



Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*

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Abstract

The hypoglycemic and hypolipidemic effects of *Lycium barbarum* fruit water decoction, crude polysaccharide extracts (crude LBP), and purified polysaccharide fractions (LBP-X) in alloxan-induced diabetic or hyperlipidemic rabbits were investigated through designed sequential trials and by measuring blood glucose and serum lipid parameters. Total antioxidant capacity was also assessed using trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assay. It was found that the three *Lycium barbarum* fruit extracts/fractions could significantly reduce blood glucose levels and serum total cholesterol (TC) and triglyceride (TG) concentrations and at same time markedly increase high density lipoprotein cholesterol (HDL-c) levels after 10 days treatment in tested rabbits, indicating that there were substantial hypoglycemic and hypolipidemic effects. Hypoglycemic effect of LBP-X was more significant than those of water decoction and crude LBP, but its hypolipidemic effect seemed to be weaker. Total antioxidant capacity assay showed that all three *Lycium barbarum* extracts/fractions possessed antioxidant activity. However, water and methanolic fruit extracts and crude polysaccharide extracts exhibited stronger antioxidant activity than purified polysaccharide fractions because crude extracts were identified to be rich in antioxidants (e.g., carotenoids, riboflavin, ascorbic acid, thiamine, nicotinic acid). *Lycium barbarum* polysaccharides (glycocojugates), containing several monosaccharides and 17 amino acids, were major bioactive constituents of hypoglycemic effect. Both

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polysaccharides and vitamin antioxidants from *Lycium barbarum* fruits were possible active principles of hypolipidemic effect.

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Introduction

Fruit from *Lycium barbarum* L. in the family Solanaceae is well-known in traditional Chinese herbal medicine and nowadays has been widely used as a popular functional food, with a large variety of beneficial effects, such as reducing blood glucose and serum lipids, anti-aging, immuno-modulating, anticancer, anti-fatigue, and male fertility-facilitating (Gao et al., 2000; Peng et al., 2001a,b; Wang et al., 2002a,b; Gan and Zhang, 2003). The earliest Chinese medicinal monograph documented medicinal use of *Lycium barbarum* around 2300 years ago. *Lycium barbarum* fruits can be used to produce various types of healthy products and foods, e.g., medicinal beverages and drinks, and healthy dietary soups (Li, 2001). Some constituents of *Lycium barbarum* fruits have been chemically investigated, especially *Lycium barbarum* polysaccharide (LBP) components. Five polysaccharides (glycoconjugates) (LbGp1-LbGp5) were isolated and structurally elucidated (Peng et al., 2001a,b; Peng and Tian, 2001).

Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis (Defronzo et al., 1992; Chait and Brunzell, 1996; Alberti et al., 1997). Control of diabetes mellitus normally involves exercise, diet and chemotherapy. Development and utilization of antidiabetic plants have attracted increasing interest (Bailey and Day, 1989; Alarcon-Aguilar et al., 1997). The plant kingdom is a wide field to search for natural effective oral hypoglycemic or hypolipidemic agents that have slight or no side effects.

More than 400 plants with glucose-lowering potential are known (Ernst, 1997). Hypoglycemic activity of nearly 100 polysaccharides from plants has been reported. Some botanical polysaccharides are considered as important bioactive components responsible for hypoglycemic effect (Yuan et al., 1998; Wang and Ng, 1999). Also, a number of plants are known to have hypolipidemic activity (Ram et al., 1996; Dhandapani et al., 2002; Sharma et al., 2003). However, there is little information about plants with both hypoglycemic and hypolipidemic effects.

Nutritional factors including antioxidants have great influence in the management of diabetes mellitus and its complications (Alberti et al., 1997; Packer et al., 2000). An imbalance between oxidative stress and antioxidative defense mechanisms in diabetics can result in cell and tissue damage and accelerate diabetic complications. Administration of appropriate antioxidants could prevent or retard diabetic complications to some extent (Packer et al., 2000). The investigation on antioxidant activity of 35 medicinal plants consumed by the indigenous peoples of the boreal forest of Canada supported the contribution of these traditional medicines in a lifestyle historically low in the incidence of diabetes (McCune and Johns, 2002).

It is well established that *Lycium barbarum* really possesses a wide variety of bioactive properties. The purpose of this study is to investigate and evaluate hypoglycemic and hypolipidemic effects of *Lycium barbarum* fruit water decoction, crude polysaccharides, and its purified polysaccharide fractions in alloxan-induced diabetic rabbits and healthy mice, to determine antioxidant activity of the fruit extracts and determine relevant antioxidant components, and to provide scientific evidence for development of *Lycium barbarum* as a potential natural oral hypoglycemic and hypolipidemic agent or functional food.

Materials and methods

Plant materials and chemicals/reagents

Dried fruits of *Lycium barbarum* were purchased in a local market. They were from Ningxia which is the well-known production area of *Lycium barbarum* in China. Sephacryl S-300 was purchased from Amersham Pharmacia Biotech (China) Ltd. and DEAE cellulose from Shanghai Reagents Company (Shanghai, China). Alloxan, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2',2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), and (*R*)-phycoerythrin (R-PE) were purchased from Sigma/Aldrich (St. Louis, MO), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) from Fluka Chemie AG (Buchs, Switzerland), and HPLC grade organic reagents from BDH (Dorset, England). All authentic standards were obtained from Sigma/Aldrich and Fluka.

Experimental animals

A total of 35 adult rabbits (26 male and 9 female) weighing from 2 to 2.2 kg were used in whole experiment. 24 male mice weighing from 18 to 22 g were used for test of reducing blood glucose. They were fed with standard laboratory diet and given tap water.

Preparation of fruit extracts

The dried fruits were ground to fine powder (~710 μm). (1) Preparation of fruit water decoction: The ground samples were put in boiling water and decocted by a traditional method for Chinese medicinal herbs. The decoction was left to cool at room temperature, filtered and then freeze-dried. The dry decoction residue was dissolved in normal saline solution and directly administered to the tested animals for investigation of hypoglycemic and hypolipidemic effects. (2) Preparation of fruit extracts for antioxidant activity assay: Water extraction and methanol extraction followed our previous method (Cai et al., 2004).

Preparation of polysaccharide (glycoconjugate) fractions

(1) Removing lipids. The dried fruit samples were refluxed three times to remove lipids with chloroform:methanol solvent (2:1) (v/v). After filtering the residues were air-dried and then refluxed again with 80% ethanol. (2) Extracting crude polysaccharides. The residue was extracted three times in hot water (90 °C) and then filtered. The combined filtrate was precipitated using 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and

vacuum-dried, giving crude polysaccharides (glycoconjugates) named crude LBP. (3) Purifying polysaccharides. Crude LBP was eluted and isolated on DEAE cellulose column (OH^- , 2.6×90 cm) with distilled water and 0.05–0.5 mol/L NaCl. The collected four fractions were dialyzed, centrifuged, and freeze-dried. The dried fractions were dissolved in water and further purified on Sephacryl S-300 column (1.6×48 cm). After centrifuging and freeze-drying, four purified polysaccharide fractions were obtained, one of which was a major fraction called LBP-X. The obtained dry crude polysaccharides (crude LBP) and purified LBP-X fraction were used in this experiment and dissolved in normal saline solution to reach the required concentration.

Alloxan-induced hyperglycemia in rabbits

Hyperglycemia and hyperlipidemia were induced by the ear vein injection of alloxan at a dose of 160 mg/kg and 180 mg/kg, respectively, dissolved in normal saline solution. Concentrations of blood glucose, serum cholesterol, and triglyceride were monitored at different time after injection of alloxan. It was confirmed that diabetic rabbits showed stable high levels of blood glucose, cholesterol, and triglyceride during experimental period.

Investigation of hypoglycemic and hypolipidemic effects of fruit extracts and polysaccharide fractions

The effects of administration of fruit water decoction, crude LBP, and LBP-X to diabetic/hyperlipidemic rabbits were investigated through designed medical sequential trials (Xu, 1979) and by measuring several biochemical parameters of tested animals (blood glucose, glucose tolerance, serum total cholesterol, triglyceride, and high density lipoprotein cholesterol). The sequential trials of opened unidirectional qualitative reaction were applied to establish effective standard of reduction of blood glucose and serum lipid levels in tested animals.

Alloxan-induced diabetic/hyperlipidemic rabbits were treated by oral infusion with fruit water decoction (0.25 g/kg · d), crude LBP (10 mg/kg · d), and LBP-X (10 mg/kg · d) dissolved in normal saline for 10 consecutive days. Control of diabetic/hyperlipidemic rabbits received normal saline (10 mg/kg · d). The doses used in this study were confirmed to be suitable and effective in tested rabbits according to our preliminary experiment. Healthy mice only used for test of reducing blood glucose were given daily the same doses of fruit water decoction, crude LBP, and LBP-X through intraperitoneal injection for 7 consecutive days.

Blood samples were drawn from rabbit ear vein and mice tail at different periods/times for various biochemical assays. For healthy mice, blood samples were taken at fasting (control, 0 day) and after 7 days *Lycium barbarum* treatment. For rabbits, blood samples were collected at normal stage (before alloxan-induced diabetes) (healthy rabbits as control), test stage (after alloxan-induced diabetes, before *Lycium barbarum* treatment), and end stage (after 10 days *Lycium barbarum* treatment). For glucose tolerance test, blood samples were collected at fasting (0 hr) and postprandially at 0.5 hr intervals for 2.0 hr during each stage.

Several biochemical parameters were determined using common assay methods. Blood glucose levels were determined by the glucose oxidase method (Stevens, 1951). Total serum cholesterol (TC) and high density lipoprotein cholesterol (HDL-c) were estimated by the method of Allain et al. (1974) and Burstein et al. (1970), respectively. Serum triglyceride (TG) levels were determined with the enzymatic method (Fossati and Lorenzo, 1982).

In vitro antioxidant activity assay

Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assay were employed in this study to assess total antioxidant capacity. The improved ABTS^{•+} method was used for TEAC assay, as described in our recent study (Cai et al., 2004). ABTS^{•+} radical cation is generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation. The ABTS^{•+} solution and the filtered sample of fruit extracts are diluted with 80% ethanol so as to give 20–80% inhibition of the blank absorbance with 0.1 mL of sample. ABTS^{•+} solution (3.9 mL) is added to 0.1 mL of the tested samples and mixed thoroughly. ORAC assay procedure was based on the method of Cao et al. (1993) with a slight modification. This assay measures the ability of antioxidant compounds in test materials to inhibit the decline of R-PE fluorescence that is induced by a peroxy radical generator, AAPH. The reaction mixture contained 1.7 ml of 75 mM phosphate buffer (pH 7.0), 100 µl of R-PE (3.4 mg/L), 100 µl of 320 nM AAPH, and 100 µl of sample. For these two methods, trolox standard solution is prepared and assayed at the same conditions. The absorbance of the resulting oxidized solution is compared to that of the calibrated trolox standard. Final results are expressed in terms of TEAC or ORAC unit (µmol trolox /100 g dry weight).

Identification of chemical constituents

GC-MS analysis was performed on a Shimadzu QP 5000 system equipped with OV-225FSOT capillary column (0.52 mm × 15 m). LBP-X was dissolved in 3% (w/w) H₂SO₄ at 120 °C and 103 kPa for 1 hour hydrolysis, and then neutralized with BaCO₃. The alditol acetate derivative was obtained to analyze monosaccharide constituents of LBP-X. Analytical conditions were based on the method of Varma et al. (1973). HPLC analysis of *Lycium barbarum* fruit extracts was conducted on a HP 1100 Series HPLC system which consists of a binary pump and three detectors (DAD, FLD and RID). Chromatographic conditions referred to Shen et al. (1992) and our recent study (Cai et al., 2004). Amino acids of LBP-X were determined using LKB-4400 AA automatic analyzer.

Statistical analysis

All results were expressed as mean ± SD. The significance of the difference between the means of test and control studies was established by student's *t*-test. *P* values less than 0.05 or 0.01 were considered significant. Data analysis of sequential trials followed the method of Xu (1979). Data from antioxidant activity assay were analyzed by one-way analysis of variance (ANOVA). Significant differences (*P* < 0.05) between means were determined using Duncan's multiple-range tests.

Results

Hypoglycemic effect of Lycium barbarum in diabetic rabbits and healthy mice

Data analysis of sequential trials in this study is shown in Table 1. Hypoglycemic effect of *Lycium barbarum* fruit water decoction, crude polysaccharides (crude LBP), and purified

Table 1

Statistical analysis and effective standards of hypoglycemic and hypolipidemic effects caused by *Lycium barbarum* fruit water decoction, crude LBP, and LBP-X in diabetic/hyperlipidemic rabbits^a

Effective standard of blood glucose decrease	≥ 3.89 mmol/L (effective)	<3.89 mmol/L (ineffective)
Effective standard of total cholesterol decrease	≥ 2.00 mmol/L (effective)	<2.00 mmol/L (ineffective)
Effective standard of triglyceride decrease	≥ 1.50 mmol/L (effective)	<1.50 mmol/L (ineffective)
False positive rate (α) or false negative rate (β)	α=β=0.01	α=β=0.05
Two pairs of linear equation ^b	U ₂ : y=2.06+0.561n L ₂ : y=-2.06+0.561n	U ₁ : y=1.32+0.561n L ₁ : y=-1.32+0.561n

^a Effective standards were established by sequential trials.

^b U: limit of receivable test drug; L: limit of refusal test drug; y: number of animals with positive reaction; n: number of tested animals.

polysaccharide fraction (LBP-X) in alloxan-induced diabetic rabbits is listed in Table 2. It was found that fruit water decoction, crude LBP, and LBP-X significantly reduced blood glucose levels in all tested diabetic rabbits and produced substantial hypoglycemic effects. Moreover, hypoglycemic effect of LBP-X was greater than those of water decoction and crude LBP. Mean decrease of blood glucose levels caused by fruit water decoction, crude LBP, and LBP-X were 8.04, 8.47, and 14.13 mmol/L, respectively (Table 2). The decrease of blood glucose levels exceeded effective standard of ≥ 3.89 mmol/L established through sequential trials (Table 1). Comparatively, blood glucose level of each diabetic rabbit treated with normal saline (control) did not decrease, but increased slightly, because mean decrease of blood glucose level was -0.70 mmol/L after normal saline treatment (Table 2).

Most botanical polysaccharides influence blood glucose levels at fasting and after administration in normal animals to a certain extent. In this study, healthy mice were daily treated with three *Lycium barbarum* extracts/fractions (fruit water decoction, crude LBP, and LBP-X). Blood glucose levels were determined at fasting (control, 0 day) and after 7 days of *Lycium barbarum* treatment. There was no significant difference in blood glucose levels of all 24 healthy mice before and after three *Lycium barbarum* extract/fraction administrations, showing that *Lycium barbarum* extracts/fractions did not influence blood glucose level of normal mice. Therefore, *Lycium barbarum* extracts/fractions could reduce blood glucose in diabetic animals but did not change blood glucose level in healthy animals. This

Table 2

Hypoglycemic effect of *Lycium barbarum* fruit water decoction, crude LBP, and LBP-X in alloxan-induced diabetic rabbits after 10 days treatment

Treatment group	Dose (mg/kg.d)	Decrease value of blood glucose (mmol/L) ^a	Hypoglycemic Effect ^b
Fruit water decoction	250	8.04 ± 2.51**	Effective
Crude LBP	10	8.47 ± 2.96**	Effective
LBP-X	10	14.13 ± 6.35**	Effective
Normal saline	10	- 0.70 ± 0.92	Ineffective

^a Mean of all tested diabetic rabbits.

^b From Table 1, decrease value of blood glucose ≥ 3.89 mmol/L represents “effective”; while decrease value of blood glucose < 3.89 mmol/L represents “ineffective”.

** $P < 0.01$.

supports the use of *Lycium barbarum* fruits as a potential source of good oral hypoglycemic agents or functional foods without side effects.

Influence of Lycium barbarum on glucose tolerance in diabetic rabbits

Glucose tolerance curves of diabetic rabbits at fasting (0 hr) and postprandially at 0.5, 1.0, 1.5, and 2.0 hr at normal stage (before alloxan-induced diabetes) (control), test stage (after alloxan-induced diabetes, before *Lycium barbarum* treatment), and end stage (after 10 days *Lycium barbarum* treatment) are shown in Fig. 1 (A–C). Glucose tolerance curves of alloxan-induced diabetic rabbits treated with three *Lycium barbarum* extracts/fractions, i.e., water decoction (Fig. 1A), crude LBP (Fig. 1B), and LBP-X (Fig. 1C) were significantly shifted downwards after 10 days treatment, compared to before treatment. This indicated that three *Lycium barbarum* extracts/fractions could clearly improve glucose tolerance of diabetic rabbits. However, it should be noted that the difference in glucose tolerance between treated and control rabbits still existed. This showed that three *Lycium barbarum* extracts/fractions could improve glucose tolerance but could not normalize it in diabetic rabbits.

Hypolipidemic effect of Lycium barbarum in hyperlipidemic rabbits

Hyperlipidemia is a common complication of alloxan-induced diabetes mellitus in experimental animals. Hypoglycemic effect of *Lycium barbarum* fruit water decoction, crude LBP, and LBP-X in alloxan-induced hyperlipidemic rabbits is shown in Table 3. When compared to control (healthy rabbits), serum total cholesterol (TC) and triglyceride (TG) increased and high density lipoprotein cholesterol (HDL-c) decreased clearly in hyperlipidemic rabbits. After the treatments of *Lycium barbarum* fruit water decoction, crude LBP, and LBP-X in hyperlipidemic rabbits for 10 consecutive days, there was a significant decrease ($P < 0.01$) in serum lipids (TC and TG), while there was an marked increase in HDL-c ($P < 0.05$). The serum lipid profiles before and after treatment showed that all three *Lycium barbarum* extracts/fractions could produce significant hypolipidemic effect in tested rabbits.

Average TC and TG reductions caused by *Lycium barbarum* extracts/fractions were 3.39 and 2.77 mmol/L for fruit water decoction, 3.82 and 1.69 mmol/L for crude LBP, and 4.27 and 3.50 mmol/L for LBP-X (Table 3). All of the TC and TG reductions in each tested hyperlipidemic rabbit exceeded effective standards established by sequential trials (≥ 2.0 and ≥ 1.5 mmol/L, respectively) (Table 1). However, each hyperlipidemic rabbit treated with normal saline (control) remained with high serum lipid levels. The average decrease of TC and TG was -0.92 mmol/L and -0.91 mmol/L (Table 3), i.e. its serum lipid levels did not decrease, but increased slightly after normal saline treatment.

In vitro antioxidant activity of Lycium barbarum

Fig. 2 shows antioxidant activity of various *Lycium barbarum* fruit extracts/fractions evaluated by TEAC assay and ORAC assay (common *in vitro* methods to assess total antioxidant capacity of plant extracts). All *Lycium barbarum* fruit extracts/fractions, as effective free radical scavengers, demonstrated antioxidant activity. The higher the TEAC or ORAC values of samples, the stronger their potential antioxidant activity. In this study, the results obtained from the TEAC assay were similar to those of the ORAC assay. The antioxidant activity of four *Lycium barbarum* extracts/fractions was significantly

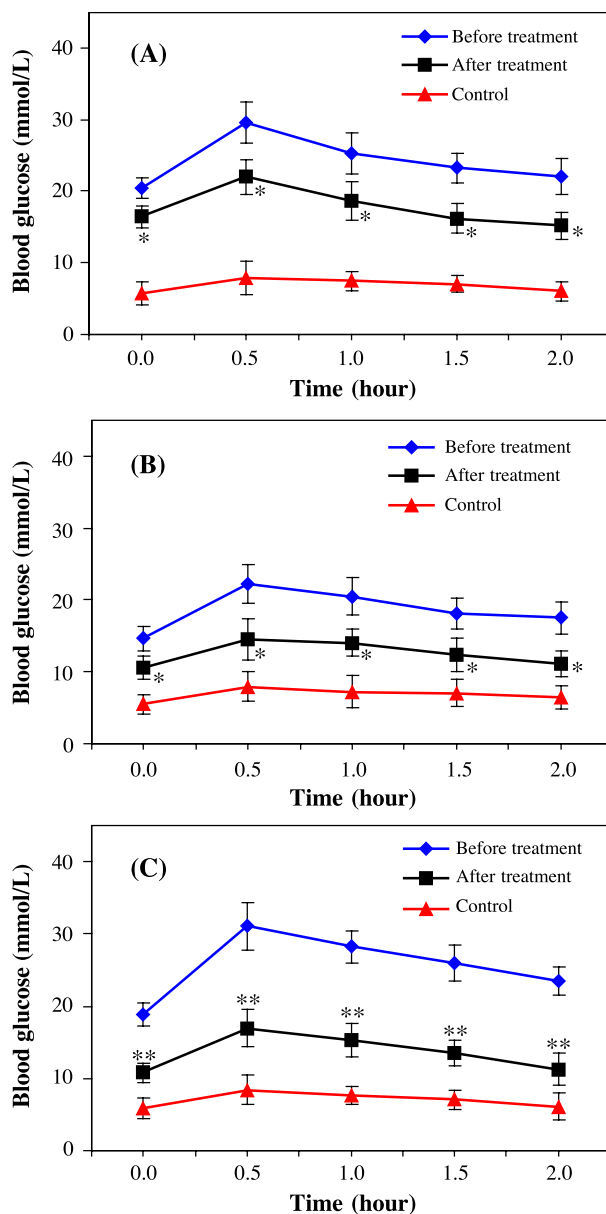


Fig. 1. Influence of *Lycium barbarum* fruit water decoction (A), crude LBP (B), and LBP-X (C) on glucose tolerance in diabetic rabbits. Control-before alloxan-induced diabetes (healthy rabbits); before treatment-after alloxan-induced diabetes and before *Lycium barbarum* treatment (modeled diabetic rabbits); after treatment-after 10 days *Lycium barbarum* treatment (treated diabetic rabbits). The results were expressed as mean \pm SD (error bars in the figure). * $P < 0.05$ and ** $P < 0.01$ vs model (before treatment).

different ($P < 0.05$), in order of methanolic extract > water extract > crude LBP extract > purified LBP fraction. A similar result has been reported for antioxidant activity of *Lycium barbarum* fruit extracts and its polysaccharide fractions (Gao et al., 2000).

Table 3

Hypolipidemic effect of *Lycium barbarum* fruit water decoction, crude LBP, and LBP-X in alloxan-induced diabetic/hyperlipidemic rabbits after 10 days treatment^a

Treatment group	Serum lipid profile (mean \pm SD) ^c		
	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)
<i>Water decoction (250 mg/kg.d)</i>			
Control (healthy rabbits) ^b	1.27 \pm 0.41	1.61 \pm 0.22	1.08 \pm 0.36
Hyperlipidemic rabbits			
Before treatment	6.54 \pm 1.71	3.89 \pm 1.01	0.54 \pm 0.29
After treatment	3.15 \pm 0.94**	1.12 \pm 0.37**	0.83 \pm 0.30*
Change value (mmol/L)	\downarrow 3.39	\downarrow 2.77	\uparrow 0.31
Change percentage (%)	\downarrow 51.8	\downarrow 71.2	\uparrow 57.4
<i>Crude LBP (10 mg/kg.d)</i>			
Control (healthy rabbits) ^b	1.14 \pm 0.34	1.54 \pm 0.61	0.99 \pm 0.41
Hyperlipidemic rabbits			
Before treatment	7.06 \pm 0.98	4.66 \pm 2.01	0.57 \pm 0.28
After treatment	3.24 \pm 0.96**	2.97 \pm 0.95**	1.35 \pm 0.57*
Change value (mmol/L)	\downarrow 3.82	\downarrow 1.69	\uparrow 0.78
Change percentage (%)	\downarrow 54.1	\downarrow 36.3	\uparrow 136.8
<i>LBP-X (10 mg/kg.d)</i>			
Control (healthy rabbits) ^b	1.51 \pm 0.46	1.62 \pm 0.58	1.13 \pm 0.47
Hyperlipidemic rabbits			
Before treatment	10.32 \pm 4.25	9.15 \pm 3.60	0.68 \pm 0.34
After treatment	6.05 \pm 1.24**	5.65 \pm 1.41**	0.95 \pm 0.42*
Change value (mmol/L)	\downarrow 4.27	\downarrow 3.50	\uparrow 0.27
Change percentage (%)	\downarrow 41.4	\downarrow 38.3	\uparrow 39.7
<i>Normal saline (control) (10 mg/kg.d)</i>			
Control (healthy rabbits) ^b	1.38 \pm 0.40	1.56 \pm 0.47	1.07 \pm 0.41
Hyperlipidemic rabbits			
Before treatment	7.26 \pm 1.54	4.45 \pm 1.27	0.58 \pm 0.30
After treatment	8.18 \pm 0.97	5.36 \pm 0.98	0.54 \pm 0.16
Change value (mmol/L)	\uparrow 0.92	\uparrow 0.91	\downarrow 0.04
Change percentage (%)	\uparrow 12.7	\uparrow 20.5	\downarrow 6.9

^a From Table 1, effective standards of hypolipidemic effect: TC decrease value \geq 2.0 mmol/L, TG decrease value \geq 1.5 mmol/L.

^b Control, healthy rabbits before alloxan-induced diabetes.

^c TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein.

* $P < 0.05$.

** $P < 0.01$; \downarrow , decrease; \uparrow , increase.

Major bioactive components in *Lycium barbarum*

Botanical polysaccharides occur naturally mainly as a glycoconjugate, i.e., a conjugate of glycan with peptides or proteins. Polysaccharides from *Lycium barbarum* are such a type of polysaccharide and are major bioactive constituents in *Lycium barbarum* fruits. Four polysaccharide fractions were isolated and purified in this study. LBP-X was a major fraction of *Lycium barbarum* polysaccharides. GC-MS

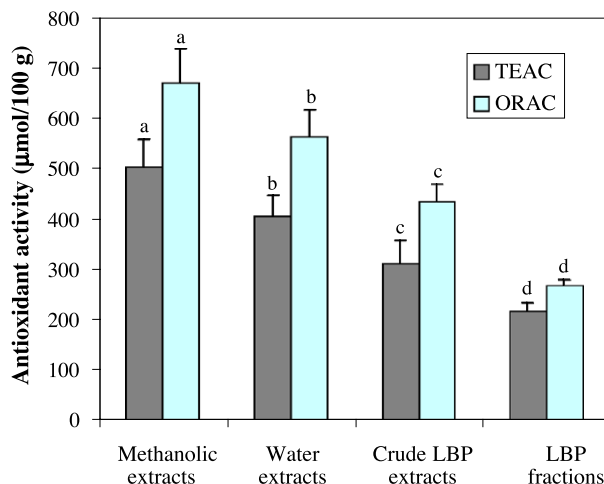


Fig. 2. Antioxidant activity (TEAC and ORAC values) of various *Lycium barbarum* fruit extracts/fractions. TEAC and ORAC values are expressed in terms of micromoles of trolox equivalents per 100 g of dry weight, as mean \pm SD from three replicate determinations. Bars topped by different letters are significantly different at $P < 0.05$.

analysis showed that LBP-X contained six monosaccharides, including rhamnose (Rha), galactose (Gal), glucose (Glc), arabinose (Ara), mannose (Man), and xylose (Xyl). Rha, Gal, Glc, Ara, Man, and Xyl were detected in a molar ratio of 4.22 : 2.43 : 1.38 : 1.00 : 0.95 : 0.38. Amino acid analysis revealed that LBP-X contained 17 amino acids. Total content of amino acids was 8.46%. Peng et al. (2001a,b) and Peng and Tian (2001) isolated five glycoconjugates and elucidated their structures (LbGp1-LbGp5), mainly composed of two to six monosaccharides and 17 amino acids. The molecular weight of these isolated glycoconjugates ranged from 23.7 to 214.8 kDa.

Also, *Lycium barbarum* fruits contained other bioactive components. The methanolic and water extracts of *Lycium barbarum* fruits were isolated and identified by HPLC. It was found that *Lycium barbarum* fruits were rich in carotene, riboflavin, ascorbic acid, thiamine, nicotinic acid, betaine, coumarin (scopoletin), zeaxanthin, cryptoxanthin, etc., most of which are antioxidants and were responsible for antioxidant properties of *Lycium barbarum*. The result was similar to those of previous findings (Gao et al., 2000; Li, 2001). Li (2001) also reported that *Lycium barbarum* fruits contain many mineral elements, e.g., Cu, Fe, Zn, Mn, Mg, Se, Ca, etc.

Discussion

Lycium barbarum, a well-known traditional Chinese medicinal herb, possesses diverse biological activities and pharmacological functions including reducing blood glucose and serum lipids. It has long been used to treat diabetes mellitus and related hyperlipidemia (Gao et al., 2000; Li, 2001). However, its pharmacological and chemical bases are not well understood.

The results of this study indicated that three tested *Lycium barbarum* extracts/fractions (fruit water decoction, crude LBP, and LBP-X) not only possessed significant hypoglycemic effect but also had remarkable hypolipidemic effect in alloxan-induced diabetic/hyperlipidemic rabbits. Before *Lycium barbarum* treatment of alloxan-induced diabetic/hyperlipidemic rabbits, the significant rise in blood

glucose was accompanied with increases in TC and TG and decrease of HDL-c. After *Lycium barbarum* treatments, blood glucose, TC, and TG of all tested rabbits were significantly decreased and at the same time HDL-c was increased. This provided evidence in favor of the view that *Lycium barbarum* could play an important role in treating diabetic/hyperlipidemic patients. Sharma et al. (2003) reported that other medicinal plant (*Eugenia jambolana*) also had hypoglycemic and hypolipidemic effects which could prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia quite often coexist. Many current oral hypoglycemic or hypolipidemic agents are synthetic drugs with certain adverse side effects (Holman and Turner, 1991; Alberti et al., 1997). Our study reveals the potential of *Lycium barbarum* for use as a natural oral agent with both hypoglycemic and hypolipidemic effects.

Some polysaccharides were identified as bioactive components responsible for hypoglycemic activity and hypocholesterolemic effect (Yuan et al., 1998; Wang and Ng, 1999). Polysaccharides are major chemical constituents of *Lycium barbarum* fruits (Peng et al., 2001a,b; Li, 2001). In this study, purified polysaccharide fraction (LBP-X) from *Lycium barbarum* fruits exhibited more significant hypoglycemic effect than its water decoction and crude LBP, implying that *Lycium barbarum* polysaccharides were major bioactive components in the hypoglycemic effect. Also, three tested *Lycium barbarum* extracts/fractions demonstrated significant hypolipidemic effect, indicating that the polysaccharides from *Lycium barbarum* fruit extracts were also bioactive components of hypolipidemic effect. However, the differences in hypolipidemic effect were markedly different from those in hypoglycemic effect. From Table 3, LBP-X led to 41.4% and 38.3% decrease in TC and TG and 39.7% increase in HDL-c, but fruit water decoction and crude LBP caused more substantial decrease in TC (51.8% and 54.1%) and TG (71.2% and 36.3%) and more significant increase in HDL-c (57.4% and 136.8%). This indicated that bioactive components in *Lycium barbarum* fruits, other than polysaccharides, also played a role in hypolipidemic activity in tested rabbits.

Antioxidant components in *Lycium barbarum* fruits were seemingly associated with hypolipidemic effect in this study. The results of total antioxidant capacity assay showed that all three tested *Lycium barbarum* extracts/fractions had antioxidant activity, but fruit water decoction and crude polysaccharide extracts exhibited stronger antioxidant activity than major purified polysaccharide fractions. This tendency was basically in accord with investigation on hypoglycemic effect of three *Lycium barbarum* extracts/fractions. Crude *Lycium barbarum* extracts were rich in antioxidant components, such as carotene, ascorbic acid, thiamine, riboflavin, nicotinic acid, zeaxanthin, cryptoxanthin, and coumarin (scopoletin), which contributed to their antioxidant properties. Among these antioxidant components, there may be some interactions and synergistic effects for antioxidant properties. Additionally, more antioxidants from *Lycium barbarum* fruit water extracts and crude LBP may play a synergistic role in their hypolipidemic effect. However, further investigation will be required to explain why water decoction and crude LBP have better hypolipidemic effect than purified polysaccharide fraction (LBP-X).

Diabetes mellitus is characterized by hyperglycemia, which usually produces many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, obesity, atherosclerosis, and even cardiovascular disease (DeFronzo et al., 1992; Alberti et al., 1997). Diabetes has been found to be associated with indices of oxidative damage. Hyperglycemia can lead to the glycation of tissue proteins. Glycation and glucose autooxidation generate hydrogen peroxides, hydroxyl radicals and protein-reactive ketoaldehydes. Hyperglycemia can also lead to increased lipid peroxidation, superoxide production, glycation of the lipoproteins, oxidative DNA damage, and so on. Antioxidants can provide defense against free

radical damage. Natural antioxidants may have beneficial implications for diabetes management. Diabetic complications can be prevented or retarded by administration of appropriate antioxidants, in addition to traditional therapeutic principles (Packer et al., 2000). High levels of TC and TG (serum lipids) are major risk factors for atherosclerosis and coronary heart disease. An increase in HDL-c is associated with a decrease in atherosclerotic and coronary risk (Chait and Brunzell, 1996). In the present study, administration of three tested *Lycium barbarum* extracts/fractions with antioxidant activity significantly reduced blood glucose and lowered both TC and TG and at same time increased HDL-c in alloxan-induced diabetic/hyperlipidemic rabbits. Li (2001) reported that *Lycium barbarum* fruits could retard and prevent atherogenesis in experimental animals. Current results have confirmed that *Lycium barbarum* polysaccharides (glycoconjugates) are major bioactive components of the hypoglycemic effect. Also, both polysaccharides and vitamin antioxidants from *Lycium barbarum* fruits are possible bioactive components of hypolipidemic effect. However, their detailed mechanism of action needs further investigation.

In summary, the present study has shown that three *Lycium barbarum* extracts/fractions demonstrate clear hypoglycemic and hypolipidemic effects in alloxan-induced diabetic/hyperlipidemic rabbits and strong antioxidant activity. Major bioactive principles identified include polysaccharides and vitamin antioxidants. This investigation is helpful for understanding mechanism of action of *Lycium barbarum* and its active ingredients, and also reveals the potential of *Lycium barbarum* for use as a natural oral agent with both hypoglycemic and hypolipidemic effects.

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