

**Research Paper**

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EVALUATION OF HYPOGLYCEMIC ACTIVITY OF THE POLYSACCHARIDES EXTRACTED FROM *LYCIUM BARBARUM***Longjun Jing^{1*}, Guowen Cui², Qiang Feng¹, Yuanshi Xiao¹**¹Qujing Normal University, Qujing, Yunnan Province, 655011, P.R. China.²Yuxi Normal University, Yuxi, Yunnan Province, 653100, P.R. China.**E-mail:** jinglongjun666@yahoo.cn, jinglongjunyn@126.com**Abstract**

A study was undertaken to evaluate the hypoglycemic activity of polysaccharide extracted from *Lycium barbarum* (LBP). The various parameters studied included body weight (bw), fasting blood glucose levels (FBG), total cholesterol (TC) and triglyceride (TG) in diabetic and normal mice. LBP treatment (20, 40 mg/ kg body weight) for 28 days resulted in a significant decrease in the concentration of FBG, TC and TG in diabetes mellitus mice. Furthermore, LBP significantly increased body weight. The data demonstrated LBP at the dose of 40 mg/kg bw exhibited the better effect.

Key words: polysaccharide, *Lycium barbarum*, hypoglycemic, activity**Introduction**

Diabetes mellitus is found in all parts of the world and is rapidly increasing in most parts of the world. As a devastating disease, diabetes is affecting approximately 3% of the population worldwide (Skyler, 2004). For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine (Akhtar and Ali, 1984). Synthetic hypoglycemic agents can produce serious side effects and in addition, they are not suitable for use during pregnancy (Pari and Saravanan, 2002). Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Lycium barbarum species are deciduous woody perennial plants, produce a bright orange-red, ellipsoid berry 1-2 cm long. The berries have been used in traditional Chinese medicine for about 1,900 years (Jin et al., 2006). They have been used for the treatment of cerebral arteriosclerosis, liver or heart diseases, hypercholesterolemia and diabetes (Zhao and Liu, 2008). The active components of *Lycium barbarum* primarily contain water-soluble polysaccharides (Chen and Mu, 2007). They could be extracted with hot water followed by precipitation with ethanol to obtain high quantity of polysaccharides (Zhi et al., 2004; Gan et al., 2004; Zhang et al., 2004). LBP have been recently studied for their physiological and pharmaceutical activities. The purpose of this study was to investigate the hypoglycemic activity of LBP in alloxan-induced diabetic mice.

Materials and Methods

Plant materials

Dried *Lycium Barbarum* (voucher No. 20080418) were purchased from a local drug market and the material was identified by Mr. King Li, a botanist of Qijing Normal University. A voucher specimen has been deposited in herbarium of Qijing Normal University.

Drugs and reagents

Alloxan was purchased from Sigma Co. (USA). Glucose Analyzer and strips were purchased from Roche Diagnostic Co. (USA). Reagents for total cholesterol (TC) and triglyceride (TG) were obtained from Beijing Chengxinde Biochemistry Reagent Company (Beijing, China). Reagents for serum insulin was purchased from Adlitteram Diagnostic Laboratories Co. (USA).

Extraction of LBP

Dried *Lycium Barbarum* was crushed in an electrical grinder and then powdered, 1000 g of this powder was immersed in tenfold dH₂O, boiled at 100 °C for 12 hr (Ayiguli *et al.*, 2007; Luo *et al.*, 2000) and then the water extract was collected. The process was repeated once, and the extracts were combined and concentrated with a vacuum rotary evaporator at 70 °C (Luo *et al.*, 2000; Gao *et al.*, 2008; Liu *et al.*, 2009). The concentrated solution was precipitated with addition of 4 times volume 95% ethanol and the precipitate was washed in turn with 100 % ethanol, 100 % Ether and acetone, polysaccharide extracted from *Lycium barbarum* was obtained by vacuum drying (Luo *et al.*, 2000). The Unico-7200 spectrophotometer (Unico Co., Shanghai, China) was used to determine the content of polysaccharides in the above extracted product at 490 nm (Ayiguli *et al.*, 2007; Wang *et al.*, 2007). The calculated extraction yield of polysaccharides was 9.43%.

Experimental animals

Male mice of original Kun-ming strain (18-22 g each) were used for the study. The study was carried out according to the "Principles of Laboratory Animal Care" (World Health Organization (WHO) Chronicle, 1985). A standard pellet diet and water were given *ad libitum*. The standard pellet diets consists of corn starch 50%, casein 21%, fish flour 10%, sucrose 8%, soybean oil 6.5%, vitamin mix 3%, mineral mix 1%, L-cystine 0.5% (the Disease Control Center, Yunnan, China). Animals were maintained under a constant 12-hr light and dark cycle and an environmental temperature of 21-23 °C with relative air humidity of 45 % to 55 %.

Preparation of alloxan-induced diabetic mice

Diabetes was induced in fasted mice (12 hr) by intraperitoneal injection of 200 mg/kg bw of alloxan, freshly dissolved in sterile normal saline immediately at a concentration of 40 g/L (Shu *et al.*, 2002; Wang *et al.*, 2004; Hu *et al.*, 2006). Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. The mice with a blood glucose level above 11 mmol/L, as well as with polydipsia, polyuria, and polyphagia were selected for the experiment (Zhang *et al.*, 2004; Yang *et al.*, 2006).

Experimental Design

Forty Male mice were randomly divided into five equal groups as follows:

- i) Normal control group(NC): normal control mice administered water daily for 28 days;
- ii) Diabetic control group(DC): diabetic control mice administered water daily for 28 days;
- iii) Diabetic + LBP (20mg/kg) group(DLL): diabetic mice administered LBP (20mg/kg) daily for 28 days.;
- iv) Diabetic + LBP (40 mg/kg) group(DLH): diabetic mice administered LBP (40 mg/kg) daily for 28 days.;
- v) Diabetic + glibenclamide (4 mg/kg) group(DG): diabetic mice administered reference drug glibenclamide (4 mg/kg) daily for 28 days (Chen and Huang, 2000; Zhang *et al.*, 2002; Shan *et al.*, 2005).

Animals of control group, NC and DC groups were subjected to forceful feeding of 0.5mL distilled water/100 g bw daily for 28 days to keep all the animals at same type of treatment condition in respect to BLP supplemented groups.

During LBP and Glib supplement for 28 days, fasting blood glucose level was measured for once every week. Blood was collected from tip of the tail vein and fasting blood glucose level was measured by using a glucose analyzer. At the same time, the body weight of each mouse was measured. On 28th day of experiment, the mice were sacrificed by decapitation under light ether anesthesia and blood was collected from dorsal aorta and serum was separated by centrifugation for 5min and was kept at -20 °C for the biochemical assay of total cholesterol (TC), and triglyceride (TG). TC and TG were determined by enzyme methods.

Acute toxicity studies

LBP was tested for its acute toxicity in male mice. The test was carried out by single oral administration of LBP at doses of 80, 240, 400 mg/kg to different groups of mice (5 mice in each group). The mortality and general behavior was observed for one hr, four hr, and intermittently for next six hr, and again at 24 hr and 48 hr. The parameters were observed are gross behavioral changes, grooming, alertness, sedation, loss of righting reflex, tremors convulsions (Mukund *et al.*, 2008).

Statistical analysis

All results were expressed as means \pm SEM for each group. Data were analysed statistically by one-way analysis of variance (ANOVA). The significance of the difference between the means of test and control studies was established by student's t-test. P values of less than 0.05 were considered significant.

Results

Acute toxicity studies

In the present study, toxicity test was carried up to high concentration of 400 mg/kg (10 times more than chosen dose). Even at this dose extract did not exhibit any sign of toxicity. Since the main purpose of this test is to get some idea on conspicuous behavioral changes and death, if any, and the LBP did not exhibit any toxic symptoms in the limited toxicity evaluation in male mice.

Effect of LBP on body weight

The alloxan-induced diabetic mice exhibited loss of body weight. Before embarking on the experiment, all the groups had no significant difference in body weight ($P > 0.05$). A significant ($P < 0.05$) decrease in body

weight was detected in the DC, DLL and DLH groups as compared to the normal control group from 7 days after alloxan injection. However, the body weights in the DLH groups were significantly ($P < 0.05$) and dose-dependently increased as compared to those of the diabetic control from 14 days after administration, which is comparable to that of the DG group. The results are shown in Figure1.

Effect of LBP on fasting blood glucose levels

The alloxan-induced diabetic mice exhibited hyperglycemia. At the beginning, a significant ($P < 0.05$) increase in FBG was detected in the diabetic groups as compared to the normal control group. But these abnormal increases in blood glucose levels significantly ($P < 0.05$) and dose-dependently lowered in the LBP-administered groups as compared to the diabetic control group from 7 days after administration. In the DG group, decrease was also significant ($P < 0.05$) from 7 days after administration. NC and DC groups did not show any significant variation on the blood glucose level throughout the experimental period ($p > 0.05$). The results are shown in Table 1.

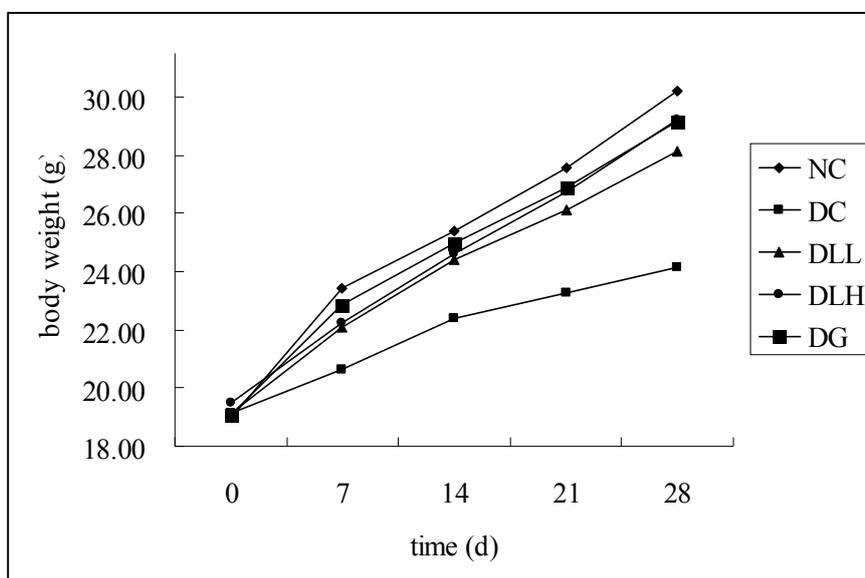


Figure 1: Effect of LBP on body weight (g) in mice

Effect of LBP on blood lipids levels

Diabetes mellitus is usually complicated with hyperlipoproteinemia. The present results showed that the TC and TG levels were significantly elevated in the diabetic control group as compared to the normal control group ($P < 0.05$). After supplementation with LBP, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels in diabetic mice. The response was better in DLH group compared to the others group. The results are shown in Table 2.

Discussion

Diabetes mellitus is a serious chronic disease. Although oral anti-hyperglycemic agents and insulin are often successful in diabetes treatment, they have prominent side effects and fail to significantly alter the course of diabetic complications. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients (Chen et al.,

2008). The present study shows that alloxan-induced diabetic mice presented obvious hyperglycemic symptoms, but LBP produces a significant antihyperglycemic effect when administered orally to alloxan-diabetic mice. The dosage of 40 mg/kg was more effective than that of 20 mg/kg.

Table 1: Effect of LBP on blood glucose Level (mmol/L) in mice

Groups	Days after dosing (day)				
	0	7	14	21	28
NC	5.03±0.14	5.02±0.16	4.98±0.17	4.92±0.08	5.04±0.11
DC	15.24±0.39 ^①	15.21±0.26 ^①	15.21±0.35 ^①	15.03±0.15 ^①	15.12±0.29 ^①
DLL	15.13±0.30 ^①	9.43±0.21 ^{①②}	8.41±0.21 ^{①②}	7.45±0.15 ^{①②}	6.79±0.16 ^{①②}
DLH	15.28±0.28 ^①	8.62±0.11 ^{①②}	7.61±0.28 ^{①②}	6.12±0.17 ^{①②}	5.23±0.16 ^{①②}
DG	15.17±0.29 ^①	8.96±0.12 ^{①②}	7.12±0.23 ^{①②}	6.35±0.24 ^{①②}	5.56±0.34 ^{①②}

n=8; (mean±S.D., g); ^①P < 0.05 as compared with normal control group.; ^②P < 0.05 as compared with diabetic control Group

Table 2: Effect of LBP on blood lipids (mmol/L) in mice

Groups	TG	TC
NC	1.58±0.03	2.64±0.04
DC	1.99±0.03 ^①	3.28±0.05 ^①
DLL	1.71±0.03 ^{①②}	3.03±0.08 ^{①②}
DLH	1.64±0.03 ^{①②}	2.75±0.11 ^{①②}
DG	1.66±0.0 ^{①②}	2.09±0.05 ^{①②}

n=8; (mean±S.D., g); ^①P<0.05 as compared with normal control group; ^②P < 0.05 as compared with diabetic control Group

Diabetes is also associated with hyperlipidemia. The serum TC and TG decreased significantly in diabetic mice after LBP supplementation. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Sharma et al., 2003). *Lycium barbarum* have been used to treat diabetes in folk tradition for a long time. From this study, we could conclude LBP possesses hypoglycemic effects and the dose of 40 mg/kg bw represents the optimal level for effecting a positive diabetic response in mice. Toxicity data have already proved that the LBP did not show any toxic reactions. So, it can be said that *Lycium barbarum* is a good natural material is a potential agent to treat diabetes, maybe the effective constituent is polysaccharide.

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