The reduction in LDL-cholesterol level by ethanolic extract, ethyl acetate soluble fraction and water soluble fraction were 43.9, 31.0, 33.4 and 24.6%, respectively.

KEY WORDS

Glycyrrhiza glabra, Dyslipidaemia, High fructose diet.

INTRODUCTION

According to the Framingham Heart Study (1), dyslipidaemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is one of the many modifiable risk factors for coronary artery disease (CAD), stroke and peripheral vascular disease. Currently available anti-dyslipidaemic agents include statins, fibrates, nicotinic acids and bile acid sequestrants (2). Since these drugs are not only costly but also have potential side effects, a search for alternative therapeutic agents was necessitated. Phytotherapies are now recognized as an alternative, as a number of plants with various metabolites have potential for therapeutic applications (3, 4).

Glycyrrhiza glabra (GG) (licorice, Fabaceae/Papilionaceae) is a plant with a rich ethnobotanical history. The roots are used as a folk medicine both in Europe and in Eastern countries. The main components are the triterpene saponins, glycyrrhizin and glycyrrhetic acid, which are believed to be partly responsible for anti-ulcer, anti-inflammatory, anti-diuretic, anti-epileptic anti-allergic and antioxidant properties of the plant as well as their ability to "fight" low blood pressure (5).

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Furthermore, GG extracts have been shown to possess antidepressant-like, memory-enhancing activities and produce antithrombotic effects. On the other hand, the root extracts are reported to exhibit antiangiogenic and antitumor activities and radio-protective effects. Besides, the isolates from GG roots viz. glabridin (an isoflavan) and isoliquiritigenin (a flavonoid), are known to be pharmacologically active compounds. Glabridin is reported to be a potent antioxidant towards LDL oxidation (6, 7); whereas isoliquiritigenin is known to exert vasore-laxant effect, anti-platelet, anti-viral, estrogenic activities and has the protective potential against cerebral ischemic injury (8). Antihyperlipilaemic and antihyper-triglyceridaemic properties of GG root have also been reported (9). *Glycyrhhiza* flavonoids provide protection to hepatocytes exposed to carbon tetrachloride, and galactosamine. The researchers pointed to the antilipid peroxidation effect of *Glycyrrhiza* as the central mechanism contributing to its protective action against carbon tetrachloride-induced hepatotoxicity. *Glycyrrhiza* has also been shown to have a significant free-radical quenching effect (10-12).

Additionally, liquorice may be useful in conventional and naturopathic medicine for both mouth ulcers and peptic ulcers (13). Non-prescription aphthous ulcer treatment Canker Melts incorporates *Glycyrrhiza* in a dissolving adherent troche. Liquorice is also a mild laxative and may be used as a topical antiviral agent for shingles, ophthalmic, oral or genital herpes.

MATERIALS AND METHODS

Plant material: The roots of *Glycyrrhiza glabra* were purchased from the local herbal merchandise and were air dried ground to powder and stored in an airtight container.

Extraction and fractionations: Coarsely powdered root was extracted with 95% ethanol by cold percolation method (4×2 l). The solvent was distilled off over boiling water-bath and the extract was concentrated under vacuum at 40-45°C in Flash rotavapour. The crude extract thus obtained (64g) was fractionated in water (200 ml), with hexane (4×200 ml) and followed by ethyl acetate (4×200 ml).

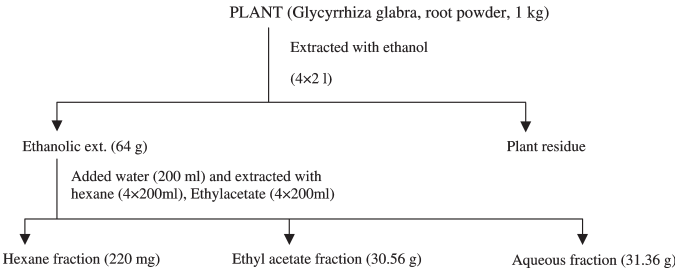

Fig 1: Preparation of ethanolic extract and further fractionation

Preparation of high-fructose diet: High-fructose diet was prepared by mixing fructose 50%; casein 19%; fat 11%; wheat, corn and gram flour mixture 15%, mineral salt 4%; cholesterol 0.5%, vitamin mixture 0.3% and methionine 0.3%.

Animals: Male Syrian golden hamster (100–120 g), obtained from lab animal division of Central Drug Research Institute (CDRI), Lucknow were used for the experiments. They were housed at a room temperature of 25±2°C, relative humidity of 75±5% and 12 h dark–light cycle. Necessary permission from the Institutional Animal Ethics Committee (IAEC) was obtained for the study and the experiments were conducted in accordance with the principles prescribed for laboratory animal use.

Evaluation of Antidyslipidaemic activity: Animals were divided into seven groups of seven hamsters each. The hamsters in Group I received normal pellet diet and served as control while the hamsters of the other groups received HFD pellets for 45 days. During this period, the rats belonging to Groups III was administered with a dose of 100 mg/kg per day of 95% ethanolic extract. Groups IV, V and VI were administered with ethyl acetate fraction, aqueous fraction and hexane fraction in dose of 100 mg/kg per day, respectively. Hamsters belonging to Group VII received fenofibrate (25 mg/

Table 1: Composition of high fructose diet (HFD)

Constituents	Amount (g/kg diet)
Fructose*	500.0
Casein*	190.0
Dalda™† Vanaspati Ghee	110.0
Wheat+Corn+Gram flour	150.0
Cholesterol*	5.0
Methionine#	3.0
Vitamin mix$	3.0
Mineral mixture£	40

*Purchased from SRL (Mumbai, India); # Purchased from HiMedia (Mumbai, India); † Commercial preparation composed of different vegetable oils; $Vitamin mix provided the following nutrients (mg/kg of dry diet): retinol, 1.8; cholecalciferol, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid, 21; pyridoxine, 3; inositol, 45; cyanocobalamin, 0.015; ascorbic acid, 240; DL - tocopherol, 51; menadione, 12; nicotinic acid, 30; paraminobenzoic acid, 15; folic acid, 1.5; biotin, 0.09. £Mineral mixture: $CaHPO_4$: 430 g; KCL: 100 g; NaCl: 100 g; MgO: 10.5 g; $MgSO_4$: 50 g; Fe_2O_3: 3 g; $FeSO_4$. 7 H_2O: 5 g; trace elements (Mn, Cu, Co, Zn, I): 10 g; quantity sufficient to 1000 g.

kg). All treatment with test articles and standards were given orally and lasted for 30 days. Group II was used as HFD control and animals were administered with 1% gum acacia for 30 days.

On 31st day blood samples were withdrawn from the retro-orbital plexus after an overnight fast. Serum was analysed for cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and lipoprotein lipase (LPL) using commercially available kits (Roche Diagnostics, USA). VLDL-C was calculated by formula VLDL=TG/5 (14).

Statistical analysis: Results are expressed as mean±SEM. The results of the study were subjected to analysis of variance (ANOVA) using Graph Pad Prizm followed by Dunnett's t-test for multiple comparisons. Values with P < 0.05 were considered to be significant.

RESULTS

Effect on body weight: After 30 days of study, the body weight of the HFD fed control animals increased significantly when compared to normal diet fed group ($P < 0.01$). Treatment with standard drugs (fenofibrate) as well as ethanolic extract (100 mg/kg) and its fractions appreciably decreased the gain in the body weight ($P < 0.01$).

Antidyslipidaemic activity: It has been reported that high-fructose diet feeding causes diet-induced alterations of lipid metabolism and decreased insulin sensitivity with alterations of hepatic pyruvate dehydrogenase (15) and hepatic very low density lipoprotein secretion (16). HFD induces hepatic stress response resulting in cholesterol and lipid dysregulation (17). The results obtained reveal that serum cholesterol, triglycerides, and LDL-C become significantly higher in the HFD fed hamsters ($p < 0.01$) compared to those in normal diet fed hamsters. The continuous treatment with the root extract of *Glycyrrhiza glabra* significantly brought down the above lipid parameters in the high fructose diet fed hamsters. Serum HDL-C levels were significantly lowered ($p < 0.01$) in HFD fed hamsters which upon treatment with ethanolic extract increased by 14.8% that is comparable to increase brought by fenofibrate (19.5%). Besides the above VLDL was significantly elevated in the HFD fed hamsters ($p < 0.01$) as compared to normal diet control hamsters. The treatment with *Glycyrrhiza glabra* root ethanolic extract and its fractions significantly brought down LDL and VLDL in the HFD fed hamsters to various degrees.

Different fractions of ethanolic extract (viz. ethyl acetate, aqueous and hexane) were studied for their antidyslipidaemic activity in HFD fed hamsters at 50 mg/kg dose. As shown in Table 2, the TG lowering activity was distributed into ethyl acetate fraction, aqueous fraction and hexane fraction as 37.2, 41.2 and 28.9%. Aqueous fraction showed maximum cholesterol lowering effect (39%) followed by ethyl acetate

Table 2: Effect of *Glycyrrhiza glabra* on high fructose diet fed (HFD) hamsters

Groups		Chol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	LPL (U/l)
I	Normal diet control	158.8±9.9	254.1±6.8	60.3±4.6	88.0±9.3	49.6±3.2	49.4±5.5
II	HFD control (1% gum acacia)	258.4±5.1#	570.9±27.9#	55.9±2.90#	168.2±13.1#	114.2±5.6#	32.6±3.70#
III	HFD+ Ethanolic GG extract (100 mg/kg p.o.)	191.6±6.8** (-25.9)	392.4±16.0* (-31.3)	64.1±3.59 (+14.8)	94.3±4.1** (-43.9)	78.5±3.2* (-31.3)	37.0±3.35* (+13.5)
IV	HFD+ ethyl acetate fraction (50 mg/kg p.o.)	160.2±8.3** (-38.0)	358.4±16.4* (-37.2)	75.1±5.72* (+34.3)	116.0±5.6** (-31.0)	71.7±3.3 (-37.2)	35.9±3.4 (+29.3)
V	HFD+ aqueous fraction (50 mg/kg p.o.)	157.6±6.5** (-39.0)	335.6±17.4* (-41.2)	71.2±6.31 (+27.3)	112..1±4.9** (-33.4)	67.1±3.5 (-41.2)	38.6±4.9 (+38.8)
VI	HFD+ Hexane fraction (50 mg/kg p.o.)	190.4±9.4** (-26.3)	405.9±19.2* (-28.9)	65.5±3.8* (+17.2)	126.9±9.6* (-24.6)	81.2±3.8 (-28.9)	30.9±2.9 (+11.0)
VII	HFD+Fenofibrate (25mg/kg/day p.o.)	178.4±9.1** (-31.0)	178.4±9.1** (-55.8)	66.8±6.96 (+19.5)	118.8±5.3** (-29.4)	50.5±3.1* (-55.8)	42.1±4.69* (+29.1)

Data are expressed as mean±SEM ($n = 7$); #$P < 0.01$ when compared with Group I (Normal diet control); *$P < 0.05$ when compared with Group II (HFD control); **$P < 0.01$ or P<0.001 when compared with Group II; Values in parentheses are denoting the percent change as compared to HFD control.

fractions (38%). Aqueous and ethyl acetate fraction exhibited equal LDL-C lowering effect (31%) which was greater than that of hexane fraction (24.6%). There was significant increase in HDL-C level in treated groups which was highest in ethyl acetate fraction (34.3%) followed by aqueous fraction (27.3%).

DISCUSSION

High level of total cholesterol is one of the major risk factors for coronary heart diseases and it is well known that hyperlipidemia and the incidence of atherosclerosis are increased in diabetes (18). The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoprotein. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications (19). Though there are a large class of hypolipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects (20). Hence efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Among the natural materials, medicinal plants hold promise in the discovery of new drugs.

Many herbs and plant products have been shown to have antihyperglycemic and antihyperlipidemic properties (21). In the present study, we carried out experiments to investigate the antidyslipidemic activity of ethanolic extract of *Glycyrrhiza glabra* root powder in the high fructose diet (HFD) fed dyslipidemic hamster model. *Glycyrrhiza glabra* (GG) root powder has been shown to possess hypocholesterolaemic and antioxidant activities in hypercholesterolaemic male albino rats (22). Feeding of high fructose diet resulted in the elevation of various parameters of lipid profile. A significant increase in the body weight was also noticed in HFD fed animals during the study period. Treatment with the standard drug, fenofibrate effectively prevented the increase in body weight to a large extent ($P < 0.01$). The repeated administration of *Glycyrrhiza glabra* ethanolic extract and its fractions for a period of 30 days resulted in a significant decrease in lipid profile in serum when compared to the dyslipidaemic HFD control. The present investigation clearly demonstrates the cholesterol- lowering effects of GG root ethanolic extract and other fractions of ethanolic extract in dyslipidaemic hamsters. In this context, the presence of phytosterols and saponins in GG root could be important in cholesterol elimination. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine (23, 24). Saponins on the other hand, are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acids making it unavailable for intestinal absorption (25, 26). Thus, the presently noted reduced cholesterol levels in dyslipidaemic animals administered with ethanolic extract and its fractions could be due to both phytosterol and saponin content of GG root. HFD diet increases both LDL-Cholesterol levels and oxidative stress that results in increased oxidized-LDL levels leading to atherosclerotic plaque formation (27). A significant decline in plasma LDL-cholesterol in treated groups could be correlated with saponin content of GG root, saponins enhance the hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aid its catabolism to bile acids (25, 28, 29).

Elevated levels of serum TG have been correlated with the development of atherosclerosis and coronary heart disease (30). While the HFD control groups exhibited significantly higher TG levels, treated groups registered a significant decline of TG in serum. Saponins which are present in the root of *G. glabra*, are known to lower TG by inhibiting pancreatic lipase activity (31).

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 (23). Thus, a simultaneous decline in both TG and VLDL-cholesterol in treated groups indicates the possible effect of saponins on one hand, and on the other hand, the effect of phytosterol content of the root on TG metabolism through a decreased absorption of dietary cholesterol. LPL activity was upregulated in treated groups which, in turn, leads to enhanced catabolism of VLDL, decreased fatty acid synthesis and catabolism, and a reduced VLDL secretion (32).

It is well documented that while low-level of HDL-cholesterol is indicative of high risk for coronary heart disease, an increase in HDL-C level is considered beneficial (33). Epidemiological studies have also shown that high HDL-cholesterol levels could potentially contribute to anti-atherogenesis, including inhibition of LDL-oxidation to protect the endothelial cells from the cytotoxic effects of oxidized LDL (34). Presently observed high level of plasma HDL-cholesterol in dyslipidaemic animals administered with GG root ethanolic extract and its fractions as compared to HFD control groups indicates the efficacies of these agents in elevating HDL-cholesterol levels. While saponins are not known to elevate HDL-cholesterol levels (25, 29, 35), ascorbic acid and flavonoids are reported to increase the HDL-cholesterol concentrations (36, 37). The GG root

contained both ascorbic acid (0.58 g %) and flavonoids (0.926 g %) that could have contributed to an increase in HDL-cholesterol concentrations in treated dyslipidaemic animals.

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