

## Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats

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### Abstract

Fruit from *Lycium barbarum* L. in the family Solanaceae is well-known in traditional Chinese herbal medicine. *Lycium barbarum* polysaccharides (LBP) have been identified as one of the active ingredients responsible for its biological activities. We isolated polysaccharides from dried *Lycium barbarum* fruits by boiling water extraction. In the study, 50 animals were divided into two groups: a nondiabetic control ( $n=10$ ) and a diabetic group ( $n=40$ ). Diabetes was induced by a single injection of streptozotocin (50 mg/kg BW; Sigma, USA) freshly dissolved in a 0.1 mol/L citrate buffer (pH 4.5) into the intraperitoneum. The normal control rats and the untreated diabetic control rats were only injected with the citrate buffer. Treated diabetic rats were administrated with LBP in drinking water through oral gavage for 30 days. At the end of experiment, oxidative indice in blood, liver and kidney of all groups were examined. The results show that administration of LBP can restore abnormal oxidative indice near normal levels. Therefore, we may assume that LBP is effective in the protection of liver and kidney tissue from the damage of STZ-induced diabetic rats and that the LBP may be of use as a antihyperglycemia agent.

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**Keywords:** *Lycium barbarum* polysaccharides; Free radical; Superoxide dismutase; Diabetic rats; Oxidative stress

### 1. Introduction

Diabetes is the most significant chronic disease and cause of death in modern society [1]. It is a group of metabolic disorders with different underlying etiologies, each characterized by hyperglycemia due to under-utilization and/or overproduction of glucose [2]. Hyperglycemia, a key clinical manifestation of diabetes mellitus, has been found to increase the generation of reactive oxygen species (ROS) [3]. Diabetes produces disturbances in lipid profiles and, especially, an increased susceptibility to lipid peroxidation [4]. Several studies have shown that tissue antioxidant status may be an important factor in the etiology of diabetes and that antioxidant treatment reduces diabetic complications [5,6].

Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active “lead”

compounds [7]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [8,9], but only a few have received scientific scrutiny.

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 [10–15]. The earliest Chinese medicinal monograph documented medicinal use of *Lycium barbarum* around 2300 years ago. *L. barbarum* fruits can be used to produce various types of healthy products and foods, e.g., medicinal beverages and drinks, and healthy dietary soups [16]. Some constituents of *L. barbarum* fruits have been chemically investigated, especially *L. barbarum* polysaccharide (LBP) components. Five polysaccharides (glycoconjugates) (LbGp1–LbGp5) were isolated and structurally elucidated [11,12]. The polysaccharides isolated from the aqueous extracts of *L. barbarum* have been identified as one of the active ingredients responsible for the biological activities. Zhang [17] report that *L. barbarum* polysaccharides have anti-decrepit effect in brain

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and heart tissues in mice by increasing the activity of superoxide dismutase (SOD). Gan and Zhang [15] report that *L. barbarum* capsule prolongs the life span of drosophila. Wang and Ng [13] report that *L. barbarum* polysaccharides exhibit anti-aging function in fruit flies and mice. *L. barbarum* polysaccharides also exert protection against time and hyperthermia-induced degeneration in cultured seminiferous epithelium [14].

The present study was undertaken to evaluate the potential antioxidative activity of the polysaccharides from *L. barbarum* fruits in the streptozotocin-induced diabetic rats.

## 2. Materials and methods

### 2.1. Chemicals

All fine chemicals including streptozotocin, nicotinamide adenine dinucleotide (NADPH), reduced glutathione (GSH), etc., were purchased from Sigma Chemical Co., USA. Thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), nitrobluetetrazolium (NBT), Triton X-100, riboflavin were purchased from NanJing Biology Technology Co., Ltd (china). All other chemicals used were of good quality and analytical grade.

### 2.2. Animals

Fifty wistar rats (6 weeks old) were used for this study. They were maintained in our air-conditioned animal facility with a 12 h light/dark cycle, and provided (unless otherwise stated) with standard food pellets and tap water ad libitum.

### 2.3. Experimental design

The animals were divided into two groups: a nondiabetic control ( $n=10$ ) and a diabetic group ( $n=40$ ). Diabetes was induced by a single injection of STZ (50 mg/kg BW; Sigma, USA) freshly dissolved in a 0.1 mol/l citrate buffer (pH 4.5) into the intraperitoneum. The control rats were only injected with the citrate buffer. Diabetes was confirmed in the STZ-treated rats by measuring the fasting blood glucose concentration 48 h post-injection. The rats with blood glucose level above 350 mg/dL were considered to be diabetic and were used in the experiment. The diabetic rats ( $n=40$ ) were randomly divided into four groups: Group II–V.

Group I ( $n=10$ ): animals were allowed to free access to a normal diet for 30 days; this group of animals served as normal control.

Group II ( $n=10$ ): the diabetic animals were allowed to free access to a normal diet for 30 days and maintained as a diabetic control group.

Group III ( $n=10$ ): the diabetic animals were put on a normal diet and treated with 50 mg/kg of the LBP, dissolved in 0.2 mL distilled water through oral gavage, daily for 30 days.

Group IV ( $n=10$ ): the diabetic animals were put on a normal diet and treated with 100 mg/kg of the LBP, dissolved in 0.2 mL distilled water through oral gavage, daily for 30 days.

Group V ( $n=10$ ): the diabetic animals were put on a normal diet and treated with 200 mg/kg of the LBP, dissolved in 0.2 mL distilled water through oral gavage, daily for 30 days.

The rationale for the selection of the doses was based on the data published by previous workers, wherein, doses of 20–1000 mg/kg have been used [9].

Body weights of mice were recorded initially, and at the end of the experiment.

At the end of the experiment, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing EDTA-sodium (1 mg/ml) and in polystyrene tubes without the anticoagulant. The EDTA-containing tubes were promptly chilled. Plasma was immediately separated by centrifugation at 3000 rpm at 4 °C for 10 min, and serum by centrifugation at 1000 rpm at room temperature for 10 min. Samples were stored at –80 °C until assayed.

The kidney and liver were carefully removed and homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The latter was next subjected to high-speed centrifugation at 15000 × *g* for 30 min at 4 °C. The resulting supernatant was used as such for assaying glutathione peroxidase (GPx), glutathione reductase (GR), SOD, malondialdehyde MDA and catalase (CAT).

### 2.4. Preparation of *L. barbarum* polysaccharide

The dried fruit of *L. barbarum* (400 g) was powdered with a blender and the ground samples were put in boiling water and decocted by a traditional method for Chinese medicinal herbs. The combined extracts were concentrated and deproteinated by the Sevag method [18] and the resulting aqueous fractions was extensively dialyzed against running distilled water for 2 days. The retentate was concentrated to 400 ml under reduced pressure and precipitated by addition of three volumes of 95% ethanol. After filtering and centrifuging, the resulting precipitate was collected and vacuum-dried at 40 °C, giving a brown powder (LBP, 1.9% from the material, i.e. 7.4 g) with a molecular weight of 24, 132. The polysaccharides consisted of D-rhamnose, D-xylose, D-arabinose, D-fucose, D-glucose, and D-galactose with molar ratio of 1:1.07:2.14:2.29:3.59:10.06 and linked together by β-glycosidic linkages.

### 2.5. Analytical method

Blood glucose levels were determined by the glucose oxidase method [19]. Plasma insulin was determined by using a rat insulin radioimmunoassay kit (Linco Research Inc., St. Charles, USA) in a gamma counter (Peckard, USA) based on the method of Ram et al. [20]. Lipid peroxidation was determined by quantifying MDA concentrations using the method described by Uchiyama and Mihara [21] and modified by Sunderman et al. [22]. The plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride concentrations were determined using an enzymatic method (Sigma Diagnostics, St Louis, MO). The serum concentration of LDL was estimated using Friedewald's method [23].

Table 1  
Effect of LBP administration on diabetic rats' body weight

	I	II	III	IV	V
Initial B.W. (g)	184.5 ± 21.4	186.5 ± 23.8	183.4 ± 16.3	183.7 ± 20.3	185.9 ± 22.4
Final B.W. (g)	242.2 ± 17.9	201.3 ± 24.5 <sup>a</sup>	205.2 ± 18.6	221.1 ± 18.2 <sup>b</sup>	239.5 ± 24.3 <sup>c</sup>

The body weights were calculated at the end of the experiment. Data represent mean ± S.D. ( $n = 10$  for each group).

<sup>a</sup>  $P < 0.01$ , compared with normal control (group I).

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.01$ , compared with diabetic control (group II).

GR was determined by the procedure described by Carlberg and Mannervik [24]. GPX activity was measured by the coupled assay method as described by Paglia and Valentine [25]. One unit of enzyme activity of both the enzymes has been defined as nmol NADPH consumed/min/mg protein based on an extinction coefficient of  $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ . Catalase was estimated as described by Aebi [26]. The specific activity of catalase has been expressed as  $\mu\text{mol H}_2\text{O}_2$  reduced/min/mg protein. SOD was assayed utilizing the method of Marklund and Marklund [27]. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of autoxidation.

## 2.6. Statistical analysis of the data

The results are presented as mean ± S.D. Statistical analysis was performed using ANOVA following Mann–Whitney  $U$ -test. A value of  $P < 0.05$  was considered to indicate a significant difference between groups.

## 3. Results

### 3.1. Body weight gain of rats

As shown in Table 1, significant difference weren't be observed in initial body weights between different groups. After 30 days of experiment, the streptozotocin-treated rats (group II) gained less body weight than did normal control rats ( $P < 0.01$ ). When compared with untreated diabetic control rats, the body weight gains were significantly increased ( $P < 0.01$ ) in three groups of LBP-treated animals ( $P < 0.05$ ,  $P < 0.01$ ) in a dose-dependent manner.

### 3.2. Change of blood glucose and plasma insulin level in diabetic rats after LBP administration

In the present model, untreated diabetic control group of rats showed significant ( $P < 0.01$ ) dose-dependent increases

in blood glucose and decrease ( $P < 0.05$ ;  $P < 0.01$ ) in plasma insulin levels after 30 days of experiment compared with the normal control group (Table 2). In LBP-treated groups of animals, we observed a significant dose-dependent decrease ( $P < 0.01$ ) in blood glucose level but group III and a significant increase ( $P < 0.05$ ;  $P < 0.01$ ) in plasma insulin level, after 30 days of experiment, compared with the untreated diabetic control group.

### 3.3. Effect of LBP administration on SOD activity and other biochemistry indice in blood in diabetic rats

Some test indice in rats of the untreated diabetic control groups were significantly ( $P < 0.01$ ) reduced in SOD activity and HDL-cholesterol level and increased ( $P < 0.01$ ) in MDA, Total cholesterol, Triglyceride, and LDL-cholesterol level compared to the rats of normal control group (Table 3) after 30 days of experiment. With 30 days of LBP administration, there was a significant difference in test indice between untreated diabetic control rats and LBP-treated ones. As shown in Table 3, LBP administration had dose-dependently reversed (restored) the abnormal test indice described above near normal level and activities.

### 3.4. Effect of LBP administration on antioxidase activity and MDA level in liver and kidney in diabetic rats

For studying the effect of LBP administration on free radical production, the activities of SOD, CAT, GPx, GR and level of MDA in liver and kidney were measured (Table 4). They presented a significant difference ( $P < 0.01$ ) in streptozotocin treatment rats (group II) when compared with normal control rats. The LBP administration had dose-dependently reduced MDA level or increased antioxidase activities ( $P < 0.05$ ,  $P < 0.01$ ) compared with untreated diabetic control rats.

Table 2  
Effect of LBP administration on blood glucose and plasma insulin level in diabetic rats

	I	II	III	IV	V
Blood glucose (mg/dl)	98.43 ± 7.65	270.76 ± 30.65 <sup>a</sup>	258.9 ± 26.43	180.43 ± 16.43 <sup>c</sup>	113.53 ± 10.72 <sup>c</sup>
Plasma insulin ( $\mu\text{U/ml}$ )	13.88 ± 14.52	4.87 ± 0.53 <sup>a</sup>	5.78 ± 0.64 <sup>b</sup>	7.04 ± 0.83 <sup>c</sup>	9.57 ± 1.13 <sup>c</sup>

Data represent mean ± S.D. ( $n = 10$  for each group).

<sup>a</sup>  $P < 0.01$ , compared with normal control (group I).

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.01$ , compared with diabetic control (group II).

Table 3  
Effect of LBP administration on SOD activity and other biochemistry indice in blood in diabetic rats

	I	II	III	IV	V
SOD (U/ml)	120.65 ± 15.53	88.87 ± 7.63 <sup>a</sup>	93.65 ± 8.73	116.7 ± 17.21 <sup>b</sup>	132.74 ± 10.74 <sup>c</sup>
MDA (nmol/ml)	2.21 ± 0.24	4.85 ± 0.53 <sup>a</sup>	4.24 ± 0.55 <sup>b</sup>	3.17 ± 0.38 <sup>b</sup>	2.53 ± 0.31 <sup>c</sup>
Total cholesterol (mmol/L)	4.78 ± 0.52	6.95 ± 0.57 <sup>a</sup>	6.05 ± 0.63	5.67 ± 0.6 <sup>b</sup>	4.95 ± 0.32 <sup>c</sup>
HDL-cholesterol (mmol/L)	0.41 ± 0.03	0.24 ± 0.02 <sup>a</sup>	0.28 ± 0.02	0.34 ± 0.02 <sup>b</sup>	0.38 ± 0.03 <sup>c</sup>
Triglyceride (mmol/L)	0.46 ± 0.03	0.75 ± 0.06 <sup>a</sup>	0.68 ± 0.07	0.54 ± 0.04 <sup>c</sup>	0.49 ± 0.04 <sup>c</sup>
LDL-cholesterol (mmol/L)	2.85 ± 0.23	3.97 ± 0.32 <sup>a</sup>	3.67 ± 0.44	3.45 ± 0.26 <sup>b</sup>	2.98 ± 0.37 <sup>c</sup>

Data represent mean ± S.D. (*n* = 10 for each group).

<sup>a</sup> *P* < 0.01, compared with normal control (group I).

<sup>b</sup> *P* < 0.05.

<sup>c</sup> *P* < 0.01, compared with diabetic control (group II).

Table 4  
Effect of LBP administration on antioxidase activity and MDA level in liver and kidney in diabetic rats

	I	II	II	IV	V
Liver					
MDA (nmol/mg protein)	6.83 ± 0.57	9.28 ± 0.45 <sup>a</sup>	8.86 ± 0.23 <sup>b</sup>	6.71 ± 0.51 <sup>c</sup>	5.05 ± 0.68 <sup>c</sup>
SOD (U/mg protein)	12.54 ± 2.54	6.89 ± 0.84 <sup>a</sup>	7.68 ± 0.93 <sup>b</sup>	8.93 ± 0.94 <sup>c</sup>	10.97 ± 6.54 <sup>c</sup>
CAT (U/mg protein)	19.43 ± 2.76	12.73 ± 2.11 <sup>a</sup>	14.52 ± 1.28	17.32 ± 2.04 <sup>c</sup>	20.84 ± 2.54 <sup>c</sup>
GPx (U/mg protein)	3.75 ± 0.45	1.07 ± 0.13 <sup>a</sup>	1.54 ± 0.16 <sup>b</sup>	2.15 ± 0.18 <sup>c</sup>	3.24 ± 0.37 <sup>c</sup>
GR (U/mg protein)	5.64 ± 0.6	3.05 ± 0.26 <sup>a</sup>	3.88 ± 0.3 <sup>b</sup>	4.32 ± 0.41 <sup>c</sup>	5.97 ± 0.42 <sup>c</sup>
Kidney					
MDA (nmol/mg protein)	1.65 ± 0.2	3.59 ± 0.12 <sup>a</sup>	3.21 ± 0.32	2.78 ± 0.22 <sup>c</sup>	1.87 ± 0.22 <sup>c</sup>
SOD (U/mg protein)	10.56 ± 1.98	4.98 ± 0.64 <sup>a</sup>	5.63 ± 0.61 <sup>b</sup>	8.76 ± 0.93 <sup>c</sup>	11.65 ± 1.8 <sup>c</sup>
CAT (U/mg protein)	14.67 ± 1.96	8.95 ± 0.67 <sup>a</sup>	12.76 ± 1.86 <sup>c</sup>	15.32 ± 2.05 <sup>c</sup>	17.86 ± 1.53 <sup>c</sup>
GPx (U/mg protein)	7.57 ± 1.06	4.11 ± 0.52 <sup>a</sup>	4.88 ± 0.55 <sup>b</sup>	5.94 ± 0.51 <sup>c</sup>	6.83 ± 0.51 <sup>c</sup>
GR (U/mg protein)	3.98 ± 0.27	1.32 ± 0.11 <sup>a</sup>	1.91 ± 0.08 <sup>b</sup>	2.54 ± 0.22 <sup>c</sup>	3.44 ± 0.26 <sup>c</sup>

Data represent mean ± S.D. (*n* = 10 for each group).

<sup>a</sup> *P* < 0.01, compared with normal control (group I).

<sup>b</sup> *P* < 0.05.

<sup>c</sup> *P* < 0.01, compared with diabetic control (group II).

#### 4. Discussion

Chronic hyperglycemia in diabetic patients or animals can cause oxidative stress, depleting the activity of the antioxidative defense system and resulting in elevated levels of oxygen free radicals [28]. Acute streptozotocin injection has been used to study cellular or tissue oxidative damage because it produces reactive oxygen species and reduces antioxidant enzyme activity, especially in pancreatic tissues [29]. In fact, streptozotocin can stimulate H<sub>2</sub>O<sub>2</sub> generation in islet cells [30] where the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase is relatively low when compared to other tissues [31]. By streptozotocin, most islet cells are impacted to death and remaining islet cells almost exhibit a significant decrease in the activity of these enzymes compared to normal rats [32]. Therefore, streptozotocin-induced diabetes in rats has been frequently used as a model for type 1 diabetes or for type 2 diabetic complications.

Increased generation of superoxide anion and other ROS, lipid peroxidation product and decreased plasma or tissue concentrations of superoxide dismutase, catalase, and glutathione are reported in both clinical and experimental diabetes [33,34]. In the present experiment, significantly decreased body weight and plasma insulin level, increased blood glucose concen-

tration were observed in streptozotocin-induced diabetic rats, suggesting that dysfunction of digestive system caused by diabetes inevitably weaken absorption of nutrition elements in food. The present study has similarly demonstrated that some lipid peroxidation products, such as total cholesterol, MDA, HDL-cholesterol, LDL-cholesterol and triglyceride in blood, significantly diverges from their normal level in diabetic rats. These results further confirm that there is a strong correlation between oxidative stress and diabetes occurrence. Antioxidants such as LBP have been reported to reduce alloxan-induced and streptozotocin-induced oxidative damage [35,36]. Similarly, we observed that administration of LBP significantly restored abnormal levels of lipid peroxidation products in blood in diabetic rats and insulin, which consequently reduces blood glucose level and raises body weight of diabetic rats. Decrease in blood glucose content of diabetic rats are partly being due to its utilization by the tissues to compromise the deleterious effects of lipid peroxidation. This suggests that LBP may improve lipid dysfunction of diabetic rats and retard development of diabetes. Therefore, we can assume that LBP acts as therapeutic agent of diabetes in traditional chinese medicine by scavenging excessive streptozotocin-induced free radicals.

Lowered activities of enzymatic antioxidants such as SOD, CAT and GPx has been well documented in streptozotocin

induced diabetic rats [37]. Other workers also reported a decrease in the activities of these antioxidant enzymes (SOD, CAT, GPx and GST) in the liver kidney of diabetic rats [38]. Our results corroborate these observations. In the present study, increased levels of MDA and decrease in the activity of SOD, CAT, GR and GPx were noticed in liver and kidney in streptozotocin induced diabetic rats. Liver and kidney are the most important organs, which play a pivotal role in regulating various physiological processes in the body. They are involved in several vital functions, such as metabolism, secretion, urination, reproduction, regulation and storage. They have great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver and kidney inflicted by hepatotoxic and nephrotoxic agents is of grave consequences [39]. In diabetic animals, free radicals can rapidly accumulated and form oxidative stress, which may impair function of liver and kidney, in which significantly decreased antioxidant activities and increased lipid peroxidation level can be observed. Previous studies have reported that there was an increased lipid peroxidation level and decreased antioxidant activities in liver, kidney, skin, lenses, heart, and aorta of diabetic rats [36,40,41]. As the alteration produced in the antioxidant activities indicate the involvement of deleterious oxidative changes, increased activities of the components of this defence system would therefore be important in protection against radical damage. Our study showed that administration of LBP tends to restore the liver peroxides near normal levels. This indicates that LBP may inhibit oxidative damage of hepatic and nephridial tissue. Antioxidant effects have been reported for some plants that contain flavonoids, phenolic compounds, ascorbic acid, and tocopherol [38]. Phytochemical results showed that *L. barbarum* polysaccharides are rich in flavonoids, phenolic compounds, ascorbic acid, and tocopherol [12,42]. It is possible that the antioxidant effect is related to this component.

As a result, it may be concluded that, probably due to its antioxidant effects, LBP is effective in the protection of liver and kidney tissue from the damage of STZ-induced diabetic rats and that the LBP may be of use as an anti-hyperglycemia agent.

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