

## Hypolipidemic Effect of *Glycine tomentella* Root Extract in Hamsters

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**Abstract:** The influence of the aqueous crude extract of *Glycine tomentella* root (Leguminosae) on lipid metabolism was investigated in hyperlipidemic hamsters. It was found that the administration of the *G. tomentella* extract (GTE) leads to a decrease of high serum cholesterol and triglyceride levels induced by high-fat diet. The GTE also increased serum high-density lipoprotein (HDL) cholesterol and decreased serum low-density lipoprotein (LDL) cholesterol. The reduction of serum triglyceride levels was accompanied by a significant decrease in the hepatic triglyceride content, while the cholesterol content was not changed. The results indicate that GTE is definitely an anti-hyperlipidemic agent, at least, in animals.

**Keywords:** Cholesterol; Triglyceride; High-fat Diet; *Glycine tomentella*; Hypolipidemic Effect; Hamsters.

### Introduction

*Glycine tomentella* Hayata (Leguminosae) has long been used in the Knimen area of Taiwan as an anti-inflammatory agent for the treatment of rheumatic illness (Ko *et al.*, 1999). Recently in Taiwan, herbal tea of *G. tomentella* root has been developed for commercial purposes. In our preliminary study, we found that antioxidant abilities of *G. tomentella* extract (GTE) correlated with their total polyphenol content based on the evaluation of different antioxidant test systems (data unpublished). These findings prompted us to investigate whether the antioxidant effect of GTE might be detectable *in vivo* in the whole animals.

Much evidence indicates that lipid peroxidation of low-density lipoprotein (LDL) contributes to the pathophysiology of atherosclerosis (Steinberg *et al.*, 1989; Diaz *et al.*, 1997). It is well known that probucol enhances regression of atherosclerotic plaques that appears to be related to its antioxidant properties of inhibiting oxidation of LDL (Carew *et al.*, 1987). Therefore, antioxidants carried within LDL particles can curtail the formation

of oxidized LDL, thus providing a paradigm for the therapeutic use of antioxidants in the treatment and prevention of atherosclerosis (Steinberg *et al.*, 1989).

In the present study, diet-induced hyperlipidemic hamsters were used to evaluate the antioxidant and hypolipidemic activities of GTE.

## Materials and Methods

### *Plant Materials*

*Glycine tomentella* plant material (root) was purchased from the local market in the Kinmen area of Taiwan. The plants were identified by the Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, Taiwan, where a plant specimen has been deposited.

Eight hundred liters of water were added to 20 kg dried root, which was powdered mechanically, and were decocted for 4 hours. After filtration, the aqueous extract was concentrated into 332 mg/ml under reduced pressure at 50°C, and stored at -20°C until use. The extract yield was about 12.5%.

The phenol groups in GTE were determined by a modification of the method of Barness *et al.* (1963). Aliquots (1 ml) of various concentrations of GTE were added to 0.1 ml Folin-Ciocalteu's reagent and 0.2 ml 10% Na<sub>2</sub>CO<sub>3</sub>. The mixtures were then placed in a water bath (100°C) for 1 minute. After boiling, the mixtures were cooled and the phenolic concentrations were measured spectrophotometrically (Hitachi U-2001; Japan) at the wavelength of 700 nm, using catechin as the standard. The concentration of phenolic groups in GTE was 35.4 µg/mg.

### *Animals and Treatment*

Male Golden Syrian hamsters (the National Laboratory Animal Breeding and Research Center, National Science Council) weighing 100–120 g at nine weeks old were used. The experimental animals were housed in an air-conditioned room of 22 ± 3°C and 12 hours of light. The hamsters were divided into a control group (n = 7), which was allowed free access to a regular diet, and diet-induced hyperlipidemic groups. The latter were fed a high-fat diet prepared by adding 4% olive oil (w/w) and 0.5% cholesterol (w/w; Sigma, St. Louis, MO) to standard diet for 4 weeks. The hamsters were used when serum cholesterol level was higher than 200 mg/dl. Hyperlipidemic animals were then divided into five groups of seven animals each, receiving different doses of GTE or probucol (Sigma, St. Louis, MO). Study drugs were administered as dietary supplements in high-fat diet. In dose-dependent experiments, 0%, 1%, 2%, 4% of GTE or 0.5% of probucol were supplemented. All diets were provided *ad libitum* for 6 weeks. Each animal was caged separately. Body weight and food consumption were recorded at weekly intervals through the study period.

After 4 weeks of a high-fat diet, blood was collected via the retro-orbital sinus of fasting hamsters under light ether anesthesia. At the end of this study, blood was collected from the abdominal aorta of fasting hamsters. After blood sampling, the liver was immediately excised,

weighted and stored at  $-80^{\circ}\text{C}$  for further analysis. Serum was collected from whole blood following centrifugation at 4700 rpm at  $4^{\circ}\text{C}$  for 15 minutes.

#### *Hepatic and Serum Lipid Analysis*

Lipid content of liver was determined by the method of Nassir *et al.* (1996). Liver samples were homogenized, and total lipid was extracted into chloroform-methanol (2:1), dried under nitrogen and resuspended in isopropanol. Triglyceride and cholesterol in the extract were measured using diagnostic kits of Randox (Randox Lab. Ltd., UK) commercial kits.

Serum total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol were also measured using diagnostic kits of Randox commercial kits. Residual serum were kept below  $-80^{\circ}\text{C}$  until analysis for LDL oxidation.

#### *LDL Oxidation*

LDL was isolated from serum by ultracentrifugation on a sodium bromide discontinuous gradient, as previously described (Bronzert and Brewer, 1977). The fraction floating at 1.020~1.055 g/ml was collected.

LDL oxidation was measured as conjugated diene production by the method of Frei and Gaziano (1993). Briefly, freshly isolated LDL was incubated at a concentration of 0.05 mg protein/ml assay volume, which includes 250  $\mu\text{l}$  of 20 mM HEPES buffer, 40  $\mu\text{l}$  of 80  $\mu\text{M}$   $\text{CuSO}_4$ , and 0.154 M NaCl (volume = 710  $\mu\text{l}$  – volume of LDL). Incubations were conducted at  $37^{\circ}\text{C}$  in a thermostatic six-cell holder in a spectrophotometer (Hitachi U-2100, Japan). Conjugated diene formation was monitored every 10 minutes as the change in 234 nm wavelength absorption as described by Esterbauer *et al.* (1989). The oxidation curve was characterized by the lag phase time (expressed in minutes), i.e. the interval between the addition of  $\text{CuSO}_4$  and the beginning of the extensive oxidation, was measured on the basis of the intercept between the baseline and the tangent to the rapid oxidation phase. Proteins were measured by the method of Lowry *et al.* (1951).

#### *Statistical Analysis*

The statistical significance was calculated by one-way analysis of variance coupled with the Dunnett test. p values less than 0.05 were taken as significant.

## **Results**

#### *Body Weight and Food Intake*

The curve of the body weight changes in each group increased equally during the experiment. There was no significant difference in the final body weight between the control group ( $176.2 \pm 19.4$  g) and each GTE- and probucol-treated groups.

There was no significant difference in the food consumption between the control group and each GTE- and probucol-treated groups. The weekly food consumption of each group throughout the study period demonstrated that there was no significant difference. The average food consumption of each group throughout the 10-week experimental period was as follows: 11.8, 11.2, 11.1, 11.6, 10.1 or 11.7 g/hamster per day in the normal, the control, the GTE 1%, 2%, 4% and the probucol 0.5%, respectively. We calculated using the average daily food intake, the dose of the GTE 1%–4% and probucol 0.5% group was estimated to be 111, 232, 404 and 58.5 mg/hamster per day, respectively.

### *Serum Lipids and Lipoprotein Analysis*

The serum total cholesterol and triglyceride concentrations in hamsters fed normal chow diet alone were stable throughout the experimental period. At the 4th and 10th week, the concentration of serum total cholesterol was  $117.8 \pm 11.9$  mg/dL and  $116.0 \pm 20.1$  mg/dL, respectively. In the high-fat diet fed group, there were about 4.9- or 8.4-fold increase in serum total cholesterol concentrations after 4 or 10 weeks of high-fat diet feeding, respectively. Administration of GTE (2% and 4%), but not probucol (0.5%), to hyperlipidemic hamsters for 6 weeks reduced the serum total cholesterol concentrations (Table 1).

**Table 1. Effect of GTE on Serum Total Cholesterol and Triglyceride in Hyperlipidemic Hamsters**

Treatment	Total Cholesterol (mg/dL)		Triglyceride (mg/dL)	
	4 Weeks	10 Weeks	4 Weeks	10 Weeks
Control	$117.8 \pm 11.9$	$116.0 \pm 20.1$	$186.0 \pm 50.3$	$216.0 \pm 79.6$
High-fat diet	$576.9 \pm 47.4^*$	$971.4 \pm 218.5^*$	$583.8 \pm 120.4^*$	$3397.6 \pm 1637.9^\ddagger$
+ GTE 1%	$518.0 \pm 116.6^*$	$754.7 \pm 285.7$	$621.8 \pm 182.8^*$	$2375.6 \pm 1958.6$
2%	$522.7 \pm 119.0^*$	$544.3 \pm 311.4^\ddagger$	$584.5 \pm 159.8^*$	$2089.0 \pm 2322.9$
4%	$519.1 \pm 132.0^*$	$645.6 \pm 198.1^\ddagger$	$494.0 \pm 183.1^*$	$996.5 \pm 1686.9^\ddagger$
+ Pro 0.5%	$514.4 \pm 123.3^*$	$783.0 \pm 350.2$	$544.7 \pm 159.0^*$	$2717.4 \pm 2763.4$

All values are means  $\pm$  SD (n = 7). \*p < 0.01 Compared with control group,  $^\ddagger$ p < 0.01 compared with high-fat diet fed group.

Pro = Probucol.

The serum triglyceride concentrations in hamsters fed normal chow diet alone were stable throughout the experimental period. At the 4th or 10th week, the concentration of serum triglyceride was  $186.0 \pm 50.3$  mg/dL and  $216.0 \pm 79.6$  mg/dL, respectively. In the high-fat diet fed group, there were about 3.1- or 15.7-fold increased in serum triglyceride concentrations after 4 or 10 weeks of high-fat diet feeding, respectively. The feeding of GTE (4%), but not probucol (0.5%), to hyperlipidemic hamsters for 6 weeks reduced the serum triglyceride concentrations (Table 1).

At the end of study, the concentrations of serum high-density lipoprotein (HDL) and LDL cholesterol in hamsters fed normal chow diet were  $80.3 \pm 15.6$  mg/dL and  $22.7 \pm 3.2$  mg/dL, respectively. In the high-fat diet fed group, there were about 1.7- and 23.8-fold increased in

serum HDL and LDL cholesterol after 10 weeks of high-fat diet feeding, respectively. Administrations of GTE (2% and 4%) for 6 weeks significantly increased serum HDL- but decreased serum LDL-cholesterol concentrations compared to those in the hyperlipidemic control. GTE treatment did not result in any appreciable changes of the LDL oxidation kinetics. However, the duration of lag phase was significantly longer in hamsters treated with probucol than in control animals (Table 2).

**Table 2. Effect of GTE on Serum LDL and HDL Concentrations and Lag Phase Time of LDL Oxidation in Hyperlipidemic Hamsters**

Treatment	HDL (mg/dL)	LDL (mg/dL)	Lag Phase Time (Minutes)
Control	80.3 ± 15.6	22.7 ± 3.2	–
High-fat diet	128.9 ± 42.8*	541.1 ± 214.6*	230.4 ± 33.1
+ GTE 1%	142.1 ± 50.3	397.9 ± 276.7	254.9 ± 51.8
2%	176.4 ± 58.5 <sup>†</sup>	309.6 ± 140.7 <sup>†</sup>	224.6 ± 35.4
4%	201.6 ± 58.51 <sup>†</sup>	248.0 ± 185.7 <sup>†</sup>	200.3 ± 32.8
+ Pro 0.5%	181.7 ± 130.3	324.4 ± 170.9	336.5 ± 95.5 <sup>†</sup>

All values are means ± SD (n = 7). \*p < 0.01 Compared with control group, <sup>†</sup>p < 0.01 compared with high-fat diet fed group.

Pro = Probucol.

#### *Liver Weights and Lipid Contents*

In the hyperlipidemic hamsters, liver weight increased significantly compared with the normal group (Table 3). Both GTE- and probucol-treated groups did not show significant change. The tissue concentrations of cholesterol and triglyceride in liver were also determined. The cholesterol and triglyceride contents in liver were significantly higher than control in hamsters fed high-fat diet (Table 3). GTE (4%) treatment, but not probucol, decreased the triglyceride content of liver. Both GTE and probucol treatment did not affect the cholesterol content of liver (Table 3).

**Table 3. Effect of GTE on Liver Weight, Hepatic Cholesterol and Triglyceride Contents in Hyperlipidemic Hamsters**

Treatment	Liver Weight (g)	Triglyceride (mg/g tissue)	Cholesterol (mg/g tissue)
Control	5.9 ± 0.5	9.7 ± 1.3	1.8 ± 0.3
High-fat diet	7.9 ± 0.8*	12.3 ± 2.6*	7.1 ± 0.8*
+ GTE 1%	8.8 ± 1.0	10.5 ± 2.9	6.6 ± 0.8
2%	7.5 ± 1.8	11.7 ± 1.8	6.9 ± 0.8
4%	7.9 ± 0.8	7.5 ± 1.6 <sup>†</sup>	7.7 ± 1.0
+ Pro 0.5%	8.4 ± 0.8	11.6 ± 7.4	6.2 ± 0.8

All values were means ± SD (n = 7). \*p < 0.01 Compared with control group, <sup>†</sup>p < 0.01 compared with high-fat diet fed group.

Pro = Probucol.

## Discussion

In the present study, the antioxidant and hypolipidaemic activities of GTE were conducted in the hyperlipidemic hamsters. Our hyperlipidemic hamsters exhibited elevations of hepatic cholesterol and triglyceride contents, serum cholesterol, triglyceride and lipoproteins concentrations. These findings are in agreement with early reports (Liu *et al.*, 1991; Piyachaturawat *et al.*, 1999).

High levels of total cholesterol and, more importantly, LDL cholesterol are major coronary risk factors (National Cholesterol Education Program Expert Panel, 1994). Administration of GTE, but not probucol, lowered both total and LDL-cholesterol in experimentally induced hyperlipidaemic hamsters.

Much evidence indicates that the lipid peroxidation of LDL contributes to the pathophysiology of atherosclerosis (Steinberg *et al.*, 1989; Diaz *et al.*, 1997). The resistance of LDL to oxidation *in vivo*, expressed by the duration of the lag-phase of the kinetic curve, was not enhanced in GTE-treated hamsters. It was suggested that GTE did not possess a free radical scavenging activity *in vivo*. Consistent with previous report, our data showed that probucol has activity of anti-LDL oxidation, independent of its cholesterol-lowering effect (Carew *et al.*, 1987).

Moreover, the reduction of serum cholesterol by GTE was associated with a marked increase of serum HDL-cholesterol, which functions to remove cholesterol from peripheral tissues to liver for final clearance (von Eckardstein *et al.*, 2001). The increase of HDL-cholesterol indicates that an action of the GTE is to accelerate the mobilization of cholesterol from the tissue to the liver. However, the decreased serum cholesterol by the GTE was not accompanied by an increase of hepatic cholesterol content, suggesting that GTE also could increase the excretion of cholesterol. The underlying mechanism of this requires further study.

Several studies showed that triglyceride are also independently related to coronary heart disease (Bainton *et al.*, 1992; National Cholesterol Education Program Expert Panel, 1994) and most anti-hypercholesterolemic drugs do not decrease triglyceride levels. In the present study, GTE decreased both the hepatic and serum triglyceride concentrations significantly.

It is well known that nicotinic acid decreases both plasma cholesterol and triglyceride levels (Robert and Bersot, 2001). Nicotinic acid decreases hepatic secretion of very low density lipoprotein (VLDL), which results in the reduction of triglyceride synthesis. LDL is derived from VLDL in the plasma. Therefore a reduction in the VLDL concentration also results in a decreased plasma LDL concentration. Furthermore, nicotinic acid also increases HDL-cholesterol levels (Robert and Bersot, 2001). Therefore, it seems reasonable to suggest that GTE exerts hypolipidemic effects as nicotinic acid does, i.e. reducing VLDL.

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