Effects of *Lycium barbarum L.* root bark extract on alloxan-induced diabetic mice

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Background: *Lycium barbarum L.* (LbL), as one of the traditional Chinese medicines, has been used to treat lung disease, hematemesis, hypertension, inflammation and diabetes for centuries. This study aims to reveal the hypoglycemic and hypolipidemic effects of LbL root bark extract on hyperlipidemic alloxan-induced diabetic mice. **Methods:** A total of 24 male, hyperlipidemic, alloxan-induced diabetic mice were divided into three groups: diabetes control, diabetes plus LbL high dose (200 mg/kg) and diabetes plus LbL low dose (100 mg/kg). One group of eight normal mice was kept as a control. The four groups of mice were administered LbL solution or dH_2O daily for 28 days. Fasting blood glucose, total cholesterol, triglycerides, body weight and serum insulin levels have been determined. **Results:** Results indicated that LbL-treated groups resulted in significant dose-dependent decreases of fasting blood glucose, total cholesterol and triglycerides. The LbL treated group also showed a tendency to improve in body weight gain. Furthermore, the serum insulin level of each group was assayed and the diabetes control group had low serum insulin levels compared with that of the normal control group. At the same time, the insulin levels were dose-dependently raised in the LbL-treated groups compared with that of the diabetes control group. **Conclusion:** The results indicate that LbL administration to diabetic mice would alleviate the increases in blood glucose and lipid levels associated with diabetes, improve abnormal glucose metabolism and increase insulin secretion by restoring the impaired pancreas β-cells in alloxan-induced diabetic mice. This would suggest that LbL has hypoglycemic and hypolipidemic potential and could be useful for diabetic therapy.

Keywords: diabetes, hypoglycemia, hypolipidemia, insulin, *Lycium barbarum L.*

Diabetes mellitus (DM) is the most common endocrine disease. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and is expected to rise to 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [1]. In spite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem [2]. Plant products are frequently considered to be less toxic and have fewer side effects compared with synthetic drugs [3]. A growing number of people are turning to alternative therapies, including herbal medicines. Traditional Chinese medicine has been performed with good clinical practice. However, the precise antidiabetic mechanisms of most herbs are unknown and need further investigation.

The dried root bark of *Lycium barbarum L.* (LbL; Chinese name: *Cortex Lycii Radicis* or Digupi), has been extensively used in traditional Chinese herbal medicine owing to its variety of biological activities against conditions such as attenuating lung disease, hematemesis, inflammation, gangrene of the extremities and DM [4–8]. However, there are few studies that have been carried out regarding the remedial mechanism of LbL.

For this research article, we analyzed the compounds of LbL extract and investigated its hypoglycemic and hypolipidemic effects. Activities of the extract were determined by comparing the changes of blood glucose, body weight, serum triglycerides (TG) and total cholesterol (TC) in alloxan-induced diabetic mice. Furthermore, the serum insulin levels of four groups of mice were assayed in order to reveal the antidiabetic mechanism of LbL.

Methods

Reagents & herbs

Glucose analyzer (GT-1640) and strips were purchased from ARKRAY Inc. (Japan). Alloxan were obtained from Sigma Co. (USA). GF254-thin-layer chromatography (TLC) plates were bought from Qingdao Haiyang Chemical Co. Other chemicals were analytic grade. Dried LbL root bark was purchased from a local drug store and compared with its picture in the Chinese medicine identified atlas [9]. At the same

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time, it was authenticated by the specialist in the Hospital of Qinhuangdao Chinese Medicine (Qinhuangdao, China).

Preparation of LbL extracts & characterization

The dried LbL was ground to a fine powder. The ground samples (1000 g) were immersed in 10× volume dH_2O, boiled at 80°C for 1 h, and then the water extract was collected. The process was repeated once, and the extracts were combined and concentrated with a rotary evaporator and vacuum-dried to yield 9.1% (w/w) of the extract. The extract was examined by TLC analysis to identify the main compounds. The LbL solution was dotted on the TLC plates, and *n*-butanol-acetic acid water (4:1:3) was used as the development system [10]. The indicators were then sprayed on the plates, respectively, and plates were heated at 105°C for 10 min in an oven. Nine kinds of indicator system were used to identify the compounds of LbL [11].

Preparation of experimental animals

Male ICR mice weighing 18–22 g were provided by the Animal Department of Beijing Institute of Traditional Medical and Pharmaceutical Sciences. The mice were housed in stainless steel cages at a controlled temperature (22 ± 2°C) and 60–65% relative humidity with a normal 12 h light and dark cycle. Eight mice were chosen randomly as a normal control (NC) group, while the rest were fed on a high-fat diet. The components of high-fat food included 10% lard, 20% sucrose, 10% vitelline powder, 0.5% sodium cholate and 59.5% regular feed. After exposure to the high-fat diets for 3 weeks, the mice were fasted overnight with free access to water and injected intraperitoneally with alloxan dissolved in normal sterile saline solution. The dosage of alloxan was 200 mg/kg of body weight. 72 h after injection the fasting blood glucose level of the mice was determined according to the glucose oxidase method, using a glucose analyzer [12]. Mice with a blood glucose level above 11.1 mmol/l were defined as diabetic. A total of 32 mice (eight normal mice, 24 alloxan-induced diabetic mice) were chosen and divided into four groups: NC, diabetes control group (DC), diabetes plus LbL low-dose group (DM+LbL LD) and diabetes plus LbL high-dose group (DM + LbL HD). The three groups of diabetic mice were fed high-fat diets continuously until completing the experiment.

Long-term effect of LbL on blood glucose level

In the DM+LbL treated groups, each mouse received LbL extract, dissolved in dH_2O, at a dose of 100 mg/kg body weight (for DM+LbL LD group) and 200 mg/kg of body weight (for DM+LbL HD group) daily by gavage for 28 days. By contrast, the control mice (NC & DC groups) received the same volume of dH_2O only. At days 0, 7, 14, 21 and 28, blood samples were collected from tail veins, following overnight fasting, and then measured. At the same time, the body weight of each mouse was recorded.

Effect of LbL on serum TG, TC & insulin levels

On day 29, the mice were fasted overnight. The blood samples were collected in a sterile tube by sino-ocular puncture under ether anesthesia, placed on the room temperature for 2 h, then centrifuged at 1500 × g for 15 min at 4°C and its supernatant immediately separated from the pellet to prepare serum. The serum was used to determine the levels of TG and TC on the automated chemistry analyzer (OLYMPUS, Japan), by following the instructions of the manufacturer (The Center of Medical Science and Technology of Capital Medical University, Beijing, China). The serum insulin level was then determined by insulin-ELISA kit (Insulin ELISA kit, Adlitteram Diagnostic Laboratories Co., USA) according to the manufacturer's instructions.

Median lethal dose experiment

Acute toxicity studies were carried out using the method reported by Lorke [13]. Median lethal dose (LD_{50}) of a toxic substance is the dose required to kill half the members of a tested animals. A total of 24 normal mice were divided into four groups, each group included six animals (three females and three males) weighing approximately 18–22 g. The mice were administered orally LBL extract in a single dose of 800, 1200, 1600 and 2000 mg/kg of body weight, respectively. The animals were then observed for gross behavioral, neurologic, autonomic and toxic effects at short intervals of time for 24 h. Food consumption, feces and urine were also examined at 2 h, and then at 6-h intervals for a total of 24 h.

Statistical analysis

Statistical analyzes were performed using the SPSS statistical software package. Data are

expressed as a mean with standard error (SE). The effects of LbL on acute-term and long-term blood glucose levels were determined using analysis of variance (ANOVA) for repeated measures. Differences in body weight, blood lipid and serum insulin were analyzed by one-way ANOVA followed by the Scheffe test to analyze specific differences. Results were considered significantly different at the level of $p < 0.05$.

Results

Compounds of LbL by TLC assay

The extract was examined by TLC analysis. The different color spots were visualized using nine kinds of indicator system (**Table 1**), which indicated there were six main compounds in LbL, including alkaloide, saponin, polysaccharide, anthraquinone, flavone and organic acid, but without hydroxybenzene, terpene, steroid and lignin.

Effect of LbL on long-term blood glucose test

Before induction of diabetic phenotype, there was no significant difference of the blood glucose levels among all groups ($p > 0.05$). In long-term blood glucose tests, the blood glucose levels of the DM + LbL LD and DM +LbL HD groups were significantly lower than that of DC group on day 7 (Figure 1). The blood glucose levels of the two LbL-treated groups were all lower than that of DC group ($p < 0.01$) after 14 days treatment. On day 28, the blood glucose levels in the LbL low dose group and high dose group decreased 36.72 and 41.89%, respectively. The NC and DC mice did not show any significant variation on the blood glucose level throughout the experimental period ($p > 0.05$).

Effect of LbL on mice body weight

Changes in body weight in control and experimental groups were shown in **Table 2**. There was no significant difference in initial body weight (before model) among the four groups ($p > 0.05$). The diet of the NC and diabetic model mice was different. After 3 weeks feeding using high-fat or normal diet, the body weight of the diabetic mice model was increased significantly compared with normal control mice. Following LbL treatment for 4 weeks, the body weight of mice in both LbL-treated groups was significantly increased compared with that of the DC group ($p < 0.01$). The mice possibly gained weight owing to increased insulin secretion, which increases fat deposition. By the end of the experiment, the body weight of the NC group was significantly increased, but the body weight of the mice from the DC group was slightly lower than NC group.

Effect of LbL on serum TC & TG levels

Serum TG and TC levels were determined on day 29 and the results are summarized in **Table 3**. The serum TG and TC levels were significantly higher in the DC group than in the NC group. TG and TC levels were decreased by LbL treatment in a dose-dependent manner. The TG levels in the DM+LbL LD and HD groups were lower than that of the DC group ($p < 0.01$) and TC levels in the DM+LbL LD and HD groups were decreased compared with that of DC group ($p < 0.05$, 0.01).

Effect of LbL on serum insulin levels

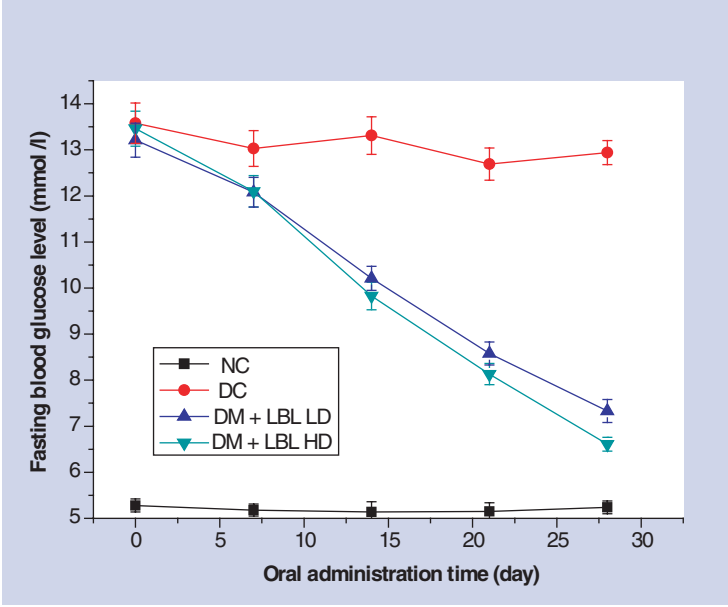
The serum insulin levels of the four groups were determined on day 29, and the results are summarized in **Figure 2**. The serum insulin level of the NC group was higher than that of DC

Table 1. Compounds of *Lycium barbarum L.* extracts by thin-layer chromatography analysis.

Indicator	Examined components	Ratio of flow (Rf)	Color
Iodine/potassic iodide	Alkaloide	0.2525	Brown
Ferric trichloride/water	Hydroxybenzene	*	*
Acetic anhydride/sulfuric acid	Terpene and steroid	*	*
Phosphomolybdic acid/ethanol	Saponin	0.2447	Dark blue
Phenol/sulfuric acid	Polysaccharide	0.2783	Brown
10% KOH	Anthraquinone	0.2473	Orange
10% NaOH	Flavone	0.2418	Yellow
Sulfuric acid/ethanol	Lignin	*	*
Bromophenol blue/ethanol	Organic acid	0.2198	Dark yellow

**Without any spots on the plates.*

Figure 1. Effect of of *Lycium barbarum L.* treatment on blood glucose in the long-term blood glucose test.



Each value represents mean ± SE of eight mice per group.
*Statistical significance versus diabetes control ($p < 0.01$).
DC: Diabetes control; DM+LbL HD: Diabetes plus LbL high dose (200 mg/kg/day); DM+LbL LD: Diabetes plus LbL low dose (100 mg/kg/day), NC: Normal control; SE: Standard error.

group, which indicates that alloxan damages the pancreas islet cells. With 28 days of LbL treatment, serum insulin level in LbL-treated groups was significantly higher than that of the DC group ($p < 0.05$), which implies that treatment with LbL improves insulin secretion in diabetic mice. In the LbL-treated high-dose group, the insulin level was higher than that of the low-dose group. The results implied that LbL improved insulin secretion in the alloxan-induced diabetic mice.

LD_{50} experiment

The behavior of the LbL-treated mice appeared normal during the experiment. No toxic effect was found after administration of up to ten-times the effective dose of the water extract and there were no deaths in any of these groups. Only food consumption was found to be increased after the administration of 8- and 10-times doses, but returned to normal after 4 h.

Discussion

LbL root bark has been used as a traditional medicine for many years in China, owing to its efficacy in treating lung disease, hematemesis, inflammation, gangrene of the extremities and DM. We used the TLC assay to identify the main compounds of LbL, which included alkaloide, saponin, polysaccharide, anthraquinone, flavone and organic acid, but without hydroxybenzene, terpene, steroid and lignin. According to previous research [10,14–15], polysaccharide and flavone are usual antidiabetic ingredients in herbs. Further work on isolation and purification of each ingredient from LbL will be carried out to identify the hypoglycemic effective compounds.

Alloxan is cytotoxic to pancreatic β-cells, so it is an effective diabetes-induction agent. It has been widely used to induce DM in experimental animal models, allowing the investigation of hypoglycemic agents for the treatment of diabetes [16,17]. Alloxan injection consistently produced symptoms of DM, including hyperglycemia, decreased insulin levels, polyuria and weight loss.

The alloxan-induced diabetic mice were treated for 28 days with LbL extract with low and high doses. Blood glucose level was reduced and the effect was shown to occur in a dose-dependent manner, which suggests that LbL has hypoglycemic properties.

Table 2. Effect of clarithromycin treatment on body weight of the mice.

Groups	Body weight (g)		
	I	*II*	*III*
NC	22.55 ± 0.68	27.73 ± 0.76	35.56 ± 0.50
DC	22.18 ± 0.64	31.36 ± 1.04	33.16 ± 0.70
DM+LbL LD	22.03 ± 0.74	31.7 ± 1.02	37.96 ± 0.76*
DM+LbL HD	22.68 ± 0.67	31.28 ± 0.86	38.06 ± 0.53*

Each value represents mean ± SE of eight mice per group.
**Statistical significance versus diabetes control ($p < 0.01$).*
I: Initial body weight; II: Body weight of mice fed with high-fat or normal diet for 3 weeks; III: Body weight of mice treated with dH_2O or LbL for 28 days.
DC: Diabetes control; DM+LbL HD: Diabetes plus LbL high dose (200 mg/kg/day); DM+LbL LD: Diabetes plus LbL low dose (100 mg/kg/day), LbL: Lycium barbarum L.*; NC: Normal control; SE: Standard error.*

Table 3. Effect of *Lycium barbarum L.* treatment on total cholesterol and triglycerol levels of the mice.

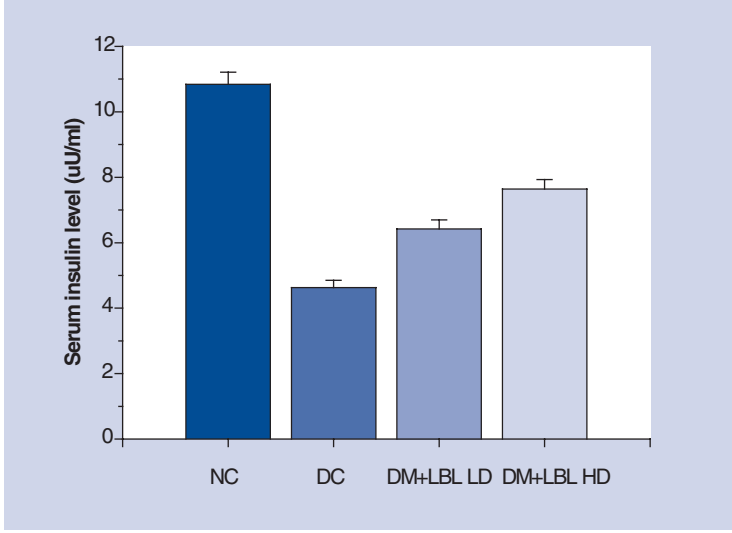
Groups	Triglycerides (mmol/l)	Total cholesterol (mmol/l)
NC	1.659 ± 0.031	2.786 ± 0.062
DC	2.050 ± 0.059	3.321 ± 0.065
DM+LbL LD	1.859 ± 0.035‡	3.086 ± 0.067*
DM+LbL HD	1.781 ± 0.054‡	3.035 ± 0.064‡

Each value represents mean ± SE of eight mice per group.
**Statistical significance vs diabetes control ($p < 0.05$).*
‡Statistical significance vs. diabetes control ($p < 0.01$).
DC: Diabetes control; DM+LbL HD: Diabetes plus LbL high dose (200 mg/kg/day); DM+LbL LD: Diabetes plus LbL low dose (100 mg/kg/day), LbL: Lycium barbarum L.*; NC: Normal control; SE: Standard error.*

The levels of body weight of diabetic animals are usually decreased [18–20]. In our study, a significant decrease in body weight was observed on day 28 after alloxan-induction compared with that of NC group. Decreased body weight observed in diabetic mice is due to excessive breakdown of tissue proteins [21]. It was demonstrated that oral administration of LbL for 28 days improved body weight loss. The result suggested that LbL could meliorate the symptom of decreasing body weight in diabetic model mice.

Diabetes is a metabolic disorder disease affecting carbohydrate, fat and protein metabolism, followed with multiorgan regression in the later period [22,23]. In the DC group, the levels of TC and TG were raised significantly in alloxan-induced diabetic mice compared with normal mice. In the LbL-treated groups, TC and TG levels were significantly decreased. This implies that LbL can prevent or be helpful in reducing the complications of lipid profiles seen in some diabetics in whom hyperglycemia and hypercholesterolemia often coexist.

Figure 2. Effect of of *Lycium barbarum L.* treatment on serum insulin level.



Each value represents mean ± SE from eight mice.
*Statistical significance versus diabetic control group ($p < 0.05$).
DC: Diabetes control; DM+LbL HD: Diabetes plus LbL high dose (200 mg/kg/day); DM+LbL LD: Diabetes plus LbL low dose (100 mg/kg/day), NC: Normal control; SE: Standard error.

Alloxan injection consistently produced characteristic symptoms of DM, including hyperglycemia, damaged pancreatic β-cell, decreased insulin levels, polyuria and weight loss, which consequently decreased utilization of glucose by the tissues [24]. In our study, we have observed that LbL decreased the level of blood glucose and increased the concentration of serum insulin in alloxan-induced diabetic mice. The possible action of LbL extract may be similar to sulfonylureas, which are also secretagogues, the possible mechanism of action and may involve closure of K+ATP channels and membrane depolarization leading to calcium influx through voltage-activated Ca^{2+} channels and insulin secretion [25,26]. Insulin regulates not only glucose metabolism, but also lipoprotein metabolism, vascular smooth muscle cell proliferation, the sympathetic nervous system and Na^{+} reabsorption [27]. Hypercholestrolemia and hypertriglycridemia have been reported to occur in diabetic rats [28]. Accumulation of TG was one of the risk factors in coronary heart disease (CHD). The research indicated that if intensive insulin therapy is administered to Type 2 diabetic patients, the LDL-c level was significantly reduced, with TG levels in chylomicrons, very low-density lipoprotein (VLDL)1 and VLDL2 all showing a decrease. At the same time, lipoprotein lipase was increased [29]. In normal conditions, the decrease of insulin activity caused hypercholestrolemia during diabetes and insulin increased the receptor-mediated removal

of LDL-c [30,31]. Insulin activated the enzyme of lipoprotein lipase and hydrolysis TG [32]. Studies have shown that elevated levels of blood lipids (TC and TG) are one of the major risk factors for CHD [33,34]. Numerous epidemiological studies have shown that hypercholesterolemias present for a longer duration leads to atherosclerosis, which in turn may precipitate cardiovascular disease [35–37]. The significant increase of the level of TG in the plasma of diabetic control mice might be due to the lack of insulin. LbL reduced the level of TG by increasing the plasma insulin level in alloxan-induced diabetic mice, so it might reduce the morbidity of cardiovascular disease.

Expert commentary

The results of the present study show that alloxan-induced diabetic mice were accompanied with an increase in blood lipid and a decrease in insulin level. The extract of LbL had hypoglycemic and hypolipidemic potential, prevented body weight loss and stimulated secretion of insulin. LD_{50} experiment results showed that LbL had no apparent toxic effect. Further investigation, including purification and identification of the antidiabetic ingredient of LbL, should be carried out.

Future perspective

Plant products are frequently considered to be less toxic and have fewer side effects than synthetic drugs, and some scientists suggest that natural products will be the main resource for diabetic treatment in the future. The root bark of LbL has been extensively used in traditional Chinese herbal medicine for treating DM, which has a significant hypoglycemic effect, but several major questions still remain unanswered. We are currently working to address these questions. Consequently, we can expect that the antidiabetic mechanisms of LbL will be fully addressed in the coming years. The study of LbL has raised awareness of the receptor as a possible target for an antidiabetic agent. We can also expect that the antidiabetic compounds of LbL will soon be isolated and identified, and that the structure of the active compound will be characterized. Such compounds may allow for the development of novel nontoxic antidiabetic agents in the near future.

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Executive summary

- The dried root bark of *Lycium barbarum L.* (LbL) has been extensively used owing to its properties against attenuating lung disease, hematemesis, inflammation, gangrene of the extremities and diabetes mellitus, but few studies have addressed the remedial mechanism of LbL.
- The dried root bark of LbL was extracted and examined and found to include alkaloid, saponin, polysaccharide, anthraquinone, flavone and organic acid, but not hydroxybenzene, terpene, steroid and lignin.
- Alloxan-induced mice were treated by administering LbL extracts in low or high doses for 28 days, the blood glucose levels were decreased by 36.72 and 41.89% in the LbL-treated low- and high-dose groups, respectively.
- Following LbL treatment for 4 weeks, the body weight of the mice was significantly increased in high- or low-dose LbL-treated diabetic groups compared with that of the diabetic control group ($p < 0.01$).
- TG and TC levels in LbL-treated low- and high-dose groups were lower than that of the diabetic control group ($p < 0.05, 0.01$).
- No toxic effect was found on administration of up to ten-times the effective dose of the LbL water extract and there were no deaths in any of the LbL-treated mice in the median lethal dose experiment.
- The extract of LbL had hypoglycemic and hypolipidemic potential, prevented body weight loss and stimulated secretion of insulin.

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