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Structural Characterization and Hypolipidemic Activity of a Polysaccharide PGEB-3H from the Fruiting Bodies of *Gastrodia elata* Blume

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Abstract

The purified *Gastrodia elata* Blume polysaccharide (PGEB-3H), was found to be a glucan with a molecular weight of 28.8 kDa and specific rotation of +206.3 (ca.0.003, H_2O). The structural characterics of PGEB was investigated by chemical methods (partial hydrolysis with acid, methylation reaction, periodate oxidation, and Smith degradation) and instrument analysis (IR and NMR). PGEB-3H was mainly composed by glucose, and had a (1→4)-α-D-glucan main chain occasionally branched with α-1,6 glycosidic linkage. PGEB-3H exhibited potential lipid-lowering effects in hyperlipidemia rats.

Keywords: *Gastrodia elata* Blume; Polysaccharide; Structure; Hypolipidemic activity

1 Introduction

Gastrodia elata Blume has been used in China as a traditional herbal medicine for nearly 2000 years [1]. There are many studies focused on the active ingredients from *Gastrodia elata* Blume regarding of

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extraction and bioactivities. An, et al. found that *Gastrodia elata* Blume significantly protects the gastric mucosa against water immersion restraint-induced gastric damage, at least in part by decreasing nitric oxide levels via suppression of inducible nitric oxide synthase mRNA expression [2]. Meantime, it attracts much attention from commerce and has been developed into an important class of healthy foods, e.g., beverages [3] and bread [4].

With the technological advance and improvement of life quality, changes in diet make the public trend to have high blood cholesterol and high blood pressure, which are characterized by high blood sugar diabetes, obesity, and hyperlipidemia. Hyperlipidemia is a risk factor for coronary heart disease, which is reported to be a major cause of deaths in several parts of the world and have been found to be unusually high in Asian populations [5]. In China, coronary heart disease has become the second leading cause of cardiovascular death. High level of blood cholesterol is believed to be a major contributing factor for heart disease [6].

Although many studies have conducted on *Gastrodia elata* Blume, the report of the structure of PGEB-3H and its hypolipidemic activity is limited. Thus, the aim of the present work was to investigate the isolation and structural characterization of PGEB-3H, a nonstarchy polysaccharide from *Gastrodia elata* Blume. Further, the potential hypolipidemic activity of PGEB-3H on a hyperlipidemia model of rats was examined, too.

2. Materials and methods

2.1. Materials

The dry bodies of *Gastrodia elata* Blume were supplied by the Kunming Research Institute of Edible Mushroom. T-series Dextran, DEAE-Cellulose A52, and Sephadex G-100 were purchased from Pharmacia Co. Trifluoroacetic acid (TFA) and monosaccharides were from E. Merck.

2.2. Preparation and structural analysis of PGEB-3H

PGEB-3H was prepared according to the method reported by Zhao [7] with a recovery of 0.797 g/kg. Paper chromatography (PC) and gas chromatography (GC) were used for identification and quantification of monosaccharide composition in PGEB-3H. The analysis procedure was performed as done by Zhao [7]. Uronic acid content was determined according to an *m*-hydroxydiphenyl colorimetric method [8]. Optical rotation was measured using a polarimeter (Model WZZ-2S). IR was recorded with a FT-Jh R10-01-A spectrophotometer. The molecular weight was determined by the HPGPC method. Methylation analysis, periodate oxidation–Smith degradation, and ^{13}C NMR were carried out as described by Gorin [9]. Molecular mechanics (MM2) was used to build the 3D-structure of PGEB-3H .

2.3. Animal test

The rats (220 ± 15 g) were housed at 25 ◦C for 10 days and then randomly assigned into five groups (6 rats/group). Normal control group were received standard laboratory diet and other groups were administered with cholesterol-enriched diet for consecutive 4 weeks. The animals had free access to food and water. PGEB-3H were fed to test groups at doses of 100, 200, 400 $mg \cdot kg^{-1} \cdot d^{-1}$. At the end of the experiment, rats were fastened for 10 h and blood samples were collected to prepare serum for the analysis of TC, TG, LDL-C and HDL-C.

2.4. Statistical analysis

Duncan test was performed by using SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA). Significant differences were set at $P < 0.05$ and $P < 0.01$.

3. Results and discussion

3.1. Physico-chemical characteristics of PGEB-3H

PGEB-3H was eluted as a symmetrical narrow peak on HPGPC with a molecular weight of 28.8 kDa. It had a specific rotation of +206.3 (ca.0.003, H_2O) was free of protein and uronic acid.

3.2. Structural features of PGEB-3H

Table 1 lists the glycosidic linkages of these monosaccharide residues. Only three types of glycosidic linkage were found. Compared with the reference [10], the result indicated that PGEB-3H was mainly consisted of 1,4-linked glucose and 1,4,6-linked glucose with an approximate molar ratio of 20:1.

Table 1. GC-MS of alditol acetate derivatives from the methylated production of PGEB-3H.

Methylated sugar	Molar ratio	Retention time (min)	MS main ion peaks (m/z)	Linkages
2,3,4,6-Me_4-Glc	1	9.13	43,45,71,87,101,117,129,145,161,205	Glc-(1→
2,3,6-Me_3-Glc	20	9.77	43,45,87,99,101,113,117,233	→4)-Glc-(1→
2,3-Me_2-Glc	1	10.58	43,101,117,261,	→4,6)-Glc-(1→

The FT-IR spectrum of PGEB-3H was shown in Fig.1. The band in the region of 3434.7 cm^{-1} is due to the hydroxyl stretching vibration of the polysaccharide. The band in the region of 2927.1 cm^{-1} is due to C-H stretching vibration and the band in the region of 1637.4 cm^{-1} is due to associated water. Absorption at 931.0 cm^{-1} was typical for D-glucose in pyranose form [11]. The high positive value of the specific rotation and the characteristic absorption at 855.9 cm^{-1} in the IR spectrum were indicative of α-glycosidic linkages between individual glucosyl residues existing in PGEB [12]. The absorptions at 1027.0, 1079.6, and 1153.2 cm^{-1} also indicated a pyranose form of the glucosyl residue.

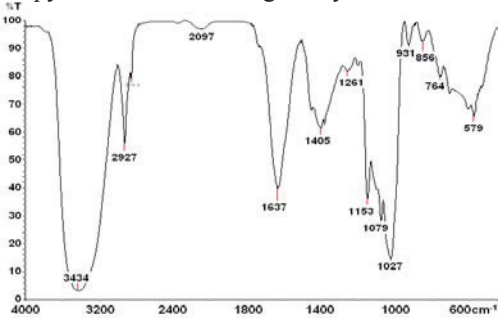

Fig.1. IR spectrum of PGEB-3H.

The ^1H NMR and ^{13}C NMR spectroscopy of PGEB-3H were shown in Fig.2. Based on the data available in the literatures, the resonance at 99.77 ppm was attributed to the anomeric carbon atoms of

glucopyranose, which also indicated that individual glucosyl residues in PGEB-3H were connected by α-glycosidic linkages. The resonances at 74.16, 73.33, 76.96, 76.18, and 60.62 ppm were assigned to C-2, C-3, C-4, C-5, and C-6 of the 1,4-linked glucosylresidues, respectively [13].

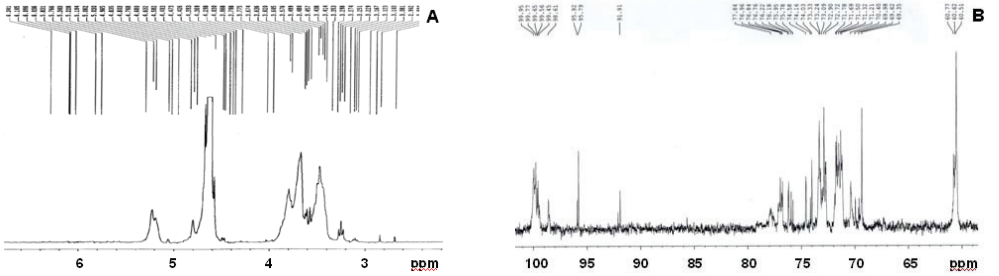

Fig.2. ^{1}H-NMR (A) and ^{13}C-NMR (B) spectrum of PGEB-3H.

In periodate oxidation test, the HIO_4 consumption and formic acid production were 1.34 mol/mol sugar residue and 0.049 mol/mol sugar residue, respectively, which was in agreement with the theoretically calculated values (1.05 mol/mol for HIO_4 and 0.045 mol/mol for formic acid) on the basis of the structural features described above. It was also suggested that sugar residues in PGEB-3H are in the IHO_4-oxidized linkages, namely 1-, 1,4-, and 1,4,6-linkage.

From the above-discussed information, the following repeating unit of PGEB-3H was established:

$$\mathrm{Glcp^{1}\text{-}\,[\text{-}(\text{-}\alpha\text{-}D\text{-}{}^{4}Glcp^{1})_{n}\text{-}\,{}^{4}Glcp^{1})\text{-}]_{x}}$$

$$\uparrow 6$$

$$\mathrm{Glcp^{1}\text{-}(\text{-}\alpha\text{-}D\text{-}{}^{4}Glcp^{1})_{m}} \quad (m+n=20)$$

The three-dimensional structure of PGEB-3H was shown in Fig.3. As can be seen from Fig.3, both partial and irregular spiral structure existed in the advanced structure of PGEB-3H.

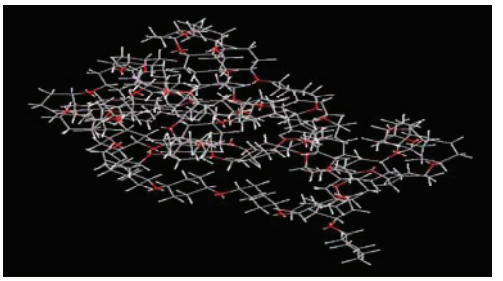

Fig.3. 3D structure of PGEB-3H.

3.3. Hypolipidemic activity of PGEB-3H

The serum lipid profile of rats is shown in Table 2. TC was almost the same in all groups, except normal and 100 mg/kg PGEB-3H group. This may due to immunosuppressive effect of high dose of PGEB-3H. For serum TG, significant differences were observed between high fat control and PGEB-3H groups. TG decreased with an increase in dose from 100 mg/kg to 400 mg/kg. There was no significant difference in LDL-C level between high fat control and test groups. However, administration of middle dose of PGEB-3H caused a 29% increase in HDL-C.

4. Conclusion

PGEB-3H had a molecular weight of 28.8 kDa and was only comprised of glucose. It had a back bone of →4)-α-D→Glu→(1 and occasionally branched with α-1,6 glycosidic linkage. The IR and NMR spectra confirmed its α form. PGEB-3H exhibited potential lipid-lowering effects in hyperlipidemia rats.

Table 2. Effect of PGEB-3H on serum lipid profile of hyperlipidemia rats

Hypolipidemic index	Normal control	High fat control	PGEB-3H groups		
			(100 mg/kg)	(200 mg/kg)	(400 mg/kg)
TC (mmol·L^{-1})	1.65±0.13^{d}	6.57±0.28^{ab}	4.64±0.65^{c}	7.08±0.18^{a}	5.96±0.65^{b}
TG (mmol·L^{-1})	0.55±0.10^{e}	1.24±0.08^{a}	0.81±0.04^{b}	0.59±0.02^{bc}	0.43±0.01^{c}
HDL-C (mmol·L^{-1})	0.77±0.06^{a}	0.58±0.00^{b}	0.63±0.04^{b}	0.80±0.04^{a}	0.66±0.03^{b}
LDL-C (mmol·L^{-1})	0.75±0.11^{c}	5.72±0.3^{ab}	5.13±0.41^{b}	5.22±0.62^{b}	6.71±0.35^{a}

Data in the same line with different letters (a,b,c,d) were significantly different *($P < 0.05$)*.

Acknowledgment

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