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Comparison of hypoglycemic and antioxidative effects of polysaccharides from four different *Dendrobium* species

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ABSTRACT

Four polysaccharides from *Dendrobium huoshanense* (DHP), *D. officinale* (DOP), *D. nobile* (DNP) and *D. chrysotoxum* (DCP), which had obvious differences in intrinsic viscosities and monosaccharide compositions, were extracted to compare their hypoglycemic and antioxidative activities in alloxan-induced diabetic mice by oral administration. The analysis of fasting blood glucose, glycosylated serum protein and serum insulin levels showed that DHP, DOP and DNP, but not DCP, possessed significant hypoglycemic effect with the decreasing order of DHP > DNP > DOP. Histopathological observation confirmed the capability of DHP, DOP and DNP to intervene the damage in pancreas tissues. The determination of superoxide dismutase, catalase, malonaldehyde and L-glutathione levels in the liver and kidney displayed that DHP, DOP and DNP had protective effects against alloxan-induced oxidative damage and the effect of DHP ranked first. These results suggested that there were significant differences in hypoglycemic and antioxidative activities between four *Dendrobium* polysaccharides, which may be contributed to their physicochemical properties.

1. Introduction

Diabetes mellitus (DM) is a chronically metabolic disorder with abnormally high levels of blood glucose (so called hyperglycemia), which is caused by the deficiency in insulin secretion and/or the decreased response of the organs to insulin [1]. The worldwide incidence of DM including type 1 and type 2 is dramatically increasing and is estimated to be more than 400 millions by 2030 [2]. Many hypoglycemic agents, such as acarbose and biguanides, are available for the treatment of diabetes, but these agents are chemically synthetic and deficient in multiple dosage regimen, high-cost, side-effects and toxicity [3]. Thus, the traditionally edible and medicinal resources have become the focus of investigation for new natural compounds with hypoglycemic activity [4].

The plants from *Dendrobium* genus belonging to Orchidaceae family have been used as a tonic in many oriental countries for more than one thousand years [5]. It has been claimed that eating or drinking the comestibles made from *Dendrobium* plants can promote sap secretion, reduce fever, enhance body's immunity, prevent cardiovascular and ophthalmic diseases and improve the quality of life [6]. Among these *Dendrobium* species, *D. huoshanense*, *D. officinale*, *D. nobile* and *D. chrysotoxum* are deemed to be superior to other *Dendrobium* species according to the records of traditional medicine and have been the most commonly used as a high-grade tonic both in traditional medicine and in diet [5]. Increasing evidence has indicated that the polysaccharides are one of main components that exert various pharmacological activities in *Dendrobium* plants [5,7]. Recent studies have showed that the polysaccharides from *D. huoshanense* can decrease blood sugar level in cataract rats induced by streptozotocin for 45 days [8] and the polysaccharides from *D. chrysotoxum* can downregulate blood sugar level in mice treated with alloxan for 7 days [9], suggesting that *Dendrobium* polysaccharides have the potential to be developed into natural hypoglycemic agents. Because above-mentioned researches used different animal models with different treatment time and polysaccharide dosages, it is difficult to compare the capability of polysaccharides from *D. huoshanense* and *D. chrysotoxum* to decrease blood sugar levels. Besides *D. huoshanense* and *D. chrysotoxum*, other *Dendrobium* species have not been reported up to now to possess anti-hyperglycemic functions. Therefore, it is

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unclear whether or not the polysaccharides from different *Dendrobium* species express the same potential to decrease blood sugar levels, which will influence the effective and proper application of polysaccharides from different *Dendrobium* species to hypoglycemic functions.

Considerable evidence has shown that oxidative stress is a major cause of chronic and degenerative diseases including diabetes [10,11]. The inextricable link between oxidative stress and hyperglycemia has been confirmed by many studies that proved that the massive production of reactive oxygen species will destroy β-cells of the pancreas, decrease insulin release and lead to hyperglycaemia [12,13]. Since the polysaccharides from *D. nobile* and *D. chrysotoxum* have been reported to display good antioxidant activity in vitro including scavenging activity of hydroxyl radicals, ABTS radicals, DPPH radicals and superoxide anions [9,14], the simultaneous evaluation of the antioxidative activity in vivo of different *Dendrobium* polysaccharides would be helpful to understand the hypoglycemic mechanism of *Dendrobium* polysaccharides from the perspective of antioxidative potential.

The present work was carried out to scientifically compare the hypoglycemic and antioxidative potential of polysaccharides from four commonly used *Dendrobium* species including *D. huoshanense*, *D. officinale*, *D. nobile* and *D. chrysotoxum* using the same animal model with the same oral administration. Because mice have been widely used to evaluate hypoglycemic, antioxidative and other bioactivities of natural compounds and herbal extracts owing to their similarity in the phenotypes developed, tractable, easier to operate, less material to consume as compared with other animals [15–18], we first established diabetic mouse model using alloxan. Then, we used alloxan-induced diabetic mice to compare the hypoglycemic and antioxidative activities of different polysaccharides based on the serum indices including blood glucose, glycosylated proteins and insulin levels, the changes in pathological morphology of the pancreas and the antioxidative indices including superoxide dismutase, catalase, malonaldehyde and L-glutathione. This work would be beneficial to elucidate the function differences of different *Dendrobium* polysaccharides and promote further research and development of functional food with *Dendrobium* polysaccharides.

2. Materials and methods

2.1. Materials and regents

Dendrobium huoshanense was collected in Anhui province of China. Other *Dendrobium* plants including *D. officinale* (or called *D. catenatum*), *D. nobile* and *D. chrysotoxum* were collected from Yunnan province of China. Alloxan was purchased from Sigma–Aldrich (USA). The colorimetry kits including superoxide dismutase (SOD), catalase (CAT), malonaldehyde (MDA) and L-glutathione (GSH) were obtained from Nanjing Jiancheng Biological Engineering Institute (China). Reagents for the determination of serum insulin were purchased from Adlitteram Diagostic Laboratories Co. (USA). Metformin was purchased from Tianjin Pacific Pharmacy Limited Company (China). All other reagents are of analytical grade.

2.2. Polysaccharide preparation

The freeze-dried stems of *Dendrobium* plants were pulverized and defatted twice with petroleum ether in Soxhlet extractor. Then the dregs were successively extracted thrice with the boiling distilled-water, each time for 0.5 h. The water extract was combined and centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was concentrated in a rotary evaporator at 60 °C under reduced pressure to small volumes and precipitated with four volumes of anhydrous ethanol for overnight. The precipitate was collected by centrifugation and washed thrice with acetone. Afterwards, the precipitate was re-suspended in distilled-water and de-proteinized using the Sevag reagent [19]. The aqueous fraction was precipitated with anhydrous ethanol at the ratio of 1:4 (v:v) for 24 h at 4 °C. The resulting precipitate was collected by centrifugation, dialyzed and lyophilized to yield four polysaccharides, namely DHP (*D. huoshanense*), DOP (*D. officinale*), DNP (*D. nobile*) and DCP (*D. chrysotoxum*).

2.3. Intrinsic viscosity determination

The viscosities of DHP, DOP, DNP and DCP were determined in deionic water with the concentration of 20 mg/mL at 25 °C using an Ubbelohde-type viscometer (0.8 mm diameter). Huggins and Kraemer equations were used to estimate the intrinsic viscosity [η] [20].

2.4. Monosaccharide composition analysis

GC-MS was used for the analysis of monosaccharide compositions according to the method of Duan et al. [21]. Briefly, five milligrams of DHP, DOP, DNP, or DCP were dissolved in 4 mL of 2 M trifluoroacetic acid (TFA) solution and hydrolyzed at 120 °C for 4 h in a sealed glass. The hydrolyzate after removing TFA was reduced with $NaBH_4$ and acetylated with acetic anhydride. The acetylated products were dissolved in 4 mL of chloroform and dried with anhydrous Na_2SO_4. The dried products were analyzed by GC–MS using an Agilent 7890 GC/5975 MS system fitted with a fused silica HP-5 capillary column (0.25 μm × 0.25 mm × 30 m).

2.5. Animal experiments

Male Kunming mice weighing 20–25 g were obtained from the Experimental Animal Center of Anhui province of China (Reg. No. SCXK 2005-001). The mice were housed in the polypropylene cages under an ambient temperature of 25 ± 2 °C, a relative humidity of 55–65% and a cycle of 12 h-dark/12 h-light. All mice were allowed free access to common rodlike diets and water for an acclimatization period of 1 week. All animal experiments were done during daytime in accordance with the animal experimentation guidelines approved by the Animal Care and Use Committee of our Institute.

For the induction of diabetes, a freshly prepared solution of alloxan (200 mg/kg b.w.) in normal saline was administered intraperitoneally to overnight fasted mice. Three days after alloxan injection, the fasting blood glucose levels were determined and the

Table 1
Intrinsic viscosities and monosaccharide compositions of different *Dendrobium* polysaccharides.

Polysaccharide[a]	[η] ($cm^3 g^{-1}$)	Monosaccharide composition (molar ratio)					
		Ara	Gal	Glc	Man	Rha	Xyl
DHP	35.05	0.03	0.11	1.00	0.07	–	–
DOP	46.14	0.04	0.12	1.00	0.13	–	–
DNP	65.88	0.07	0.10	1.00	0.14	0.06	0.04
DCP	127.00	–	0.04	1.00	0.42	–	–

[a] DHP, DOP, DNP and DCP denote different polysaccharides obtained from *D. huoshanense*, *D. officinale*, *D. nobile* and *D. chrysotoxum*, respectively.