



Structure characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Lycium barbarum* L.

Shan Zou¹, Xian Zhang¹, Wenbing Yao, Yuge Niu, Xiangdong Gao^{*}

School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Article history:

Received 4 December 2009

Received in revised form 18 January 2010

Accepted 20 January 2010

Available online 25 January 2010

Keywords:

Lycium barbarum L.

Polysaccharide

Structure analysis

Hypoglycemic activity

ABSTRACT

An acidic polysaccharide LBP-1 isolated from the fruit of *Lycium barbarum* L. was purified by ion-exchanged column. Structure characterization and *in vitro* hypoglycemic activity of LBP-1 were determined. Chemical analysis indicated LBP-1 was composed with rhamnose, arabinose, xylose, galactose, mannose, galacturonic acid = 1.00:7.85:0.37:0.65:3.01:8.16 with average molecular weight of 2.25×10^6 Da. The structure features of LBP-1 were investigated by FT-IR, GC-MS, ¹H-NMR and ¹³C-NMR. The backbone were mainly composed of (1,5)-linkage arabinose, (1,4)-linkage galacturonic acid, -(1)-mannose-(3,6)-linkage and terminated with -(1)-mannose. LBP-1 protected the pancreatic islets cells from oxidative damage and enhanced cell survival ratio significantly. Moreover, its application inhibited the development of insulin resistance in HepG2 cells. This paper indicates the beneficial effect of LBP-1 and explores a potential hypoglycemic functional food and pharmaceuticals with definitely structure.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Fruit from *Lycium barbarum* Linnaeus, commonly called “Wolfberry” in English, is a traditional Chinese herbal medicine, which is popular as a functional dietary supplement in Western countries now. Variety beneficial effects of *Lycium barbarum* L. have been demonstrated such as hypoglycemia, immunomodulation, anti-hypertension, lipotropic, anti-aging, antioxidant, anticancer, anti-apoptotic, anti-fatigue and so on (Chen, Tan, & Chan, 2008; Li, Ma, & Liu, 2007; Luo, Cai, Yan, Sun, & Corke, 2004). Several functional components including carotenoids, flavonoids and polysaccharides in *Lycium barbarum* L. berries correlated with their bioactivities have been chemically investigated. However, the polysaccharide constituents still remained uncertain because of its complex monosaccharide composition with a wide range molecular weight (M_w).

Diabetes mellitus is a significant chronic disease in modern society characterized by high blood glucose level. Given that currently available drugs for diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Yasunori et al., 2002), renewed attention to alternative medicines and natural therapies has stimulated a new wave of research inter-

est in traditional practices. The plant kingdom is a wide field to search for new drugs and biologically active compounds that have slight or no side effect. Previous publications indicated that some plant polysaccharide, isolated from *Cordyceps mycelia*, *Astragalus membtanaceus*, *Aloe vera*, Green tea, pumpkin and *Physalis sikekengi* L., were found to have hypoglycemic activity (Akira, Sahar, Amal, & Engy, 2009; Chen, Zhang, & Xie, 2005; Fu, Tian, Cai, Liu, & Li, 2007; Li et al., 2006; Mao et al., 2009; Tong, Liang, & Wang, 2008). The medicinal use of *Lycium barbarum* L. for curing diabetes was documented in Chinese medicinal monograph around 2300 years ago and the *in vivo* hypoglycemic effects of fruit water decoction, crude polysaccharides and purified polysaccharides from *Lycium barbarum* L. have been reported (Amagase, Sun, & Borek, 2009; Li, 2009; Luo et al., 2004). In recent studies, five polysaccharides (glycoconjugates) (LbGp1–LbGp5) were isolated with the molecular weight (M_w) ranged from 92,500 to 237,000 Da (Qi et al., 2001; Wang et al., 2009; Zhao, Alexeev, Chang, Greenburg, & Bojanowski, 2005). However, the macromolecular structure characterization has not been elucidated due to its complicated monosaccharide composition.

In this study, a new high-molecular-weight polysaccharide fraction from *Lycium barbarum* L. fruit (LBP-1) was purified, and its components and structure characterization were determined by chemical analysis. The hypoglycemic activity of LBP-1 was also investigated *in vitro* to provide scientific evidence for development

^{*} Corresponding author. Tel.: +86 025 8327 1298; fax: +86 025 8327 1249.

E-mail address: Xiangdong_gao@yahoo.com.cn (X. Gao).

¹ Both authors contributed equally to this paper.

of LBP-1 as a functional food and a potential alternative medicine in anti-diabetes with clearer structure characterization.

2. Materials and methods

2.1. Materials

The fruit bodies of *Lycium barbarum* L. were obtained from the Agriculture and Forestry Research Institute of Ningxia Huizu Autonomous Region, People's Republic of China. Trichloromethane, sodium hydroxide, sodium chloride, acetone, and hydrochloric acid were purchased from Nanjing chemical reagent Co. Ltd. Prind. Deuterated water (D_2O) and alloxan was purchased from Sigma-Aldrich (St. Louis, USA). Insulin and pioglitazone hydrochloride were produced by Novo Nordisk (North Carolina, USA) and Hengrui (Lianyungang, China), respectively. Other chemicals and reagents were analytical grade.

2.2. Cell line and culture

RINm5F (rat insulinoma cells line) and HepG2 cells (a human hepatoma cell line) were supplied from Chinese Academy of Sciences and maintained in monolayer culture at 37 °C and 5% CO_2 in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2 g/L $NaHCO_3$, 0.06 g/L penicillin and 0.1 g/L Streptomycin. At 90% confluence, the cells were trypsinized with 0.25% trypsin-0.02% EDTA in PBS solution for 30 s and resuspended in complete culture medium.

2.3. Extraction, isolation and purification

Ground dry fruit samples (100 g) were refluxed to remove lipids with chloroform methanol solution by using Soxhlet's extractor for 2 h. After filtering, the residues refluxed again with 80% ethanol for 2 h to remove oligosaccharide. Dry residual (20 g) were extracted three times in 80 °C hot water. The concentrated extract solution was precipitated by three times volume of 95% ethanol. The precipitate was collected by centrifugation, washed successively with ethanol and acetone, and then dried at reduced pressure, giving crude polysaccharides. Crude polysaccharide applied to the DEAE column (5 × 50 cm) and eluted with 0.4 M NaCl. Fractions were collected and enriched according the results of analysis by the phenol-sulfuric acid procedure (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The collected fraction was dialyzed in distilled water for 24 h and freeze-drying, one purified polysaccharide was obtained named LBP-1.

2.4. Characterization of LBP-1

2.4.1. Purity and molecular weight determination

The purity and molecular weight of LBP-1 was determined by size-exclusion HPLC chromatography instrument (Agilent1100, USA) with a gel-filtration chromatographic column of Shodex KS-805 (SHOWA DENKO K.K., Japan) at 35 °C. Sample was dissolved in distilled water and passed through from 0.45 μm filter, applied to gel-filtration column, eluted with distilled water at a flow rate of 1.0 ml/min and detected by a refractive index detector. Standard dextrans with different molecular (21,400, 41,100, 84,400, 133,800, 2,000,000 Da) were passed through the column, and a standard curve was plotted according to the retention time and the logarithm of their respective molecular weights. The molecular weight of LBP-1 was calculated by comparison to the standard curve.

2.4.2. Analysis of monosaccharide composition by HPLC with pre-column derivative and GC

PMP derivatization of monosaccharides was carried out as described previously with proper modification (Daotian & Roger, 1995; Lv et al., 2009). Briefly, 6 standard monosaccharides mixture solution or the hydrolyzed samples of LBP-1 were dissolved in 0.6 M aqueous NaOH (50 μl) and a 0.5 M methanol solution (100 μl) of PMP was added. Samples were allowed to react for 30 min at 70 °C, then cooled to room temperature and neutralized with 50 μl of 0.3 M HCl. The resulting solution was added with H_2O (1 ml) and extracted with chloroform (1 ml). The process was repeated three times and then aqueous layer was filtered through a 0.45 μm membrane. The analytical column used a RP-C18 column (4.6 mm × 250 mm, 5 μm , waters, USA). The wavelength for UV detection was 250 nm. Elution was carried out at a flow rate of 1.0 ml/min at 35 °C. The mobile phase was consisted of acetonitrile and ammonium acetate solution (pH 5.5) with the volume ratio of 78:22. The injection volume was 20 μl .

LBP-1 was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C for 8 h, turning into monosaccharide compositions (Susumu, Shigeo, Kazuaki, Akiko, & Tsuneo, 1981). The residual solution was concentrated, the excess of acid was removed by 1% hydrochloric acid methanol solution, and the residual were acetylated by acetic anhydride. The alditol acetates were analyzed by gas chromatography (GC) on a Hewlett-Packard model 6890 instrument equipped with a capillary column (HP-5.5% phenyl methyl siloxane 30 × 0.25 × 0.25 μm) and a flame-ionization detector, and temperature programmed from 150–220 °C to 280 °C at 30 °C/min.

2.4.3. Infrared spectral analysis

The infrared spectrum (IR), as KBr pellets, was recorded by a Nicolet-170X spectrophotometer in the range of 4000–400 cm^{-1} .

2.4.4. Methylation analysis

The methylation of LBP-1 (10 mg) was performed four times with methyl iodide in DMSO according to the Needs' method (Needs & Selvendran, 1993). Formic acid hydrolysis of methylated polysaccharide was performed by heating with formic acid (3 ml) for 6 h at 100 °C, and then the residual were hydrolyzed with 2 M trifluoroacetic acid (2 ml). Methylated products were reduced with $NaBH_4$ and acetylated with acetic anhydride. The resulting mixture of alditol acetates was analyzed by GC-MS. Linkages of partially methylated alditol acetates were carried out on the basis of the retention time and fragmentation pattern. The molar ratios of each sugar were calibrated using the peak areas and response factor of the flame-ionisation detector in GC.

2.4.5. Nuclear magnetic resonance spectroscopy

1H -NMR and ^{13}C -NMR spectrums were recorded by Bruker DRX-400NMR Spectrometer. LBP-1 was dissolved in D_2O and examined at 500 MHz 30 °C.

2.5. Hypoglycemic activity of LBP-1 in vitro

2.5.1. RINm5F proliferation assay

RINm5F cells, seeded in 96-well microplate with a density of 10^4 cells/ml and allowed to attach for 6 h at 37 °C. Each well was added 10^{-2} M alloxan to induce RINm5F damage. After 2 h damage, cells were treated in the absence or presence of different concentrations of LBP-1 (100, 200, 300, 400 and 500 $\mu g/ml$) for 24 or 48 h. Cell proliferation was measured by MTT assay. MTT solution (20 μl) was added to each well and incubated at 37 °C for 4 h. After adding stop solution DMSO (100 $\mu l/well$), the absorbance at 490 nm was measured by a Multiskan Spectrum Microplate Spectrophotometer (Thermo, Finland).

2.5.2. Glucose consumption assay

HepG2 cells, the human hepatocarcinoma cell line, cultured in 96-well microplate with density of 500 cells per well and maintained in Dulbecco's modified Eagle's medium containing 2% fetal calf serum, supplemented with 60 mg/L penicillin, 100 mg/L streptomycin, 3.57 g/L HEPES and 3.7 g/L NaHCO₃ in a humidified atmosphere with 5% CO₂ at 37 °C overnight before treatment. Subsequently, HepG2 cells incubated with or without insulin (5×10^{-7} M) for 16 h, washed once with serum-free medium for 20 min, and then treated with LBP-1 at different concentrations (1, 5, 10, 50 and 100 µg/ml) in the presence of 10^{-9} M insulin for 24 h. The glucose concentrations in cell culture supernatant of each group were determined by Glucose assay kit (Rongsheng, Shanghai) following the manufacturer's instruction. The result is expressed as glucose consumption (C_{cons}). Pioglitazone (8 mg/L) and medium were positive and negative control, respectively.

2.5.3. Statistical analysis

All values were expressed as mean \pm SD. Statistical significance was determined by analysis of variance (ANOVA) followed by Student's *t* test. $P \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Isolation and purification

The crude polysaccharide, a yield of 2.24%, was isolated from *Lycium barbarum* L. powder, with removed lipid and oligosaccharide. An ion-exchange chromatography was used for purifying crude polysaccharide and eluted with 0.4 M NaCl to yield one major peak. The homogeneous of LBP-1 as one symmetrical peak was shown on HPLC with a gel-filtration chromatographic column. No significant absorbance was shown at or near 280 nm in the UV-visible spectral profiles and the protein content was 5.67% determined by Bradford's method. Optical rotation value was +77.1° for LBP-1.

3.2. Molecular weight of LBP-1 and monosaccharide composition

HPLC was applied to determine the average molecular weight of LBP-1, which was estimated to be 2.25×10^6 Da in reference to standard dextrans. Concerning monosaccharides quantitative and qualitative determination, the use of GC is preferred because of its sensitivity. According to retention time of the alditol acetate derivatives in GC, LBP-1 consisted of five different monosaccharides, including rhamnose, arabinose, xylose, galactose, mannose and the molar ratio of 1.00:7.85:0.37:0.65:3.01 (Fig. 1). Fig. 2 showed galacturonic acid is a major component of LBP-1. The galacturonic acid content was 44.61%, determining colorimetrically by the 3,5-dimethylphenol method. Given the information above, we found that LBP-1 consisted of rhamnose, arabinose, xylose, galactose, mannose, galacturonic acid and the molar ratio of 1.00:7.85:0.37:0.65:3.01:8.16. The monosaccharide content is rhamnose 4.23%, arabinose 30.36%, xylose 1.43%, galactose 3.02%, mannose 13.97% and galacturonic acid 44.61%, respectively.

3.3. Structure characterization of LBP-1

IR spectrum of LBP-1 showed absorption bands at 3433, 2955, 2917, 2862, 1633, 1408, 1380, 1325, 1261, 1149–1018, 947 and 637 cm⁻¹. It exhibited a broadly-stretched intense peak at around 3433 cm⁻¹ characteristic of hydroxyl group. The two peaks at 2955 and 2862 cm⁻¹ were assigned to C–H stretching bands of CH₂. A weak stretching band at 2917 cm⁻¹ was ascribed to C–H₂ stretching vibration. Two strong bands at 1633 and 1408 cm⁻¹ were assigned to the absorbance of the deprotonated carboxylic group (COO⁻) (Manrique & Lajolo, 2002) and no absorbance band at 1700 cm⁻¹ which belonged to carboxylic acid groups indicated that the LBP-1 was predominantly in salt form. The absorption at 1325 cm⁻¹ was possibly due to non-symmetrical and symmetrical CH₃ bending, respectively. The band at 1261 cm⁻¹ was ascribed to non-symmetrical C–O–C stretching vibration. The bands in the region 1149–1018 cm⁻¹ were corresponded to C–O–C and C–O–H glycosidic linkage (Kacuráková, Capek, Sasinková, Wellner, &

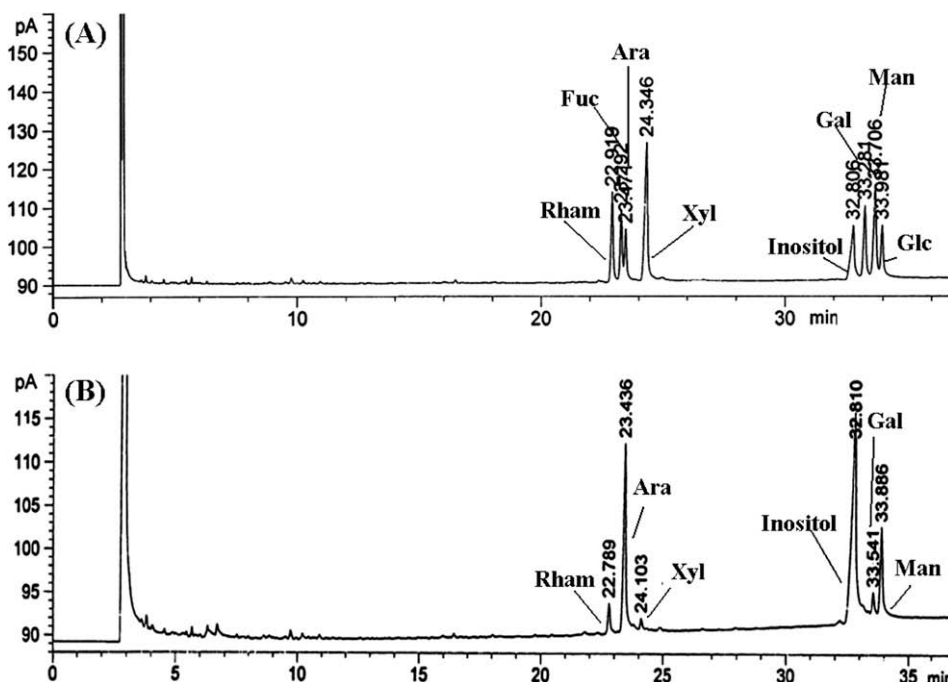


Fig. 1. GC chromatogram of monosaccharide composition of LBP-1. The upper profile (A) represents the monosaccharide standard. The bottom profile (B) represents the LBP-1 tested. LBP-1 is the purified high-molecular-weight polysaccharides from *Lycium barbarum* L. fruiting bodies.

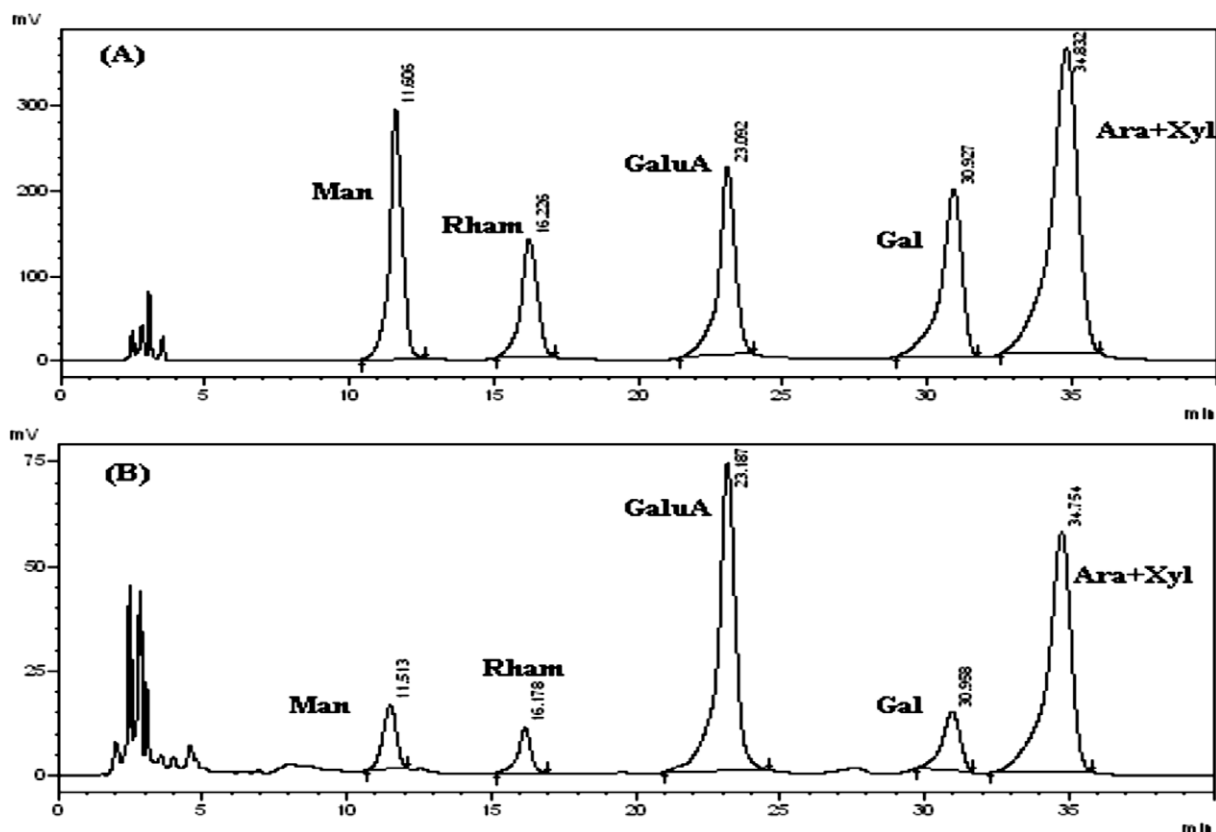


Fig. 2. The HPLC chromatograms of PMP derivatives of six standard monosaccharides (A) and component monosaccharides released from LBP-1 (B). The polysaccharide was hydrolyzed with TFA at 100 °C for 8 h and then was labeled with PMP. The HPLC analysis was carried out as described in the experimental section. Peaks: 1. mannose; 2. rhamnose; 3. galacturonic acid; 4. galactose; 5. arabinose and xylose.

Ebringerová, 2000; Zhao, Yang, Yang, Jiang, & Zha, 2007). The weak peak at 947 cm⁻¹ was characterized by deoxysaccharide C–H₂ stretching band and the peak at 637 cm⁻¹ resulted from O–H out-of-plane vibration (Chiovitti et al., 1997).

Methylation linkage analysis of LBP-1 was summarized in Table 1. Methylated product of LBP-1 was hydrolyzed with acid, converted into alditol acetates and analyzed by GC and GC–MS. According to mass spectrums, LBP-1 furnished three types of methylated derivatives, named 2,4-Me₂-Man, 2,3,4,6-Me₄-Man and 2,3-Me₂-Ara in a relative molar ratio of 1.00:0.29:2.25 (Zha, Luo, Luo, & Jiang, 2007). The signal of uronic acid was not shown in GC–MS analysis, which was due to the possibility that the uronic acid in LBP-1 have been transformed into methyl esters and subsequently degraded by β -elimination under highly alkaline environments in the repeated methylation in Needs' method (Jansson, Kenne, Liedgren, Lindberg, & Lönngrén, 1976; Yang & Montgomery, 2001). This result implied a good correlation between terminal and branched residues, and LBP-1 had a 1,5-linkage arabinose main chain with 1,6-linkage mannose and 1,3-linkage mannose as side chains in the native polysaccharide.

The analysis of the ¹H- and ¹³C-NMR spectra (Figs. 3 and 4) were based on component analysis, linkage analysis and

literature values. In the spectrums of LBP-1, signal at around δ 2.20 (¹H-NMR) and δ 36.00 ppm (¹³C-NMR) was assigned to the internal acetone in D₂O solvent. The ¹H-NMR showed corresponding to methyl protons at 1.16 and 1.23 ppm and the other proton signal was assigned the α -D-galacturonic acid (GaluA) H-1 at 5.05 ppm (Habibi et al., 2005; Velasco et al., 1998). The chemical shifts from 3.75 to 4.39 ppm, showing the overlapping peaks, were assigned to protons of carbons C-2 to C-5 (or C-6) of the glycosidic ring. From the ¹³C-NMR, the main (1 \rightarrow 5)-linkage- α -L-arabinose units were obviously characterized by five strong signals at 110.27, 79.49, 75.72, 83.57 and 69.62 ppm, which originated from C-1, C-2, C-3, C-4 and C-5 (Golovchenko, Ovodova, Shashkov, & Ovodov, 2002). The signals identified at 101.68, 70.89, 71.61 and 80.64 ppm could be assigned to C-1, C-2, C-3, C-4 and C-5 of (1 \rightarrow 4)-linkage- α -D-galacturonic acid. The signal at 178.15 ppm was due to C-6 of α -D-galacturonic acid (Polle, Ovodova, Shashkov, & Ovodov, 2002).

The NMR signal of rhamnose, xylose and galactose was no indication owing to their low content (less than 5%), and the overwhelmed NMR signal of mannose by baseline noise may be attributed to poor water solubility of LBP-1. The shift of C-4 from 70.30 to 80.64 ppm (C-4) indicated that there might be existence of (1 \rightarrow 4)-linkage- α -D-galacturonic acid and the signal shift of

Table 1
GC–MS of alditol acetate derivatives from the methylated production of LBP-1.

Methylated sugar	Molar ratios	MS main fragments (m/z)	Linkages
2,3,4,6-Tetra-O-Me-Man	0.29	43, 45, 71, 87, 101, 115, 129	Man-(1 \rightarrow
2,4-Di-O-Me-Man	1.00	43, 58, 71, 87, 99, 117, 129, 159, 173, 189, 233	\rightarrow 3,6)-Man-(1 \rightarrow
2,3-Di-O-Me-Ara	2.25	43, 87, 101, 117, 129, 189	\rightarrow 5)-Ara-(1 \rightarrow

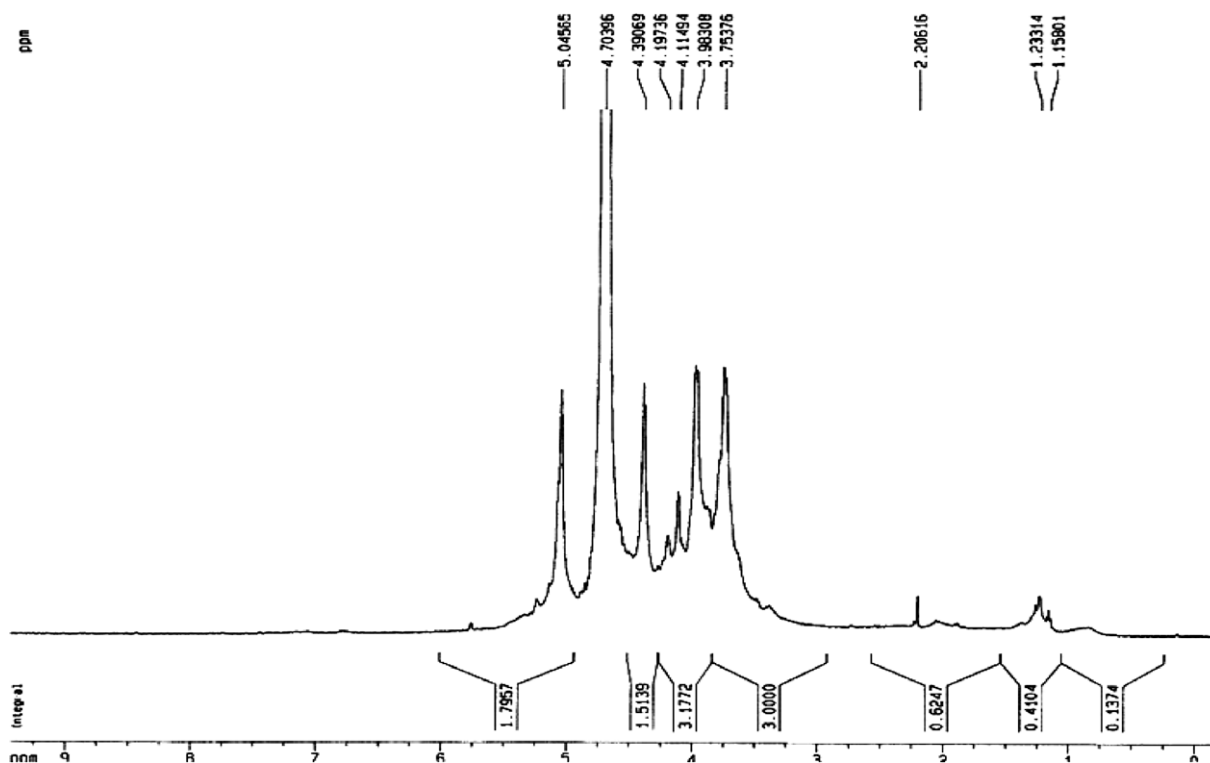


Fig. 3. ^1H -NMR spectrum of LBP-1. LBP-1 from the fruits of *Lycium barbarum* L. in D_2O on a Varian 500 NMR spectrometer using internal acetone (δ_{H} 2.20) as references. Numerical value is in d (ppm).

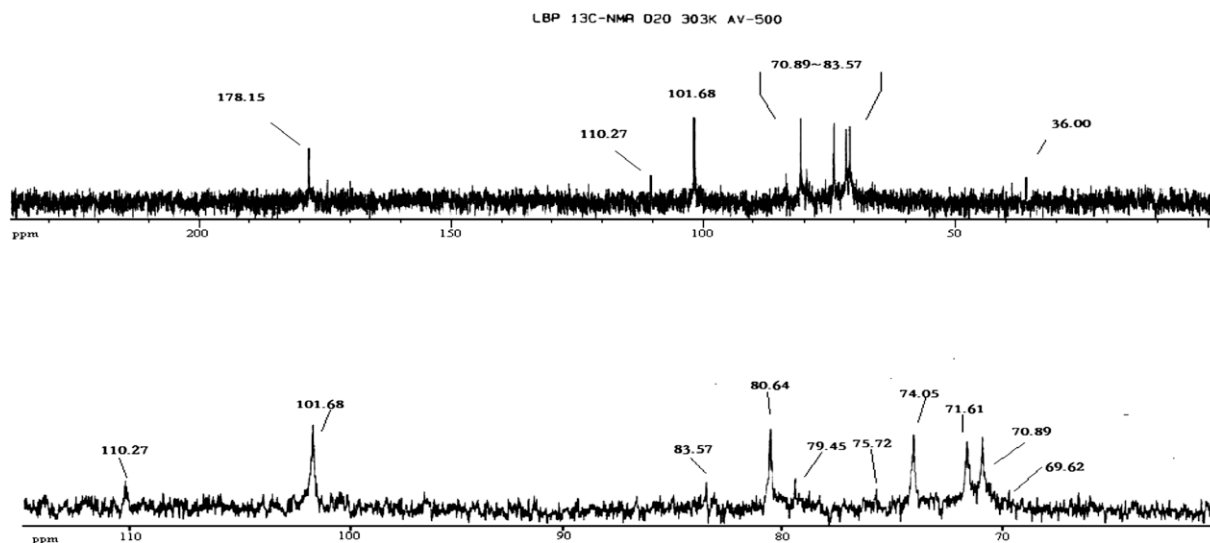


Fig. 4. ^{13}C -NMR spectrum of LBP-1. LBP-1 from the fruits of *Lycium barbarum* L. in D_2O on a Varian 500 NMR spectrometer using internal acetone (δ_{C} 36.00) as references. Numerical value is in d (ppm).

arabinose C-5 proved α -L-arabinose was (1 \rightarrow 5)-linkage (Omaira, Maritza, Lilian, & Gladys, 2005). The molar ratio of -(1)-mannose-(3,6)- and -(1)-mannose was 1.00:0.29, which demonstrated the mannose was not only terminal sugar from methylation.

In conclusion, the LBP-1 had a backbone chain mainly composed of (1,5)-linkage- α -L-arabinose and possibly (1,4)-linkage- α -D-galacturonic acid with branch chain of -(1)-mannose-(3,6)-. The terminal sugar was mainly -(1)-mannose and other monosaccharide.

3.4. Hypoglycemic activity of LBP-1 in vitro

Diabetes is complicated metabolic disorder characterized by high blood glucose level due to decreasing of utilized glucose in blood by body cells. The causes of diabetes are multiple. The supply of insulin may be decreased by a decrease in pancreatic β cell mass and/or function disturbances of β cells (Yasunori et al., 2002). Alloxan (AXN) chemical agent have damaging effect on insulin producing β cell of pancreas due to superoxide free radical causing

lipid peroxidation, damaging the structure of cell membrane, and leading to high glucose level by hypoinsulinism.

As a first step towards understanding the mechanism of the hypoglycemic activity of LBP-1, we investigated its protective effect against AXN-induced RINm5F cells damage *in vitro*. Fig. 5 showed LBP-1 had protection effect on RINm5F damaged by AXN, with dose-dependent manner at the concentrations from 0 to 500 $\mu\text{g/ml}$ LBP-1 significantly enhanced the cell survival ratio to 97.5% and 126.4% after 24 h and 48 h compared with model survival ratio to 67.0% and 72.0%. The result implied that the polysaccharide LBP-1 can nearly rehabilitate the pancreatic islets cells completely after damage, and even reverse them to the normal state. The result suggest that LBP-1 may decrease high glucose level by protecting pancreatic β cells against disturbances or increasing the number of β cells to raise insulin secretion.

To further elucidate the hypoglycemic effect of LBP-1 and its mechanisms, we simulated the internal hyperglycemia state *in vitro* to investigate whether LBP-1 is a potential component to alleviate insulin resistance partially which is ubiquitous in type 2 diabetes. Insulin resistance (IR) is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from liver cells, muscle and fat. Different cells respond to insulin differently. Some cells are more resistant than others, as some cells are incapable of becoming very resistant. The liver becomes resistant first, followed by the muscle tissue and lastly the fats. Insulin resistance in liver cells results in impaired glycogen synthesis and a failure to suppress glucose production (Xie et al., 2006). In our experiments, we used HepG2, a human hepatoma cell line, with high concentration insulin (5×10^{-7} M) to establish an insulin-resistant cell model, which expressed an insensitive response to normal-concentration insulin (10^{-9} M). Fig. 6 showed high-concentration insulin-treated HepG2 cells suffered an obvious glucose consumption decrease compared with the normal cells ($P \leq 0.01$), showing the insulin-resistant model was simulated successfully *in vitro*. After we treated the IR cells with LBP-1 for 24 h,

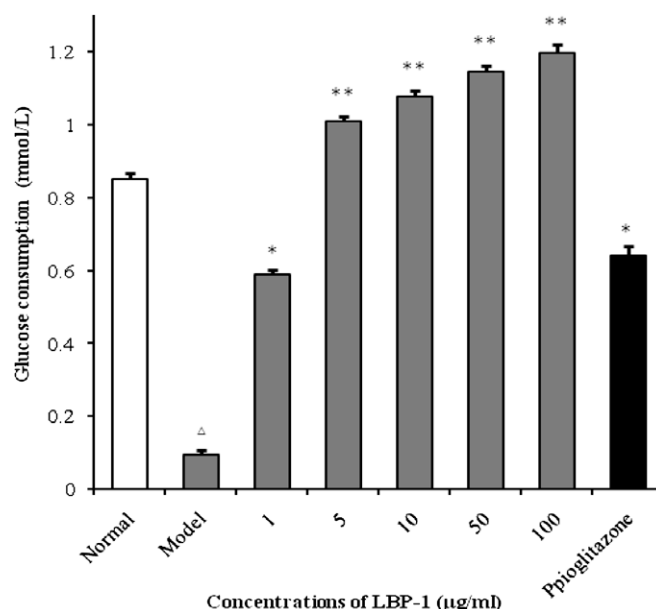


Fig. 6. Effect of LBP-1 on sensitivity to exogenous insulin in insulin-resistant HepG2 cells. The cells were pretreated with 5×10^{-7} M insulin to simulate IR micro-environment. LBP-1 was added at different concentrations (1, 5, 10, 50 and 100 $\mu\text{g/ml}$) in the presence of 10^{-9} M insulin for 24 h. The glucose concentrations in cell culture supernatant of each group were determined by Glucose assay kit and the absorbance at 505 nm was measured in a multiskan spectrum. The result was expressed as glucose consumption. $\Delta P \leq 0.01$ vs. normal control (without insulin and LBP-1 treatment); $*P \leq 0.05$, $**P \leq 0.01$ vs. model control (without LBP-1 treatment) by ANOVA followed by Student's *t*-test.

the glucose consumption increased significantly in a dose-dependent manner at the concentrations from 1 to 100 $\mu\text{g/ml}$. The results suggest that LBP-1 can make the IR cells more sensitive to the normal insulin level resulting in the increasing glucose consumption in culture supernatant.

The experiment results demonstrated that LBP-1 can protect the pancreatic islets cells in RINm5F cells and alleviate the insulin resistance in HepG2 cells, which may contribute to one of its hypoglycemic activities.

4. Conclusion

The acidic polysaccharide LBP-1 with the molecular weight of 2.25×10^6 Da was consisted of rhamnose, arabinose, xylose, galactose, mannose, galacturonic acid in the molar ratio of 1.00:7.85:0.37:0.65:3.01:8.16. It had a backbone composed of (1,5)-linkage α -L-arabinose and possibly (1,4)-linkage α -D-galacturonic acid with branch chain of -(1)-mannose-(3,6)-linkage and main terminal sugar of -(1)-mannose. *In vitro* hypoglycemic effect assay indicated that LBP-1 may decrease high glucose level by protecting pancreatic β cells from oxidative damages and alleviating the insulin resistance on liver cells. Hypoglycemic activity of *Lycium barbarum* L. fruit may be correlative with polysaccharides, a mainly functional composition, considered as a potential candidate for developing a new beneficial food or a new anti-diabetic agent. The correlation between structure and hypoglycemic activity of LBP-1 and increasing water solubility will be further investigated in future work.

Acknowledgements

This research was supported by grants from the National Natural Science Foundation of China (Grant Nos. 30672479 and

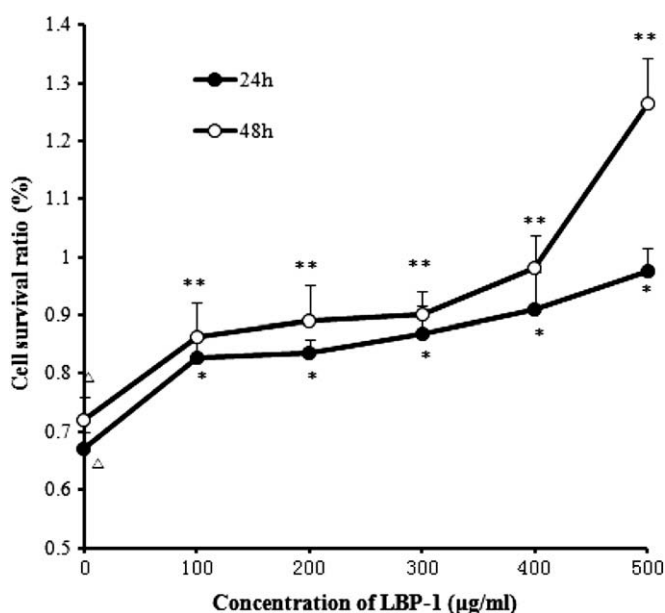


Fig. 5. Protective activity of LBP-1 against AXN-induced RINm5F cells damage *in vitro*. RINm5F cells were damaged by AXN (10 mmol/L) and subsequently stimulated with LBP-1 (from 0 to 500 $\mu\text{g/ml}$) for 24 h and 48 h, respectively. Proliferation was measured by MTT assay and the absorbance at 490 nm was measured in a multiskan spectrum. The protective effect of LBP-1 was expressed as cell survival ratio = A_{490} of each group/ A_{490} of normal group. Values are the mean \pm SD, ($n = 6$). $*P \leq 0.05$, $**P \leq 0.01$ vs. medium control, $\Delta P \leq 0.05$ vs. normal control by ANOVA followed by Student's *t*-test.

30873201), and a specialized Research Fund for the Doctoral Program of Higher Education (No. 20060316001).

References

- Akira, Y., Sahar, H., Amal, K., & Engy, A. W. (2009). Possible hypoglycemic effect of *Aloe vera* L. high molecular weight fractions on type 2 diabetic patients. *Journal of the Saudi Pharmaceutical Society*, 16, 209–215.
- Amagase, H., Sun, B. X., & Borek, C. (2009). *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nutrition Research*, 29, 19–25.
- Chen, Z. S., Tan, B. K. H., & Chan, S. H. (2008). Activation of T lymphocytes by polysaccharide–protein complex from *Lycium barbarum* L.. *International Immunopharmacology*, 8, 1663–1671.
- Chen, H. X., Zhang, M., & Xie, B. J. (2005). Components and antioxidant activity of polysaccharide conjugate from green tea. *Food Chemistry*, 90, 17–21.
- Chiovitti, A., Bacic, A., Craik, D. J., Munro, S. L. A., Kraft, G. T., & Liao, M. L. (1997). Cell-wall polysaccharide from Australian red algae of the family *Solieriaceae* (Gigartinales, Rhodophyta): Novel, highly pyruvated carrageenans from the genus *Callophycus*. *Carbohydrate Research*, 299, 229–243.
- Daotian, F., & Roger, A. (1995). Monosaccharide composition analysis of oligosaccharides and glycoproteins by high-performance liquid chromatography. *Analytical Biochemistry*, 227, 377–384.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350–356.
- Fu, C. L., Tian, H. J., Cai, T. Y., Liu, Y., & Li, Q. H. (2007). Some properties of an acidic protein-bound polysaccharide from the fruit of pumpkin. *Food Chemistry*, 100, 944–947.
- Golovchenko, V. V., Ovodova, R. G., Shashkov, A. S., & Ovodov, Y. S. (2002). Structural studies of the pectic polysaccharide from duckweed *Lemna minor* L.. *Phytochemistry*, 60, 89–97.
- Habibi, Y., Mahrouz, M., & Vignon, M. R. (2005). Isolation and structural characterization of protopectin from the skin of *Opuntia ficus-indica* prickly pear fruits. *Carbohydrate Polymers*, 60, 205–213.
- Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B., & Lönngren, L. (1976). A practical guide to the methylation analysis of carbohydrates. *Chemical Communication*, 18, 1–74.
- Kacuráková, M., Capek, P., Sasinková, V., Wellner, N., & Ebringerová (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, 43, 195–203.
- Li, X. M. (2009). Protective effect of *Lycium barbarum* polysaccharide on streptozotocin-induced oxidative stress in rats. *International Journal of Biological Macromolecules*, 40, 461–465.
- Li, X. M., Ma, Y. L., & Liu, X. J. (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology*, 111, 504–511.
- Li, S. P., Zhang, G. H., Zeng, Q., Huang, Z. G., Wang, Y. T., Dong, T. T. X., et al. (2006). Hypoglycemic activity of polysaccharide, with antioxidant, isolated from cultured *Cordyceps mycelia*. *Phytomedicine*, 13, 428–433.
- Luo, Q., Cai, Y. Z., Yan, J., Sun, M., & Corke, H. (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sciences*, 76, 137–149.
- Lv, Y., Yang, X. B., Zhao, Y., Ruan, Y., Yang, Y., & Wang, Z. Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection. *Food Chemistry*, 112, 742–746.
- Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology*, 25, 99–107.
- Mao, X. Q., Yu, F., Wang, N., Wu, Y., Zou, F., Wu, K., et al. (2009). Hypoglycemic effect of polysaccharide enriched extract of *Astragalus membranaceus* in diet induced insulin resistant C57BL/6J mice and its potential mechanism. *Phytomedicine*, 16, 416–425.
- Needs, P. W., & Selvendran, R. R. (1993). Avoiding oxidative degradation during sodium hydroxide/methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydrate Research*, 245, 1–10.
- Omaira, G., Maritza, M., Lilian, S., & Gladys, L. (2005). 1D- and 2D-NMR spectroscopy studies of the polysaccharide gum from *Spondias purpurea* var. *Lutea*. *Food Hydrocolloids*, 19, 37–43.
- Polle, A. Y., Ovodova, R. G., Shashkov, A. S., & Ovodov, Y. S. (2002). Some structural features of pectic polysaccharide from tansy, *Tanacetum vulgare* L.. *Carbohydrate Polymers*, 49, 337–344.
- Qi, C. H., Huang, L. J., Zhang, Y. X., Tian, G. Y., Ru, X. B., & Shen, B. F. (2001). Chemical structure and immunoactivity of the glycoconjugates and their glycan chains from the fruit of *Lycium barbarum* L.. *Chinese Journal of Pharmacology and Toxicology*, 15, 185–189 (in Chinese, with in English abstract).
- Susumu, H., Shigeo, S., Kazuaki, K., Akiko, H., & Tsuneo, T. (1981). Analysis of the monosaccharide compositions of total non-dialyzable urinary glycoconjugates by the dithioacetal method. *Journal of Chromatography*, 226, 341–350.
- Tong, H. B., Liang, Z. Y., & Wang, G. Y. (2008). Structural characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Physalis alkekengi* L.. *Carbohydrate Polymers*, 71, 316–323.
- Velasco, J., Moll, H., Knirel, Y. A., Sinnwell, V., Moriyón, I., & Zahringer, U. (1998). Structural studies on the lipopolysaccharide from rough strain of *Ochrobactrum anthropi* containing a 2,3-diamino-2,3-dideoxy-D-glucose disaccharide lipid A backbone. *Carbohydrate Research*, 306, 283–290.
- Wang, C. C., Chang, S. C., & Chen, B. H. (2009). Chromatographic determination of polysaccharides in *Lycium barbarum* Linnaeus. *Food Chemistry*, 116, 595–603.
- Xie, W. D., Wang, W., Su, H., Xing, D. M., Pan, Y., & Du, L. J. (2006). Effect of ethanolic extracts of *Ananas comosus* L. leaves on insulin sensitivity in rats and HepG2. *Comparative Biochemistry and Physiology Part C*, 143, 429–435.
- Yang, B. Y., & Montgomery, R. (2001). B-Elimination of glucosyluronic residues during methylation of an acidic polysaccharide from *Erwinia chrysanthemi* CU 643. *Carbohydrate Research*, 332, 317–323.
- Yasunori, K., Yasuhiko, I., Masashi, K., Kishiho, N., Akira, S., Yutaka, S., et al. (2002). Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Research and Clinical Practice*, 55, 65–85.
- Zha, X. Q., Luo, J. P., Luo, S. Z., & Jiang, S. T. (2007). Structure identification of a new immunostimulating polysaccharide from the stems of *Dendrobium huoshanense*. *Carbohydrate polymers*, 69, 86–93.
- Zhao, H., Alexeev, A., Chang, E., Greenburg, G., & Bojanowski, K. (2005). *Lycium barbarum* glycoconjugates: Effect on human skin and cultured dermal fibroblasts. *Phytomedicine*, 12, 131–137.
- Zhao, M. M., Yang, N., Yang, B., Jiang, Y. M., & Zha, G. H. (2007). Structural characterization of water-soluble polysaccharides from *Opuntia monacantha* cladodes in relation to their anti-glycated activities. *Food Chemistry*, 105, 1480–1486.