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Hypoglycaemic effect of *Artemisia sphaerocephala* Krasch seed polysaccharide in alloxan-induced diabetic rats

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Summary

The purpose of this study was to examine the hypoglycaemic activity of a new polysaccharide extracted from *Artemisia sphaerocephala* Krasch seed in alloxan-induced diabetic rats. The *Artemisia* seed polysaccharide (ASP) was administered orally for 4 weeks and the blood glucose changes were determined in fasted rats. Plasma insulin, cholesterol and triglycerides levels were also determined. The ASP at a dose of 200 mg/kg body weight (bw) produced a significant decrease in blood glucose levels in diabetic rats (P <0.01). In the other hand, the effect of the ASP on the plasma cholesterol were also significant in diabetic rats (P <0.05). Furthermore, there was a significant effect of ASP on plasma triglycerides in both normal and diabetic groups. In order to characterise the active principle(s), which could be responsible for the therapeutic effect, a preliminary phytochemical analysis of the ASP was performed. The monosaccharides of ASP were composed of L-Ara, D-Xyl, D-Lyx, D-Man, D-Glc, D-Gal. Their molar proportions were 1, 4.98, 1.69, 27.86, 3.76 and 13.92, respectively.

Key words: Artemisia sphaerocephala krasch; polysaccharide; hypoglycaemia

Introduction

Diabetes mellitus (DM) is a common disorder associated with markedly increased morbidity and mortality rate. DM, which affects a large number of people around the globe, can be defined as a group of metabolic diseases characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, resulting in impaired function in carbohydrate, lipid and protein metabolism. Pharmacological treatment of DM is based on oral hypoglycaemic agents and insulin, but these approaches currently used in clinical practice either do not succeed in restoring normoglycaemia in most patients or fail after a variable period of time. Moreover, continuous use of the synthetic anti-diabetic drugs causes side effects and toxicity [1, 2]. Therefore, seeking natural and non-toxic anti-diabetic drugs is necessary for diabetic therapy.

Artemisia sphaerocephala Krasch (Asteraceae) is a perennial shrub, which is widely distributed in desert areas of Gansu and Nei Monggol in China. The surface of its seeds is covered by a layer of gum, Artemisia seed polysaccharide [3]. Artemisia seed is a common Chinese medicine that has been used traditionally. In Chinese medicine, it has the function of detumescent and is used to treat many disases, such as parotitis and abdominal distention [4]. In addition, the seed has been used in folk medicine by diabetic patients. However, its pharmacological activity remains unknown. Therefore, in this study we want to evaluate the hypoglycaemic effect of ASP in alloxan-induced rats.

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Material and methods

Preparation of ASP

The seeds of *Artemisia sphaerocephala* Krasch were collected from the Nei Monggol region of China. *Artemisia* polysaccharide conjugates were extracted from the *Artemisia* seeds, and impurities such as colour and proteins were removed by procedures as follows [5, 6].

Artemisia seeds powders were extracted with hot water (70 °C) three times (1:20, w/v). The *Artemisia* extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at 4 °C for 24 h, and then centrifuged (10 min, 5000 rpm). The sediment was washed with ethanol, acetone, and ether alternately three times to exclude lipid groups. The precipitate was vacuum freeze-dried, and crude *Artemisia* polysaccharide conjugates were obtained. Crude *Artemisia* polysaccharide conjugates were dissolved in water and decoloured by H_2O_2, then proteined were removed by the Sevage join papain method. Further more, this crude polysaccharide was suspended in water, dialysed to eliminate small molecules. The sugar part of the high molecular weight was separated by gel permeation chromatography (ultrahydrogel^{TM} 500 (7.8 × 300 mm)). The pure polysaccharide was collected and precipitated with 95% (v/v) ethanol and then lyophilised. The amount of saccharide was determined by using the sulfuric acid phenol method with glucose as the standard and optical rotation measure method [7]. The sugar part of the high molecular weight was collected and precipitated with 95% (v/v) ethanol and then lyophilised [8, 9].

Determination of molecular weight (scattering of light method)

The molecular weight was determined by BI-200SM dynamic and static light scattering apparatus [7]. The purified ASP was diluted with deionised water to the final concentrations of 0.400 mg/ml, 0.200 mg/ml, 0.133 mg/ml, 0.100 mg/ml. After filtration (0.2Ìm) and de-dusting, the molecular weight was determined on a molecular weight analyzer (BI-200SM/9000AT, goniometer/autocorrelator and BI-MwA; Brookhaven Instruments Corporation) at 488 nm wavelength.

Determination of monosaccharides by Ion Chromatograph (IC)

The monosaccharides composition of ASP was determined by a Dionex 2500 ion chromatograph equipped with a pulsed amperometric detector, Dionex Ionpac PG10 guard column (2 mm × 50 mm) and PA10 separating column (2 mm × 250 mm). ASP (10 mg) was hydrolysed with trifuoroacetic acid (4 ml, 4 mol/L). ASP trifuoroacetic acid hydrolyte, was evaporated continuously by a rotary evaporator at 45 °C for pH up to neutrality, and then dissolved with deionised water to 66 nmol/L solution [7]. Eluent is 20 mmol l^{-1} NaOH at flow-rate 0.22 ml min^{-1}. Injection volume is 20 µl. Simultaneously, standard monosaccharide aqueous solution (66 nmol/ml) was determined.

Preparation of alloxan-induced diabetic rats

Male Wistar rats (200 ± 20 g) were housed in standard conditions and fed with commercial diet and water ad libitum. Diabetes was induced in fasted rats (12 h) by intraperitoneal injection of 150 mg/kg bw of alloxan, freshly dissolved in sterile normal saline immediately before at a concentration of 30 g/L. The diabetic state was assessed by measuring the non-fasting serum glucose concentration 72 h after alloxan treatment. The rats with a serum glucose level above 11 mmol/L, as well as with polydipsia, polyuria, and polyphagia were selected for the experiment [10].

Treatment groups

The diabetic animals were classified at random into five groups of eight rats. Group 1 as a control received 1.5 ml of sterile normal saline (vehicle). Group 2 was given a standard oral hypoglycaemic agent, glibenclamide (2 mg/kg bw), in the same vehicle, while groups 3–5 received ASP at different dosages (50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw), respectively. In addition, as normal glycaemic control, group 6 received 1.5 ml of sterile normal saline (vehicle), and group 7 received ASP (100 mg/kg bw). The ASP were redissolved in 1.5 ml of sterile normal saline and administered orally by a canule.

Criteria of observations

Collection of blood samples

For the purpose of the estimation of serum glucose, lipid profile and plasma insulin, the blood samples of fasted rats were collected from retrobulbar venous plexus immediately with capillary tubes under ether anaesthesia and with 0.1M EDTA as anticoagulant. Blood samples were allowed to clot for 30 min and serum was separated by centrifugation.

Studies performed in rats

The glucose-oxidase-oxygen method was used for the determination of the plasma glucose level.

Plasma triglycerides and total cholesterol levels are determined enzymatically by specific kits.

Insulin concentrations were measured in serum by radioimmunoassay method using a Beta matic counter [11].

Statistical analysis

All results were expressed as means ± 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 or 0.01 were considered significant.

Results

The molecular weight of ASP was about 1.42×10^5. IC analysed standard monosaccharides and ASP hydrolysate (figure 1).

Figure 1 shows that ASP was composed of six monosaccharides: L-Ara, D-Xyl, D-Lyx, D-Man, D-Glc, D-Gal, and their molar ration is 1: 4.98: 1.69: 27.86: 3.76: 13.92.

The ASP hypoglycaemic effect obtained in alloxan-induced diabetic rats with moderate hyperglycaemia, resembled that observed in laboratory animals. Doses dependent anti-hyperglycaemic activity was also observed with ASP in alloxan-induced diabetic rats. The effects of ASP on plasma glucose concentration in alloxan induced diabetic rats are summarised in table 1. The stronger hypoglycaemic effect was obtained at 200 mg/ml bw dosage of the oral administration, and the percentage reduction of blood glucose was 56.0%. Synchronously, a significant ($P < 0.01$) reduction in blood glucose of 13.3% and 51.0% was observed at the doses of 50 and 100 mg/kg bw, respectively.

Plasma insulin level were significantly higher in high blood glucose groups which received ASP at doses of 200 mg/kg bw ($P < 0.01$) and 100 mg/kg

Figure 1

Ion chromatography spectrogram of ASP Hydrolysate IC.

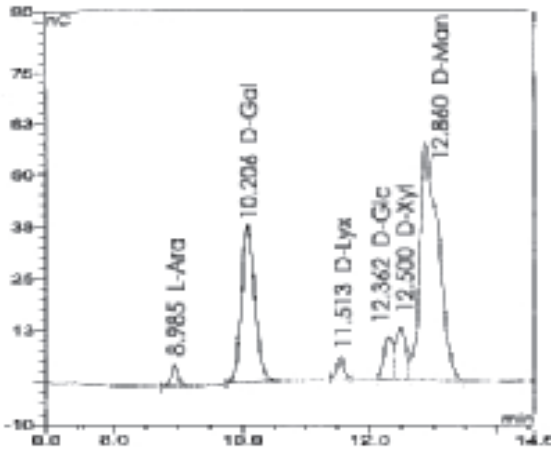

Table 1

Effect of ASP on plasma glucose in alloxan-induced diabetic rats.

Groups	Dose (mg/[kg·bw])	BG (mmol/L)
Control	–	2.87 ± 0.77
Alloxan	150	18.29 ± 2.38[a]
Alloxan + ASP	150 + 200	8.04 ± 2.05[ac]
Alloxan + ASP	150 + 100	8.96 ± 1.03[ace]
Alloxan + ASP	150 + 50	15.86 ± 4.80[ae]
Alloxan + Glibenclamide	150 + 2	8.26 ± 1.43[ac]

Values are in mean ± S.E.M., [a] p <0.01, [b] p <0.05 vs Control.
[c] p <0.01, [d] p <0.05 vs Alloxan. [e] p <0.01, [f] p <0.05 vs Glibenclamide.

Table 2

Effect of ASP on serum insulin in alloxan-induced diabetic rats.

Groups	Dose (mg/[kg·bw])	Insulin (mIV/L)
Control	–	26.67 ± 0.81
Alloxan	150	12.54 ± 0.81[a]
Alloxan + ASP	150 + 200	20.36 ± 1.69[bcf]
Alloxan + ASP	150 + 100	16.25 ± 2.35[ad]
Alloxan + ASP	150 + 50	13.37 ± 0.50[a]
Alloxan + Glibenclamide	150 + 2	15.73 ± 1.93[ad]
ASP	100	27.43 ± 1.34

Values are in mean ± S.E.M., [a] p <0.01, [b] p<0.05 vs Control.
[c] p <0. 01, [d] p <0.05 vs Alloxan. [e] p <0.01, [f] p <0.05 vs Glibenclamide.

bw (P <0.05) than in the diabetic control group, but the effect of 50 mg/kg bw dosage did not reach the level of statistical significance.

Hyperlipidaemia is a common complication of lipidaemia mellitus in experimental animals. The effect of ASP on serum total cholesterol (TC) and triglyceride (TG) in alloxan-induced diabetic rats is shown in table 3. When compared to diabetic controls, TC and TG decreased remarkably. This suggests that ASP may have a hypolipidaemic effect in alloxan-induced diabetic rats.

Table 3

Effect of ASP on TC and TG in alloxan-induced diabetic rats.

Groups	Dose (mg/[kg·bw])	TC (mmol/L)	TG (μmol/L)
Control	–	2.35 ± 0.20	0.50 ± 0.12
Alloxan	150	3.07 ± 0.21[a]	1.21 ± 0.13[a]
Alloxan + ASP	150 + 200	2.82 ± 0.20[acf]	0.63 ± 0.07[ce]
Alloxan + ASP	150 + 100	2.96 ± 0.27[af]	0.45 ± 0.04[ace]
Alloxan + ASP	150 + 50	3.01 ± 0.49[b]	0.76 ± 0.17[bd]
Alloxan + Glibenclamide	150 + 2	2.76 ± 0.39	0.73 ± 0.04[b]
ASP	100	2.72 ± 0.24[b]	0.38 ± 0.03[b]

Values are in mean ± S.E.M., [a] p <0.01, [b] p <0.05 vs Control.
[c] p <0.01, [d] p <0.05 vs Alloxan. [e] p <0.01, [f] p <0.05 vs Glibenclamide.

Discussion

Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increasing demand of patients to use natural products with anti-diabetic activity. Insulin cannot be used orally and continuous use of the synthetic anti-diabetic drugs causes side effects and toxicity [12]. Due to its hypoglycaemic and hypolipidaemic effects in alloxan-induced diabetic rats, ASP as a wild plant polysaccharide has an anti-diabetic potential.

Soluble fibres, especially the galactomannan, can form an unstirred water layer in the gut, which decreases absorption of sugars and lipids. This effect has been related to the property of this polymer to form an unstirred water layer which, by decreasing the rate of gastric emptying, and resisting the convective effects of intestinal contractions, decreases sugar absorption by the small intestine. Thus, to some extent, soluble fibres can be used to prevent the postprandial increase of glucose, making it useful for the treatment of diabetes at certain levels [13–15]. In this experiment, ASP, composed mainly of galactose and mannan, was found to possess significant hypoglycaemic activity, and this activity was compatible with the suggested mode of action of galactomannan in decreasing the rate of gastric emptying and delaying the absorption of glucose from the small intestine.

The viscous structure of polysaccharides has been suggested to be important by interfering with

the absorption in the digestive tract, and thereby increasing the excretion of cholesterol [16]. ASP has been found to possess high viscosity, ie 1800 times that of gelatin [4]. So the high viscosity of ASP may response to its hypocholesterolaemic effect. On the other hand, the hypotriglyceridaemic effect might be due to a delayed absorption of triglyceride in the small intestine caused by the high viscosity of the intestinal contents [16].

In conclusion, these results indicate the possible usefulness of ASP on the decrease in serum glucose and in cholesterol and triacylglycerol levels in plasma for diabetes treatment. These effects may involve alterations in gastrointestinal transit times and intestinal absorption rates. But, further studies are needed to explain the exact mechanisms behind its hypoglycaemic effect.

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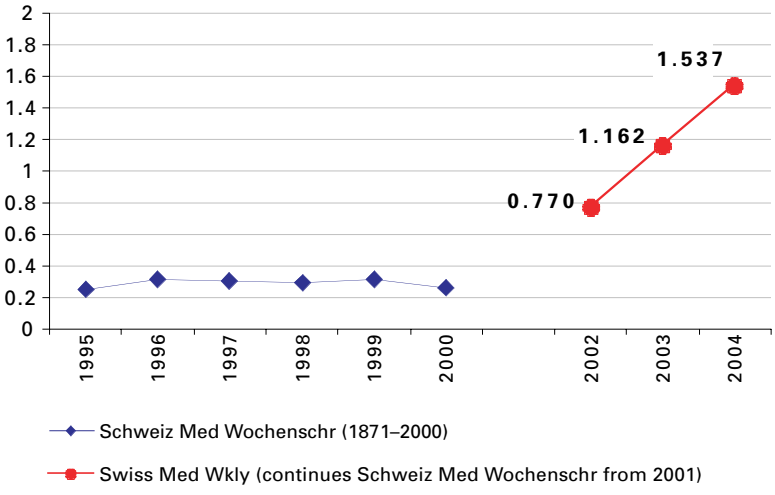

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