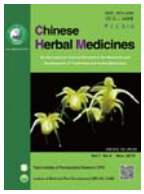

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Original article

Hypoglycemic and Hypolipidemic Effects of *Lycium barbarum* Polysaccharide in Diabetic Rats

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ABSTRACT

Objective To study the antidiabetic effects and the underlying molecular mechanisms of *Lycium barbarum* polysaccharide (LBP) and its DEAE cellulose elution fraction LBP-IV in diabetic rats induced by high fat diet (HFD) and streptozotocin (STZ). **Methods** After ig administration of LBP-IV [50, 100, and 200 mg/(kg·d)] and LBP [100 mg/(kg·d)] once daily for consecutive 4 weeks to diabetic rats, the glucose and lipids in blood, mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK), sterol regulatory element binding-protein-1c (SREBP-1c), and fatty acid synthase (FAS) in liver were determined. **Results** Ig administration of LBP and LBP-IV significantly decreased the levels of blood glucose, HbA1c, TC, TG, and LDL-C, as well as the hepatic mRNA expression of PEPCK, SREBP-1c, and FAS, whereas significantly increased the oral glucose tolerance of diabetic rats. **Conclusion** The findings suggest that the antidiabetic effects of LBP and LBP-IV are associated with the decreased hepatic mRNA expression of PEPCK, SREBP-1c, and FAS in HFD-STZ induced diabetic rats.

Key words

antidiabetic effect; diabetic rat model; *Lycium barbarum* polysaccharides

1. Introduction

Fruit of *Lycium barbarum* L. is an important Chinese materia medica (CMM) and nowadays has been widely used as a popular functional food with vital biological activities. The main chemical components were *L. barbarum* polysaccharides (LBP), zeaxanthin, and other constituents with small molecules, such as betaine, cerebroside, β-sitosterol, *p*-coumaric acid, and various vitamins. LBP possesses a range of biological activities, including anti-fatigue/endurance, glucose control in diabetics, anti-oxidant property, immunomodulation, and antitumor activity (Jin et al, 2013; Zhang et al, 2012). Recently, many studies have focused on the isolation, preparation, and structural characterization of LBP and its purified constituents (Shan et al, 2010; Wang et al, 2010; Liang et al, 2011; Ke et al, 2011; Peng et al, 2012; Zhu et al, 2013), as well as their anti-oxidant activity (Luo et al, 2004; Li et al, 2007; Ma et al, 2009; Wang et al, 2010; Liang et al, 2011; Jia et al, 2012) and antidiabetic effects (Luo et al, 2004; Ma et al, 2009; Rui et al, 2009; Shan et al, 2010; Jing et al, 2013). The results demonstrated that LBP and its purified constituents not only have anti-oxidant activity *in vitro* (Wang et al, 2010; Liang et al, 2011; Ke et al, 2011) and *in vivo* (Luo et al, 2004; Ma et al, 2009; Liang et al,

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2011; Jia et al, 2012), but also have the ability to alleviate the insulin resistance of liver cells *in vitro* (Shan et al, 2010; Jing et al, 2013), decrease the blood glucose and lipid levels of alloxan-induced diabetic rabbits (Luo et al, 2004) and mice fed by high fat diet (HFD) (Ma et al, 2009), and protect kidney from STZ-induced damage in diabetic rats (Rui et al, 2009).

It is known that the reactive oxygen species (ROS) produced by cytochrome P450 2E1 (CYP2E1) is an important causative factor for insulin resistance in diabetes and related conditions (Bloch et al, 2005; Kathirvel et al, 2009). LBP can alleviate CCl_4-induced acute hepatic damage by down-regulation of nuclear factor kappa-B and CYP2E1 activity in mice liver (Jia et al, 2012), increase insulin sensitivity by up-regulation of myocyte glucose transporter four of diabetic rats (Zhao et al, 2005). LBP-4 (the major active component of LBP) can significantly increase the activities of anti-oxidant enzymes such as SOD, CAT, and GSH-Px, inhibit protein kinase C over-activation in kidneys of STZ-induced diabetic rats. And the antidiabetic mechanism of LBP-4 was demonstrated to be associated with the decreased phosphorylation of ERK1/2 signal transduction pathway (Rui et al, 2009). Although numerous studies have been published on humans and animals examining the health aspects of LBP, to the best of our knowledge, there are little report about the antidiabetic mechanism of LBP and its purified constituents in experimental type 2 diabetic rats *in vivo*. In this work, we investigated the therapeutic effects of LBP and its DEAE cellulose elution fraction LBP-IV in diabetic rats induced by HFD and STZ and the underlying molecular mechanism.

2. Materials and methods

2.1 Reagents

Fruits of *Lycium barbarum* L. were purchased from Yinchuan City Herb Market (Ningxia, China). Streptozotocin (STZ) was purchased from Sigma (USA). Trizol and PCR primers were purchased from Invitrogen (USA). ReverTra Ace qPCR RT Kit and TransStart Green qPCR Super Mix were purchased from Toyobo (Japan). The test kits of glucose, total cholesterol (TC), triglyceride (TG), glycosylated hemoglobin (HbA1c), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) were purchased from Nanjing Jiancheng Bioengineering Institute (China). Cellulose DE-52 and SephadexG-100 were purchased from GE Healthcare Bio Sciences AB (Sweden). The other chemicals used were of reagent grade from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Preparation for LBP and LBP-IV

Lycium barbarum polysaccharide (LBP) was extracted with hot distilled water (1:10) at 100 ^{o}C for 2 h under constant stirring. The extract was filtered, evaporated under vacuum, and precipitated by adding 3-fold volume of ethanol. The precipitate was washed with acetone and alcohol, and then dried under vacuum to give crude LBP. The contents of carbohydrate and protein in LBP extract were determined by phenol-sulfuric acid method using *D*-glucose as standard sample and by Bradford method using BSA as standard sample, respectively.

The decoloration and deproteinization of LBP were performed by continuous stirring 1% crude LBP aqueous solution with D101-I nonpolar macroporous resin (7:1) at room temperature for 3 h followed by filtration and lyophilization. Then LBP (5 g dissolved in 50 mL water) was dialyzed (MWCO8000) against distilled water for 48 h and separated by DEAE cellulose-52 column (40 cm × 8 cm) with the step gradient elution of water, 0.05, 0.10, and 0.50 mol/L NaCl in turn at a flow rate of 5 mL/min. There were four eluted fractions designated as LBP-I, LBP-II, LBP-III, and LBP-IV, respectively. LBP-IV was dialyzed in distilled water for 24 h, and dried by lyophilization. The purity and molecular weight of LBP-IV were determined by Sephadex G-100 gel-filtration column (50 cm × 0.5 cm) at a flow rate of 0.5 mL/min eluent (deionized water). Column calibration was performed using standard dextrans with different molecular weights (10 000, 40 000, 70 000, 100 000, 500 000, and 2 000 000, respectively). The standard curve represented the linear relationship of the retention time and the logarithm of their respective molecular weights. The molecular weight of LBP-IV was calculated by comparing with the standard dextrans with different molecular weights.

2.3 Diabetic rats induced by HFD and STZ

Male Wistar rats [weighing (200 ± 20) g, purchased from Provincial Disease Prevention and Control Center of Hubei] were maintained in SPF animal room at temperature of (22 ± 2) ^{o}C, with humidity of (60 ± 5)% and 12 h/12 h day/night cycle. All procedures were approved by Ethic Committee of Hubei University, and complied with health guidelines for the care and use of laboratory animals. The diabetic rat model was built by HFD and STZ as follows (Reed et al, 2000). Rats in diabetic group were fed with HFD diet (composed of 10% lard oil, 10% white sugar, 5% yolk power, 1% cholesterol, and 74% regular diet) for continuous 6 weeks, and then the rats were ip injected with 40 mg/kg STZ (dissolved in citrate buffer, pH 4.4). Rats in normal control group were fed with regular diet and injected with same volume of citrate buffer. One week after STZ injection, the diabetic rats with blood glucose level ≥ 16.7 mmol/L were selected for the further study. Animals were kept on their respective diet till the end of the study.

The diabetic rats were randomly divided into five groups (n = 8) as follows: diabetic control group (treated with saline alone), LBP group (treated with 100 mg/kg body weight of LBP), LBP-IV 50, 100, and 200 groups (treated with 50, 100, and 200 mg/kg body weight of LBP-IV, respectively). The rats were ig administered with LBP and LBP-IV (dissolved in saline) respectively once daily for continuous 4 weeks. The blood samples collected from orbit and HbA1C were determined in EDTA-blood samples using commercial assay kit (Nanjing Jiancheng Bioengineering Institute, China). Blood samples were centrifuged at 7000 r/min for 10 min at 4 ^{o}C to

separate the serum. Serum glucose and serum lipid were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China). The rat liver was immediately removed, weighed, and stored at −80 °C for real-time PCR assay.

2.4 Oral glucose tolerance test

At the day before sacrificed, rats in various groups were fasted for 16 h and then ig administered with glucose (2 g/kg body weight) to undergo an oral glucose tolerance test. The blood samples were collected from caudal vein at 0 (prior to the glucose load), 30, 60, 90, and 120 min after glucose administration. The concentration of blood glucose was determined by glucometer.

2.5 Real time quantitative RT-PCR for mRNA expression of PEPCK, SREBP-1c, and FAS

Total RNA was extracted from liver tissue with Trizol reagent and 2 μg of RNA was reversely transcribed to cDNA by RT-PCR using 1 μL of Primer Mix, 1 μL of RT Enzyme Mix, 4 μL of 5 × RT buffer, and nuclease-free water in a final volume of 20 μL. The cDNA was amplified by real-time fluorescence quantitative PCR as follows. The cDNA samples were pre-denatured by heating to 95 °C for 5 min, followed by 30 PCR cycles of denaturation (95 °C for 10 s), annealing, (52−55 °C for 25 s), and extension (72 °C for 25 s). The PCR results were analyzed using Rotogene 6.0 software. 36B4 was used as the internal control. The relative mRNA expression of PEPCK, SREBP-1c, and FAS was normalized to 36B4 expression level in each sample. The primer sequences and the PCR conditions were depicted in Table 1.

Table 1 Primer sequences of tested genes

Genes	Primer sequences	GenBank ID
PEPCK	F: AGTCCCATCACTTCCTGGAAGA	18534
	R: GGTGCAGAATCGCGAGTT	
SREBP-1c	F: GGAGCCATGGATTGCACATT	14104
	R: AGGCCAGGGAAGTCACTGTCT	
FAS	F: GACCCTGACTCCAAGTTATTCGA	20787
	R: CGTCAAGCGGGAGACAGACT	
36B4	F: TTCCCACTGGCTGAAAAGGT	11837
	R: CGCAGCCGCAAATGC	

2.6 Statistical analysis

Results are presented as $\bar{x} \pm s$, and the comparison among groups was performed by One-way ANOVA, followed by paired-sample T-test to compare the mean values between normal control and after treatment. $P < 0.05$ was considered statistically significant.

3. Results

3.1 DEAE elution fraction of LBP and molecular weight of LBP-IV

The contents of carbohydrate and protein were 72.5% and 7.0% in crude LBP, and 89.8% and 5.9% in purified LBP, respectively. The color and other proteins removal rates were 85.4% and 40.5%, respectively. The separation of purified LBP by DEAE cellulose-52 column presented four elution fractions designated as LBP-I, LBP-II, LBP-III, and LBP-IV, which was eluted in turn by water, 0.05, 0.10, and 0.50 mol/L NaCl (Figure 1A). The contents of LBP-I−IV in total purified LBP were 9.2%, 17.9%, 15.0%, and 57.8%, respectively. No significant absorbance was observed at 260 nm in the UV spectrum. LBP I−IV were further separated by Sephadex G-100 column and only one elution fraction presented (Figure 1B). The average molecular weight of LBP-IV was calculated to be 23 000 based on its retention time and equation of the standard curve.

3.2 Hypoglycemic and hypolipidemic effects of LBP and LBP-IV in diabetic rats

The effects of LBP and LBP-IV on blood glucose and lipids of diabetic rats were shown in Table 2. The levels of HbA1c, blood glucose, TG, TC, and LDL-C of diabetic control group were markedly higher those of normal control group ($P < 0.01$). Treatment by LBP (100 mg/kg) and LBP-IV (200, 100, and 50 mg/kg) significantly decreased the levels of HbA1c, BG, TG, TC, and LDL-C of diabetic rats as compared to the diabetic control group ($P < 0.05$ or 0.01), whereas no obvious effect was found on serum HDL-C level. In addition, there was no significant difference about the levels of blood glucose and blood lipid between LBP and LBP-IV 100 groups ($P > 0.05$).

3.3 Effects of LBP and LBP-IV on glucose tolerance in diabetic rats

The effects of LBP and LBP-IV on oral glucose tolerance of rats were shown in Figure 2A (blood glucose *vs* time curve) and Figure 2B (area under the curve, $AUC_{0\text{-}120\ min}$). After ig administration of glucose (2 g/kg), the level of blood glucose and $AUC_{0\text{-}120\ min}$ of diabetic control group significantly increased compared to the normal control group ($P < 0.01$). Treatment by LBP and LBP-IV significantly decreased the blood glucose levels and $AUC_{0\text{-}120\ min}$ of diabetic rats compared to the diabetic control group ($P < 0.05$ or 0.01). No obvious difference about oral glucose tolerance was found between LBP and LBP-IV 100 groups ($P > 0.05$).

3.4 Effects on hepatic mRNA expression of PEPCK, SREBP-1c, and FAS in diabetic rats

The effects of LBP and LBP-IV on hepatic mRNA expression of *PEPCK, SREBP-1c*, and *FAS* in diabetic rats were shown in Figure 3. The hepatic mRNA expression levels of *PEPCK, SREBP-1c*, and *FAS* in the diabetic control group were significantly higher than those in the normal control group ($P < 0.01$). After the treatment of diabetic rats by LBP and LBP-IV once a day for continuous 28 d, the mRNA expression levels of the genes mentioned above were obviously

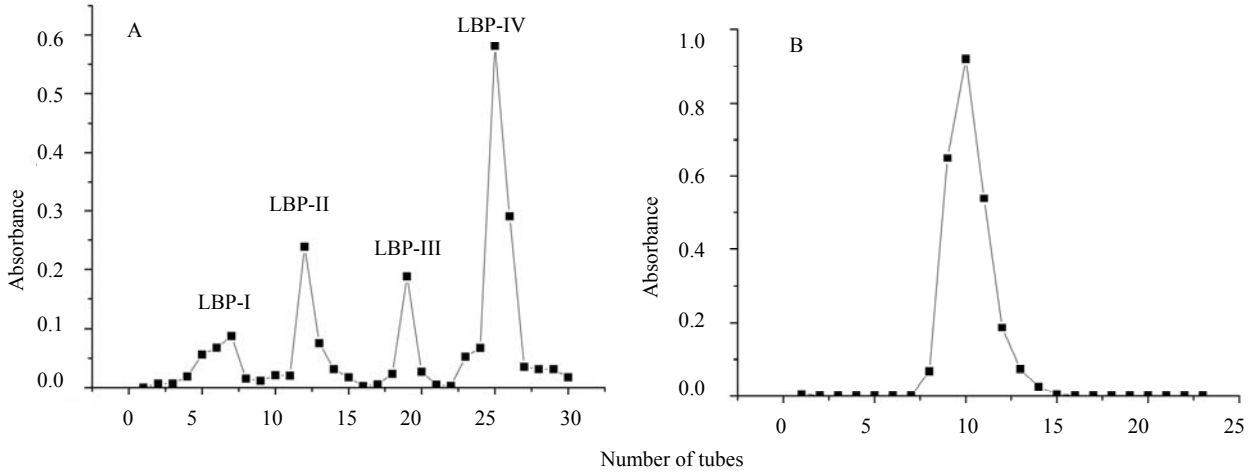

Figure 1 Elution profile of LBP on DEAE cellulose-52 column (A) and LBP-IV on Sephadex G-100 column (B)

A: eluent was distilled water, 0.05, 0.10, and 0.5 mol/L NaCl solution in turn with a flow rate of 5 mL/min (300 mL/tube);

B: eluent was distilled water with a flow rate of 0.5 mL/min (2 mL/tube).

Table 2 Effects of LBP and LBP-IV on blood glucose and lipids of diabetic rats

Groups	Doses/(mg·kg^{-1})	FBG/(mmol·L^{-1})	HbA1c/%	TG/(mmol·L^{-1})	TC/(mmol·L^{-1})	HDL-C/(mmol·L^{-1})	LDL-C/(mmol·L^{-1})
NC		3.96 ± 0.96	5.86 ± 0.45	0.96 ± 0.17	1.70 ± 0.19	1.69 ± 0.31	0.31 ± 0.12
DC		14.95 ± 3.08$^{\#\#}$	7.50 ± 0.64$^{\#\#}$	1.77 ± 0.10$^{\#\#}$	2.20 ± 0.19$^{\#\#}$	1.71 ± 0.34	1.37 ± 0.22$^{\#\#}$
LBP	100	10.12 ± 2.97$^{\#\#**}$	6.82 ± 0.56$^{\#\#**}$	1.25 ± 0.30$^{\#**}$	1.85 ± 0.32**	1.83 ± 0.44	0.34 ± 0.09**
LBP-IV	200	6.47 ± 1.89$^{\#**}$	6.44 ± 0.80$^{\#\#**}$	0.81 ± 0.19**	1.75 ± 0.40**	1.92 ± 0.51	0.33 ± 0.11**
	100	9.45 ± 1.60$^{\#\#**}$	6.66 ± 0.80$^{\#\#**}$	1.06 ± 0.24**	1.83 ± 0.24**	2.04 ± 0.36	0.35 ± 0.09**
	50	11.41 ± 2.25$^{\#\#*}$	7.21 ± 1.04$^{\#\#}$	1.20 ± 0.42**	1.94 ± 0.43	2.01 ± 0.37	0.35 ± 0.09**

$^{\#}P < 0.05$ $^{\#\#}P < 0.01$ DC *vs* NC; $^{*}P < 0.05$ $^{**}P < 0.01$ DC treated by LBP or LBP-IV *vs* DC; same as below

NC: normal control; DC: diabetic control; LBP: diabetic rats treated by 100 mg/kg LBP

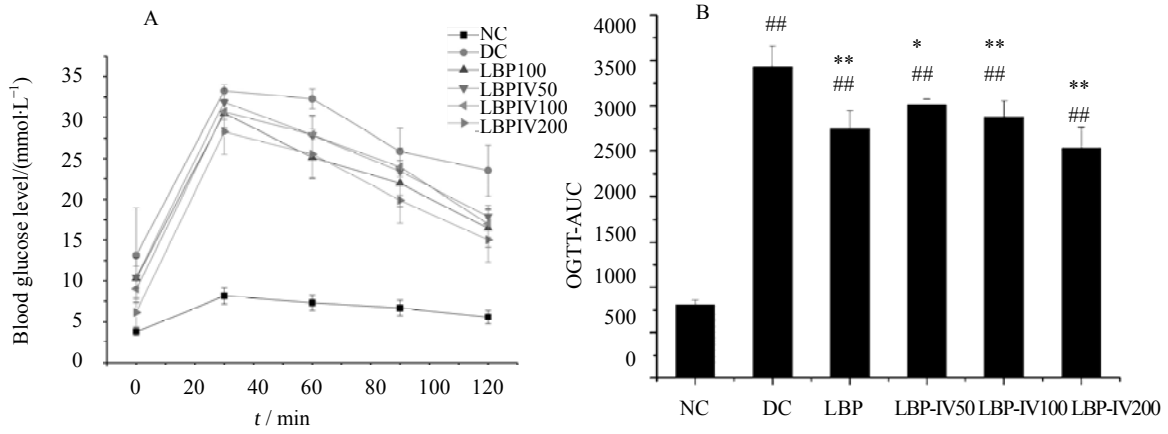

Figure 2 Blood glucose-time curves (A) and $AUC_{0\text{-}120\ min}$ (B) of rats after ig administration of LBP and LBP-IV ($\bar{x} \pm s$, $n = 8$)

decreased as compared to the diabetic control group ($P < 0.05$ or 0.01). And the inhibition of the purified LBP on the mRNA expression of *PEPCK*, *SREBP-1c* and *FAS* was greater than that of LBP-IV100 groups ($P < 0.05$).

4. Discussion

Both hyperglycemia and hyperlipidemia have been linked to diabetic mellitus (DM) and its complications. Some botanical polysaccharides were identified as bioactive components responsible for hypoglycemic activity and hypocholesterolemic effect (Kumar et al, 2005; Liu et al, 2013; Zhu et al, 2013). Polysaccharides are major chemical constituents of *L. barbarum* fruits (Luo et al, 2000). The present work demonstrated that the ig administration of LBP and LBP-IV not only significantly reduced the blood glucose and lipid (TG, TC, and LDL-C) levels, but also increased the glucose tolerance of diabetic rats induced by HFD and STZ significantly. The inhibition of LBP and LBP-IV on hyperglycemia and hyperlipidemia of diabetic rats suggested that both LBP and LBP-IV should be the effective extracts of *L. barbarum* to treat DM.

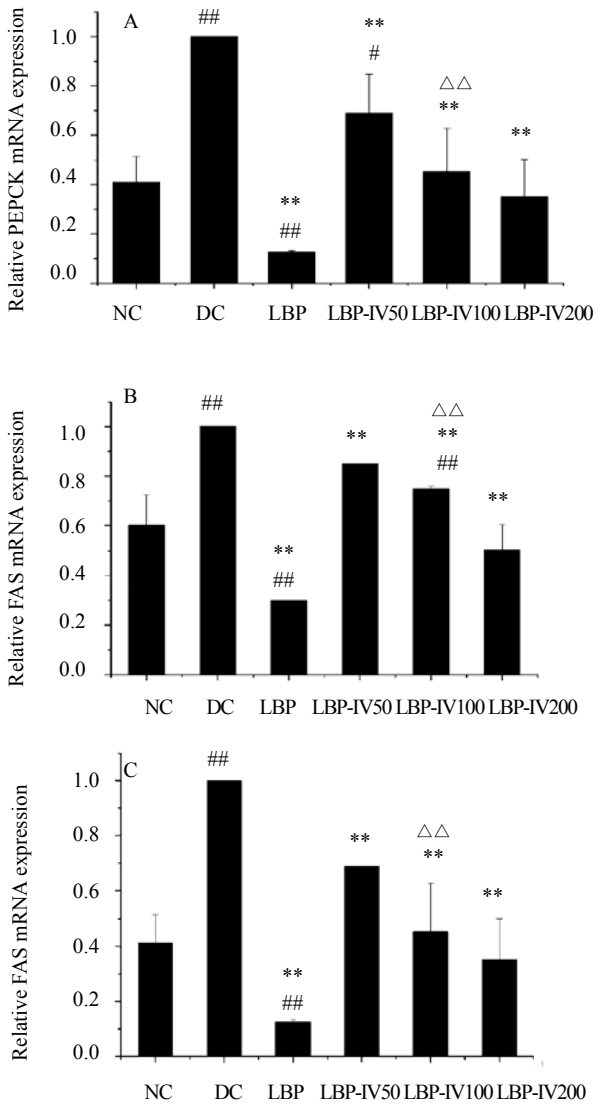

Figure 3 Effects on mRNA expression of PEPCK (A), SREBP-1c (B), and FAS (C) in rat liver after ig administration of LBP and LBP-IV ($\bar{x} \pm s$, $n = 8$)

△△ $P < 0.01$ DC treated by LBP-IV 100 *vs* DC treated by LBP

The liver is the major source of glucose when food supply is limited, through hepatic glycogenesis and gluconeogenesis. Abnormal activation of hepatic gluconeogenesis contributes to hyperglycemia (Sharma et al, 2011). It is well known that PEPCK is the rate-limiting enzyme of gluconeogenetic process. The expression of PEPCK in patients with type 2 DM was significantly increased, accompanied by the increase of gluconeogenesis, and further resulted in a higher FBG level (Dong et al, 2009; Rees et al, 2009). The present study demonstrated that LBP and LBP-IV significantly decreased the hepatic mRNA expression level of PEPCK in diabetic rats. Therefore, the results suggested that the hypoglycemic effects of LBP and LBP-IV in diabetic rats were associated with their inhibition on hepatic gluconeogenesis.

The transcriptional effect of insulin is mediated by sterol regulatory element binding protein-1c (SREBP-1c), which is a transcription factor from the basic-helix-loop-helix leucine zipper (bHLH/Zip) transcription factor family. SREBP-1c controls the synthesis of triglyceride and fatty acid via regulating the expression of lipogenic genes including fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Ferré and Foufelle, 2007; Ruth and Andre, 2004; Hitoshi, 2001). The present work indicated that the ig administration of LBP and LBP-IV significantly inhibited the hepatic mRNA expression levels of SREBP-1c and FAS in diabetic rats. This finding should be a possible hypolipidemic mechanism of LBP and LBP-IV in diabetic rats.

In the present work, we also compared the hypoglycemic and hypolipidemic effects of equal dosage of LBP and LBP-IV on diabetic rats. Although the inhibition of the purified LBP on mRNA expression of *PEPCK*, *SREBP-1c*, and *FAS* was greater than that of the equal dosage of LBP-IV, there was no significant difference in the levels of blood glucose and blood lipids of diabetic rats between LBP (100 mg/kg body weight) group and LBP-IV (100 mg/kg body weight) group. The results indicated that LBP could be used directly as a potential candidate for developing a new beneficial food or a new antidiabetic agent without further purification. However, further investigation will be required to explain why LBP have more powerful inhibition on the hepatic mRNA expression of PEPCK, SREBP-1c, and FAS in diabetic rats than its purified polysaccharide fraction (LBP-IV).

5. Conclusion

In the present work, we have found that ig administration of LBP and LBP-IV significantly could decrease the levels of blood glucose, HbA1c, TC, TG, and LDL-C, as well as the hepatic mRNA expression of *PEPCK*, *SREBP-1c*, and *FAS*, whereas significantly increase the oral glucose tolerance of diabetic rats. The findings suggest that the antidiabetic effect of LBP and LBP-IV in HFD-STZ-induced diabetic rats be associated with the decreased hepatic mRNA expression of *PEPCK*, *SREBP-1c*, and *FAS*. In addition, we have also found that the inhibition of the purified LBP on the mRNA expression of *PEPCK*, *SREBP-1c*, and *FAS* is greater than that of the equal dosage of LBP-IV, but there is no significant difference in the levels of blood glucose and blood lipids of diabetic rats between them. These results suggest that the purified LBP could be used directly as a potential candidate for developing a new beneficial food or a new antidiabetic agent without further DEAE separation.

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