

Full Length Research Paper

Effects of polysaccharide from *Lycium barbarum* in alloxan-induced diabetic mice

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A study was undertaken to evaluate the effects of polysaccharide from *Lycium barbarum* (LBP) in alloxan-induced diabetic mice. The various parameters studied included body weight, fasting blood glucose levels, 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 fasting blood glucose (FBG), total cholesterol (TC) and triglyceride (TG) in diabetes mellitus mice. Furthermore, LBP significantly increased body weight (bw). The data demonstrated LBP at the dose of 40 mg/kg bw exhibited the optimal effect.

Key words: Polysaccharide from *Lycium barbarum*, effect, diabetic.

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 *L. 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 effect of LBP in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Plant materials

Dried *L. barbarum* was purchased from a local drug market and the material was identified by Mr. King Li, a botanist of Qujing Normal University. A voucher specimen has been deposited in herbarium of Qujing 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 were purchased from Adlitteram Diagnostic Laboratories Co. (USA).

Extraction of LBP

Dried *L. barbarum* was crushed in an electrical grinder and then powdered; 1000 g of this powder was immersed in tenfold dH₂O,

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boiled at 100°C for 12 h (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. The concentrated solution was precipitated with addition of 4 times volume 95% ethanol and the precipitation was washed in turn with 100% ethanol, 100% ether and acetone; polysaccharide from *L. 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).

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*. Animals were maintained under a constant 12-h light and dark cycle and an environmental temperatures of 21 - 23°C.

Preparation of alloxan-induced diabetic mice

Diabetes was induced in fasted mice (12 h) by intraperitoneal injection of 200 mg/kg bw of alloxan, freshly dissolved in sterile normal saline immediately before at a concentration of 40 g/L. 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 (20 mg/kg) group (DLL): diabetic mice administered LBP (20 mg/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.

Animals of control group, NC and DC groups were subjected to forceful feeding of 0.5 mL 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 by balance. 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 5 min 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 continuously for 1, 4 h and intermittently for next 6 h and again at 24 and 48 h. The parameters 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 (N = 8). 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 were shown in Figure 1.

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 decreased 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

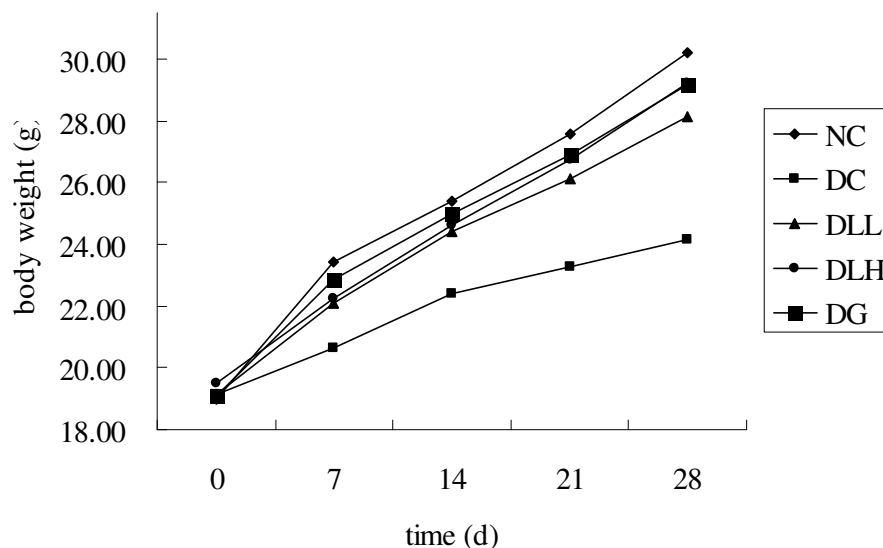


Figure 1. Effect of LBP on body weight (g) in mice.

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 ^x	15.21±0.26 ^x	15.21±0.35 ^x	15.03±0.15 ^x	15.12±0.29 ^x
DLL	15.13±0.30 ^x	9.43±0.21 ^{xy}	8.41±0.21 ^{xy}	7.45±0.15 ^{xy}	6.79±0.16 ^{xy}
DLH	15.28±0.28 ^x	8.62±0.11 ^{xy}	7.61±0.28 ^{xy}	6.12±0.17 ^{xy}	5.23±0.16 ^{xy}
DG	15.17±0.29 ^x	8.96±0.12 ^{xy}	7.12±0.23 ^{xy}	6.35±0.24 ^{xy}	5.56±0.34 ^{xy}

N = 8; (mean ± S.D., g); ^x(P < 0.05) as compared with normal control group; ^y(P < 0.05) as compared with diabetic control group.

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 were shown in Table 1.

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 were 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 oral administration to alloxan-diabetic mice. The dosage of 40 mg/kg is more effective than that of 20 mg/kg.

The levels of serum lipids are usually elevated in diabetes mellitus (Pushparaj et al., 2000). This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides (Taskinen, 1987). However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency and this results in hypertriglyceridemia (Eidi and Eidi, 2009). The effect of

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 ^x	3.28±0.05 ^x
DLL	1.71±0.03 ^{xy}	3.03±0.08 ^{xy}
DLH	1.64±0.03 ^{xy}	2.75±0.11 ^{xy}
DG	1.66±0.0 ^{xy}	2.097±0.05 ^{xy}

N = 8; (mean ± S.D., g); ^x(P < 0.05) as compared with normal control group; ^y(P < 0.05) as compared with diabetic control group.

the LBP on diabetic hypercholesterolemia and hypertriglyceridemia could be through its control of hyperglycemia. In this study, the serum TC and TG have been 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).

L. barbarum has 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 in the limited toxicity evaluation in male mice. So, it can be said that *L. barbarum* is a good natural material to develop new agent to treat diabetes. It can also be said that the effective constituent may be polysaccharide.

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