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Hypocholesterolaemic and antioxidant effects of *Glycyrrhiza glabra* (Linn) in rats

Nishant P. Visavadiya and Amaravadi V. R. L. Narasimhacharya

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

The hypocholesterolaemic and antioxidant effects of *Glycyrrhiza glabra* (GG) root powder were examined in hypercholesterolaemic male albino rats. A 4-week administration of GG root powder (5 and 10 gm% in diet) to hypercholesterolaemic rats resulted in significant reduction in plasma, hepatic total lipids, cholesterol, triglycerides and plasma low-density lipoprotein and VLDL-cholesterol accompanied by significant increases in HDL-cholesterol levels. Furthermore, significant increases in fecal cholesterol, neutral sterols and bile acid excretion along with an increase in hepatic HMG-CoA reductase activity and bile acid production were observed in these animals. The root powder administration to hypercholesterolaemic rats also decreased hepatic lipid peroxidation with a concomitant increase in superoxide dismutase (SOD) and catalase activities and total ascorbic acid content. Thus, the hypocholesterolaemic and antioxidant effects of GG root appeared to be mediated via (i) accelerated cholesterol, neutral sterol and bile acid elimination through fecal matter with an increased hepatic bile acid production and (ii) improving the activities of hepatic SOD, catalase and increasing the ascorbic acid content. The normo-cholesterolaemic animals when fed with GG root powder at 10 gm% level, registered a significant decline in plasma lipid profiles and an increase in HDL-cholesterol content. The antioxidant status of these animals also was improved upon treatment.

Keywords: Antioxidants / Cholesterol metabolism / *Glycyrrhiza glabra* / Hypercholesterolemia / Lipid peroxidation

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1 Introduction

It is widely accepted that hypercholesterolemia, elevated low-density lipoprotein (LDL) cholesterol concentration and triglycerides (TG) are major risk factors for development of atherosclerosis and cardio-vascular diseases [1, 2]. For a decade now, the circulating concentrations of free radicals are regarded as the central issue in atherosclerosis, as these convert LDL into oxidized-LDL, which plays a key role in atherosclerosis [3]. Currently available hypocholesterolaemic agents include statins, fibrates, nicotinic acids and bile acid sequestrants [1]. 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 [4, 5].

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 [6]. Furthermore, GG extracts have been shown to possess antidepressant-like, memory-enhancing activities and produce antithrombotic effects [7–9]. On the other hand, the root extracts are reported to exhibit antiangiogenic and antitumor activity [10] and radio-protective effects [11]. Besides, the isolates from GG roots, glabridin (an isoflavan) and isoliquiritigenin(a flavonoid), are known to be pharmacologically active compounds. Glabridin is reported to be potent antioxidant towards LDL oxidation [12, 13] whereas isoliquiritigenin is known to exert vasore-

Correspondence: Dr. Amaravadi V. R. L. Narasimhacharya, Department of Biosciences, Sardar Patel University, University Road, Vallabh Vidyanagar-388120, Gujarat, India
E-mail: n.bs@spu.ernet.in, narasimhacharya@yahoo.com
Fax: +91-2692-226-865/236-475

Abbreviations: GG, *Glycyrrhiza glabra*; **HC**, hypercholesterolaemic animals; **HGG-I**, hypercholesterolaemic animals administered with 5 gm% *Glycyrrhiza glabra* root powder; **HGG-II**, hypercholesterolaemic animals administered with 10 gm% *Glycyrrhiza glabra* root powder; **LDL**, low-density lipoprotein; **NC**, normal controls; **NGG-I**, normal animals administered with 5 gm% *Glycyrrhiza glabra* root powder; **NGG-II**, normal animals administered with 10 gm% *Glycyrrhiza glabra* root powder; SOD, superoxide dismutase; **TG**, triglycerides

Table 1. Composition of the diet (gm%)

Ingredient	NC	NGG-I	NGG-II	HC	HGG-I	HGG-II
Crude protein	22.12	22.12	22.12	22.12	22.12	22.12
Crude	55.67	55.67	55.67	55.67	55.67	55.67
Crude fat	4.06	4.06	4.06	4.06	4.06	4.06
Crude fiber	3.76	3.76	3.76	3.76	3.76	3.76
Mineral mixture	5.64	5.64	5.64	5.64	5.64	5.64
Cholesterol	–	–	–	0.5	0.5	0.5
Sodium taurocholate	–	–	–	1.0	1.0	1.0
Glycyrrhiza glabra root powder	–	5.00	10.00	–	5.00	10.00

laxant effect, anti-platelet, anti-viral, estrogenic activities and has the protective potential against cerebral ischemic injury [14]. Although antihyperlipilaemic and antihypertriglyceridaemic properties of GG root have been reported [15], no detailed reports are available on the synergistic effects of GG root on hyperlipidemia/hypercholesterolemia and oxidative stress in hypercholesterolaemic animal models and its role on body cholesterol metabolism. The objectives of the present study are to examine the effects of GG root powder feeding on hepatic cholesterol metabolism in hypercholesterolaemic animals, to relate them to plasma, hepatic and fecal sterol concentrations, and, to determine the hepatic lipid peroxidation and antioxidant status.

2 Materials and methods

2.1 Root powder preparation and analysis

The GG roots were purchased from the local herbal merchandise and were authenticated by our faculty taxonomist Dr. A. S. Reddy. Roots were air-dried, ground to powder and stored in an airtight container. The root powder was extracted in petroleum ether to remove fat and subjected to acid and alkaline treatment and the fiber content was estimated by gravimetric analysis [16]. Phytosterol and saponin contents of the root were estimated using ferric chloride – sulfuric acid and Vanillin-sulfuric acid methods, respectively [17, 18]. The polyphenol and flavonoid contents of the root were analyzed using Folin-Ciocalteu and Vanillin-sulfuric acid reagents, respectively [16, 19]. The total ascorbic acid content was estimated using 2,4-dinitrophenyl hydrazine reagent [20].

2.2 Experimental design

The 3-month-old male Albino rats (Charles Foster) weighing 150 – 200 gm were used for the present investigation. The animals were provided standard diet (Pranav Agro, Vadodara, India) and water *ad libitum*. Animals were housed individually in well-ventilated animal unit (26 ± 2°C, humidity 62%, and 12-h light/dark cycle). The Institutional Animal Ethics Committee approved the present investigation.

After a 10-day adaptation period, 48 animals were divided into six groups of 8 rats each as follows: NC,normal controls; NGG-I, normal animals administered with 5 gm% GG root powder; NGG-II- normal animals administered with 10 gm% GG root powder; HC, hypercholesterolaemic animals; HGG-I, hypercholesterolaemic animals administered with 5 gm% GG root powder; HGG-II- hypercholesterolaemic animals administered with 10 gm% GG root powder. Hypercholesterolemia was induced by addition of 0.5 gm% cholesterol and 1.0 gm% sodium taurocholate to the basal diet. The GG root powder was incorporated at 5 gm% and 10 gm% level in the diet. The composition of basal diet and the diet to induce hypercholesterolemia/hyperlipidemia is shown in Table 1. Prior to the termination of experiment, a 24-h fecal matter was collected from the individual cages for fecal analysis of cholesterol, neutral sterol and bile acid. At the end of experiment, animals were deprived of food overnight and sacrificed under light ether anesthesia. Blood was collected by cardiac puncture and plasma was separated by centrifugation. Liver was excised and both plasma and liver were kept frozen until analyzed.

2.3 Plasma and hepatic lipid profiles

Plasma total lipid (TL) content was estimated by sulfophosphovanillin method [21]. Plasma cholesterol (TC) and TG were estimated by ferric perchlorate-sulfuric acid and GPO methods, respectively [22, 23]. HDL-cholesterol (HDL-C) was extracted using phosphotungstate-magnesium chloride reagent from plasma [24] and estimated [22]. LDL-cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and atherogenic index (AI) were calculated [25]. The liver TL was extracted in chloroform:methanol (2:1) [26] and estimated by gravimetric analysis. TC and TG were extracted [18] and estimated [22, 23].

2.4 Hepatic HMG-CoA reductase and bile acid profile

Hepatic HMG-CoA reductase (EC 1.1.1.34) activity was measured in terms of the ratio of HMG-CoA to mevalonate

Table 2. Phytoconstituents of *Glycyrrhiza glabra* root powder (triplicate values; mean ± SD)

Phytoconstituents	mg/gm dry material
Crude fiber	128.00 ± 2.00 (12.8%)
Phytosterols	29.97 ± 1.89 (2.997%)
Saponins	37.75 ± 2.35 (3.775%)
Polyphenols	32.20 ± 1.91 (3.220%)
Flavonoids	9.26 ±1 .09 (0.926%)
Total ascorbic acid	5.80 ± 0.05 (0.580%)

[27]. Colorimetric determinations were carried out for both HMG-CoA and mevalonate using hydroxylamine reagent at alkaline and acidic pH, respectively. The ratio of HMG-CoA to mevalonate is inversely proportional to the enzyme activity, *i. e*. the increase in ratio corresponds to a decrease in enzyme activity. The alkaline-ethanolic extract of hepatic bile acid was acidified and estimated using vanillin-phosphoric acid reagent [28].

2.5 Fecal cholesterol, neutral sterol and bile acid content

The fecal cholesterol, neutral sterols and bile acid were extracted using alkaline methanol medium [29] and fecal cholesterol and neutral sterols were estimated [22, 30]. A portion of the extract was acidified and used for bile acid estimation [28].

2.6 Hepatic lipid-peroxidation and antioxidant profile

The hepatic lipid peroxidation (malondialdehyde concentration) was determined by thiobarbituric acid (TBA) assay [31]. Catalase (EC 1.11.1.6) was assayed spectrophotometrically as decomposition of H_2O_2 at 240 nm [32] and superoxide dismutase (SOD; EC 1.15.1.1) activity was measured using nitrobluetetrazolium reduction method [33]. Total ascorbic acid (TAA) contents were estimated [20].

2.7 Statistical analysis

Results are expressed as means ± SEM. Significant differences among the groups were determined by one-way ANOVA using 10th version of SPSS with Duncan's test as *post hoc* analysis. Differences were considered significant if $p < 0.5$.

3 Results

3.1 Phytoconstituents in GG root

The quantitative phytochemical analysis of GG root indicated that the root contained 12.8 gm% fiber, 2.99 gm% phytosterol, 3.77 gm% saponin, 3.22 gm% polyphenol, 0.92 gm% flavonoid and 0.58 gm% ascorbic acid (Table 2).

3.2 Food intake, body weight, and liver weight

No significant variations were found among different groups with respect to body weight and food intake. While the HC group registered an increase in liver weight (by 25%) as compared to that of NC group, both HC and NC animals administered with GG root powder demonstrated a decline in liver weight (Table 3).

3.3 Plasma and hepatic lipid profiles

The plasma lipid profiles of NGG-I, NGG-II, HGG-I and HGG-II groups revealed a dose-dependant response

Table 3. Final body weight, food intake and liver weight in experimental animals ($n = 8$)

Group	Food intake	Body weight	Liver weight
NC	16.00 ± 0.43	239.50 ± 1.58[a)]	8.18 ± 0.20[b)]
NGG-I	16.06 ± 0.33 (+ 0.37)	220.87 ± 3.06[c)] (−7.77)	7.98 ± 0.20[b)] (−2.44)
NGG-II	16.12 ± 0.28 (+ 0.75)	227.62 ± 3.80[b)] (−4.96)	7.88 ± 0.22[b)] (−3.66)
HC	16.12 ± 0.34 (+ 0.75)	233.12 ± 4.28[a)] (−2.66)	10.22 ± 0.28[a)] (+24.93)
HGG-I	16.00 ± 0.26 (−0.74)	230.25 ± 4.10[a)] (−1.23)	8.12 ± 0.23[b)] (−20.54)
HGG-II	16.06 ± 0.30 (−0.37)	240.12 ± 2.30[a)] (+3.00)	8.06 ± 0.24[b)] (−21.13)
One-way FAVOVA	0.065	4.857	13.979
df	5, 42	5, 42	5, 42
p	NS	0.01	0.0001

a) Values = gm, mean ± SEM.
b) Figures in parentheses indicate percent increase (+) or decrease (−).
c) Comparisons for the percentage were taken between groups NC *vs*. NGG-I; NC *vs*. NGG-II; NC *vs*. HC; HC *vs*. HGG-I; HC *vs*. HGG-II. Means in the column not sharing a common superscript are significantly different (Duncan, $p < 0.05$).

Table 4. Effect of *Glycyrrhiza glabra* (root powder) feeding on plasma lipid profiles ($n = 8$)

Group	TL	TC	TG	HDL-C	LDL-C	VLDL-C	AI
NC	319.83 ± 6.20[c)]	119.16 ± 2.92[b)]	43.14 ± 1.28[b)]	65.53 ± 1.13[d)]	45.00 ± 3.41[b)]	8.62 ± 0.25[b)]	1.81 ± 0.05[b)]
NGG-I	320.58 ± 4.78[c)] (+0.23)	114.34 ± 3.35[b)] (−4.04)	41.13 ± 1.34[b)] (−4.65)	79.63 ± 1.30[b)] (+21.51)	26.49 ± 3.49[c)] (−41.13)	8.22 ± 0.26[b)] (−4.64)	1.43 ± 0.04[c)] (−20.99)
NGG-II	313.70 ± 3.87[c)] (−1.91)	109.04 ± 3.47[c)] (−8.49)	30.21 ± 1.26[c)] (−29.97)	84.69 ± 1.32[a)] (+29.23)	18.30 ± 3.95[c)] (−59.33)	6.04 ± 0.25[c)] (−29.93)	1.28 ± 0.04[d)] (−29.28)
HC	730.91 ± 9.50[a)] (+128.53)	353.66 ± 3.93[a)] (+196.79)	52.51 ± 1.73[a)] (+21.71)	45.56 ± 1.38[e)] (−30.47)	297.59 ± 4.64[a)] (+561.31)	10.50 ± 0.34[a)] (+21.80)	7.81 ± 0.25[a)] (+331.49)
HGG-I	350.41 ± 6.55[b)] (−52.05)	124.82 ± 3.60[b)] (−64.70)	38.81 ± 1.92[b)] (−26.09)	73.72 ± 1.59[c)] (+61.80)	43.33 ± 4.14[b)] (−85.43)	7.76 ± 0.38[b)] (−26.09)	1.69 ± 0.06[b)] (−78.36)
HGG-II	342.66 ± 6.68[b)] (−53.13)	107.63 ± 4.93[c)] (−69.56)	31.02 ± 1.32[c)] (−40.92)	77.12 ± 1.45[b)] (+69.27)	24.31 ± 4.51[c)] (−91.83)	6.20 ± 0.26[c)] (−40.95)	1.38 ± 0.05[c)] (−82.33)
One-way FANOVA	641.688	678.483	30.686	104.507	729.211	30.706	474.527
df	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42
p	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

a) Values = mean ± SEM; plasma = mg/100 mL.
b) Figures in parentheses indicate percent increase (+) or decrease (−).
c) Comparisons for the percentage were taken between groups NC *vs*. NGG-I; NC *vs*. NGG-II; NC *vs*. HC; HC *vs*. HGG-I; HC *vs*. HGG-II.
Means in the column not sharing a common superscript are significantly different (Duncan, $p < 0.05$).

Table 5. Effect of *Glycyrrhiza glabra* (root powder) feeding on hepatic lipid profile, HMG-CoA reductase activity and bile acid content ($n = 8$)

Group	TL	TC	TG	HMG-CoA reductase	Bile acid
NC	32.37 ± 2.35[d)]	1.97 ± 0.09[d)]	2.94 ± 0.12[c)]	2.69 ± 0.08[c)]	3.73 ± 0.07[d)]
NGG-I	29.87 ± 2.01[d)] (−7.72)	2.04 ± 0.09[d)] (+3.55)	2.80 ± 0.09[c)] (−4.76)	2.53 ± 0.06[c)] (+5.94)	3.57 ± 0.07[d)] (−4.28)
NGG-II	28.87 ± 1.75[d)] (−10.81)	1.83 ± 0.07[d)] (−7.10)	2.67 ± 0.10[c)] (−9.18)	2.54 ± 0.04[c)] (+5.57)	3.74 ± 0.08[d)] (+0.26)
HC	130.5 ± 3.27[a)] (+303.15)	34.70 ± 1.30[a)] (+1661)	25.51 ± 1.30[a)] (+767.68)	7.02 ± 0.12[a)] (−160.96)	6.77 ± 0.09[c)] (+81.50)
HGG-I	68.12 ± 4.12[b)] (−47.80)	13.68 ± 1.11[b)] (−60.57)	19.46 ± 1.04[b)] (−23.71)	3.00 ± 0.07[b)] (+57.26)	11.10 ± 0.07[b)] (+63.95)
HGG-II	48.12 ± 2.33[c)] (−63.12)	8.99 ± 0.73[c)] (−74.09)	18.32 ± 0.91[b)] (−28.18)	2.50 ± 0.04[c)] (+64.38)	11.35 ± 0.08[a)] (+67.65)
One-way FANOVA	203.255	280.192	175.258	508.469	1930.211
df	5, 42	5, 42	5, 42	5, 42	5, 42
p	0.0001	0.0001	0.0001	0.0001	0.0001

a) Values = mean ± SEM; Liver = mg/gm
b) Figures in parentheses indicate percent increase (+) or decrease (−).
c) Comparisons for the percentage were taken between groups NC *vs*. NGG-I; NC *vs*. NGG-II; NC *vs*. HC; HC *vs*. HGG-I; HC *vs*. HGG-II.
d) HMG-CoA reductase activity is inversely proportional to the ratio HMG CoA/mevalonate.
Means in the column not sharing a common superscript are significantly different (Duncan, $p < 0.05$).

(Table 4). The NGG-II group animals registered significant decline ($p < 0.05$) in TC (8%), TG (30%), LDL-C (59%), VLDL-C (30%) and AI (29%) and a rise ($p < 0.05$) in HDL-C (29%) as compared to NC group. Both HGG-I and HGG-II groups registered further significant decreases ($p < 0.05$) in plasma TL (52; 53%), TC (65; 70%), TG (26; 41%), LDL-C (85; 92%) VLDL-C (26; 41%) and AI (78; 82%) and an increase ($p < 0.05$) in HDL-C (62; 69%) as compared to HC group (Table 4). Both NGG-I and NGG-II groups did not reveal any significant variation in hepatic lipid profiles as compared to NC group. However, both hypercholesterolaemic groups (HGG-I, HGG-II) exhibited a significant decline ($p < 0.05$) in hepatic TL (48p%; 63%), TC (61p%; 74%) and TG (24p%; 28%) upon GG root powder administration (Table 5).

3.4 Hepatic cholesterol metabolism

The hepatic HMG-CoA reductase activity and bile acid contents were marginally affected in NGG-I and NGG-II groups as compared to NC group. However, in HGG-I and

Table 6. Effect of Glycyrrhiza glabra (root powder) feeding on fecal cholesterol, fecal neutral sterols, and fecal bile acid excretion ($n = 8$)

Group	Cholesterol	Neutral sterols	Bile acid
NC	1.91 ± 0.04[c)]	4.93 ± 0.10[c)]	5.76 ± 0.15[c)]
NGG-I	2.00 ± 0.04[c)] (+ 4.71)	4.95 ± 0.07[c)] (+ 0.40)	5.83 ± 0.10[c)] (+ 1.21)
NGG-II	1.99 ± 0.04[c)] (+ 4.18)	5.05 ± 0.07[c)] (+ 2.43)	5.85 ± 0.09[c)] (+ 1.56)
HC	6.02 ± 0.12[b)] (+ 215.18)	8.39 ± 0.10[b)] (+ 70.18)	10.63 ± 0.52[b)] (+ 84.54)
HGG-I	8.12 ± 0.09[a)] (+ 34.88)	11.19 ± 0.08[a)] (+ 33.37)	17.67 ± 0.29[a)] (+ 66.22)
HGG-II	8.20 ± 0.07[a)] (+ 36.21)	11.24 ± 0.09[a)] (+ 33.96)	18.23 ± 0.26[a)] (+ 71.49)
One-way FANOVA	1573.325	1068.596	436.218
df	5, 42	5, 42	5, 42
p	0.0001	0.0001	0.0001

a) Values = mean ± SEM; fecal = mg/gm
b) Figures in parentheses indicate percent increase (+) or decrease (−).
c) Comparisons for the percentage were taken between groups NC *vs*. NGG-I; NC *vs*. NGG-II; NC *vs*. HC; HC *vs*. HGG-I; HC *vs*. HGG-II. Means in the column not sharing a common superscript are significantly different (Duncan, $p < 0.05$).

HGG-II groups a significant raise ($p < 0.05$) in HMG-CoA reductase activity (57 and 64%, respectively) and bile acid contents (64 and 68%, respectively) were noted (Table 5).

3.5 Fecal analysis

The NGG groups did not reveal any significant variation in fecal cholesterol, neutral sterol and bile acid contents as compared to NC group. Both the HGG-I and HGG-II groups revealed a significant increase ($p < 0.05$) in fecal cholesterol (35, 36%, respectively), neutral sterol (33, 34%, respectively) and bile acid excretion (66, 71%, respectively) as compared to HC group (Table 6).

3.6 Hepatic lipid peroxidation and antioxidant profile

A decreased ($p < 0.05$) hepatic lipid peroxidation (8, 11%, respectively) with a rise ($p < 0.05$) in ascorbic acid content (23, 26%, respectively) was noted in NGG-I and NGG-II groups as compared to NC group. However, hepatic catalase and SOD activities did not show any significant alteration in these groups. The HGG-I and HGG-II groups revealed further decrease ($p < 0.05$) in lipid peroxidation (35%) with a further raise ($p < 0.05$) in catalase (37, 41%, respectively) and SOD (23, 32%, respectively) activities and also an increase in ascorbic acid (52, 55%, respectively) levels, as compared to HC group (Table 7).

4 Discussion

The present investigation clearly demonstrates that the cholesterol-lowering effects of GG root in hypercholesterolaemic rats is related to an increased excretion of cholesterol, neutral sterols, bile acid and an increase in hepatic bile acid content. In this context, the presence of phytosterols (2.997 gm%), saponins (3.775 gm%) and fiber (12.8 gm%) in GG root could be important in cholesterol elimination and an increase in hepatic bile acid content in GG root fed hypercholesterolaemic rats. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine [34, 35]. 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 [36, 37]. Thus, the presently noted reduced cholesterol levels in hypercholesterolaemic animals administered with GG root powder could be due to both phytosterol and saponin content of GG root. The accelerated fecal excretion of cholesterol, neutral sterol and bile acid in these animals could also be a response to the relatively higher fiber content of the root. This view is in accordance with an earlier report suggesting that the cholesterol lowering effect of fibers is primarily due to an increased excretion of cholesterol and bile acids [38]. The significant decreases in hepatic cholesterol in HGG-I and HGG-II groups also indicate the possible influence of GG root fiber as dietary fibers interfere with cholesterol absorption and enterohepatic bile circulation and result in depletion of hepatic cholesterol pools [39, 40]. A significant decline in plasma LDL-cholesterol in these groups could be correlated with the fiber and saponin content of GG root as both fibers and saponins enhance the hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aid its catabolism to bile acids [36, 40, 41].

Elevated levels of plasma TG have been correlated with the development atherosclerosis and coronary heart disease [2]. While the HC group exhibited significantly higher TG levels, both HGG-I and HGG-II groups registered a significant decline of TG in plasma and hepatic tissue indicating the hypotriglyceridaemic effect of GG root. Both dietary

Table 7. Effect of *Glycyrrhiza glabra* (root powder) feeding on concentrations of malondialdehyde (MDA), activities of catalase and superoxide dismutase and total ascorbic acid in liver ($n = 8$)

Group	Lipid peroxidationnm MDA/gm	Catalasenm H_2O_2 decomposed/s/gm	Superoxide dismutaseUnit/mg protein	Total ascorbic acidμg/gm
NC	10.25 ± 0.21[b)]	17.74 ± 0.54a	4.18 ± 0.10[a)]	137.46 ± 3.88[c)]
NGG-I	9.40 ± 0.14[c)] (−8.29)	18.22 ± 0.49[a)] (+2.70)	4.27 ± 0.11[a)] (+2.15)	169.01 ± 2.69[a)] (+22.95)
NGG-II	9.17 ± 0.17[c)] (−10.53)	18.50 ± 0.54[a)] (+4.28)	4.27 ± 0.12[a)] (+2.15)	173.25 ± 2.63[a)] (+26.03)
HC	14.30 ± 0.22[a)] (+39.51)	8.58 ± 0.37[c)] (−51.63)	2.63 ± 0.13[c)] (−37.08)	100.91 ± 3.88[d)] (−26.58)
HGG-I	9.36 ± 0.20[c)] (−34.54)	11.77 ± 0.39[b)] (+37.17)	3.23 ± 0.12[b)] (+22.81)	153.16 ± 2.48[b)] (+51.77)
HGG-II	9.35 ± 0.20[c)] (−34.61)	12.14 ± 0.31[b)] (+41.49)	3.48 ± 0.09[b)] (+32.31)	156.22 ± 3.24[b)] (+54.81)
One-way FANOVA	92.340	85.830	32.286	69.100
df	5, 42	5, 42	5, 42	5, 42
p	0.0001	0.0001	0.0001	0.0001

a) Values = mean ± SEM.
b) Figures in parentheses indicate percent increase (+) or decrease (−).
c) Comparisons for the percentage were taken between groups NC *vs*. NGG-I; NC *vs*. NGG-II; NC *vs*. HC; HC *vs*. HGG-I; HC *vs*. HGG-II. Means in the column not sharing a common superscript are significantly different (Duncan, $p < 0.05$).

fibers and saponins are known to lower TG by decreasing hepatic lipogenesis and inhibiting pancreatic lipase activity, respectively [41, 42]. Furthermore, the decline in VLDL-cholesterol levels in HGG 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 [34]. Thus, a simultaneous decline in both TG and VLDL-cholesterol in HGG groups indicates the possible effect of both fiber and saponin 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. An increased HMG-CoA reductase activity in both HGG-I and HGG-II groups compared to that of HC group appears to constitute a metabolic alteration occurring in hepatic tissue as a response to dietary fiber and saponin; such a compensatory increase in hepatic cholesterol synthesis is reported to occur when intestinal cholesterol absorption is impaired or when bile acid synthesis is stimulated [36, 38, 39]. Thus, the increased HMG-CoA reductase activity in HGG-I and HGG-II groups alone, and not in HC, NC, NGG-I and NGG-II groups clearly indicates the compensatory inductive effect of GG root on cholesterol synthesis.

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 [43]. 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 [44]. Presently observed high level of plasma HDL-cholesterol in both hyper- and normo-cholesterolaemic animals administered with GG root powder as compared to HC and NC groups indicates its efficacy in elevating HDL-cholesterol levels. While dietary saponins and fibers are not known to elevate HDL-cholesterol levels [36, 39, 41], ascorbic acid and flavonoids are reported to increase the HDL-cholesterol concentrations [45, 46]. The GG root contained both ascorbic acid (0.58 gm%) and flavonoids (0.926 gm%) that could have contributed to an increase in HDL-cholesterol concentrations in both GG root powder administered to hyper- and normo-cholesterolaemic animals.

High cholesterol diet increases both LDL cholesterol levels and oxidative stress that results in increased oxidized-LDL levels leading to atherosclerotic plaque formation [47]. Several studies suggest that naturally occurring antioxidants such as polyphenols, flavonoids and vitamin-C in diet may play a role as anti-atherogenic agents [45, 46, 48, 49]. In addition to ascorbic acid and flavonoids, the GG root also contained polyphenols (3.22 gm%). While polyphenols and flavonoids scavenge hydroxyl and superoxide anions [48, 50], ascorbic acid and flavonoids were shown to synergistically decrease lipid peroxidation and improve lipid profile [45]. In this context, it is pertinent to note that the *Glycyrrhiza* root extract has been shown to possess antioxidant activity *in vitro* [51] and glabridin, one of the major components of the root is reported to be a potent antioxidant that prevents LDL oxidation [12, 13]. Epidemiological studies have revealed that diets containing polyphenols and flavonoids also stimulated catalase and SOD activities and decreased malondialdehyde concentration [49, 52]. Administration of GG root powder to hypercholesterolaemic animals significantly decreased lipid peroxidation with a concomitant increase in catalase, SOD activities and ascorbic acid levels. Thus, the elevated levels of hepatic antioxidants in GG root powder administered animals might be due to the presence of polyphenols and flavonoids in the diet. Additionally, the root could be a potent source for ascorbic acid as it suppressed lipid peroxidation and reduced the levels of malondialdehyde.

The present investigation therefore, suggests that the hypolipidaemic/hypocholesterolaemic effect of GG root is mediated through an increased cholesterol turnover via higher fecal sterol excretion. The increased hepatic antioxidant activities in GG root powder administered animals indicate a decreased oxidative susceptibility in these animals. These hypolipidaemic/hypocholesterolaemic and antioxidant effects of GG root powder could be attributed to aforementioned phytoconstituents.

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5 References

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