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Modulatory effects of one polysaccharide from *Acanthopanax senticosus* in alloxan-induced diabetic mice

Jianfang Fu[a,1], Jufang Fu[b,1], Yang Liu[c], Ruiyu Li[c], Bin Gao[a], Nanyan Zhang[a], Boliang Wang[d], Yizhan Cao[d], Kaoshan Guo[c,*], Yanyang Tu[d,*]

[a] *Department of Endocrinology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China*
[b] *Department of Nursing, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China*
[c] *Second Affiliated Hospital, Xingtai Medical College, Xingtai 054000, China*
[d] *Department of Emergency, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China*

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ABSTRACT

One water-soluble polysaccharide ASP was purified from *Acanthopanax senticosus* and its physicochemical properties were confirmed by the combination of chemical and instrumental analysis. ASP administered orally at three doses (100, 200 and 400 mg/kg body weight) could significantly decrease the concentration of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) cholesterol levels except for high density lipoprotein (HDL) cholesterol level and the relative ratio (HDL/TC) in alloxan-induced diabetic mice, compared with the diabetic controls without drug treatment, comparable with that of diabetic mice treated with metformin. Furthermore, ASP could obviously increase the body weight and serum insulin level and reduce the fasting blood glucose (FBG) levels, especially at dose of 200 mg/kg. The data demonstrated ASP at the certain did often exhibit the optimal protective effect in alloxan-induced diabetic mice. It is promising that ASP may serve as a drug candidate or a healthcare food for diabetic therapy or protection.

1. Introduction

Acanthopanax senticosus Harms, belonging to the Araliaceae family, is a shrub distributed widely in China, Korea and Japan. In China, various parts of the plant, including roots, rhizomes, stems, and leaves, have been used as a traditional Chinese medicine for invigorating the liver and kidney, replenishing the vital essence, strengthening bone, stimulating appetite and improving memory for a long history time (Zhang, Huang, Ye, & Qin, 2011). In the previous studies, the major active components of *Acanthopanax senticosus* include volatile compounds, lignans, coumarins, saponins, flavones, polysaccharides, vitamin, minerals, etc., and these chemical compounds are related to diverse biological activities, such as anti-inflammatory, antitumor, hepatoprotective, anti-ulcer, anti-allergic, anti-irradiation, neuroprotective, antifatigue, immuno-enhancing, antidepressive, hypoglycemic, antioxidant effects and so on (Huang et al., 2011).

Diabetes is a metabolic disease as a result of absolute or relative lack of insulin and chronic hyperglycemia. The main clinical manifestations of diabetes include polydipsia, polyuria, polyphagia and weight loss, accompanied by high blood sugar and urine glucose. World Health Organization divide diabetes mellitus into four types by etiology, namely type I diabetes, type II diabetes, secondary diabetes mellitus and gestational diabetes mellitus. Although the symptoms of each type of diabetes are similar or even identical, but the leading cause of disease in different populations and their distribution is different. Different types of diabetes can lead to insufficient quantities of insulin produced by pancreatic β cells to lower blood sugar levels, resulting in the occurrence of hyperglycemia. Type II diabetes mellitus often appear in adults (particularly obese patients), leading to their weight loss and which was accompanied by hyperglycemia, glucose intolerance, obesity, lipid abnormality, hyperinsulinemia, and insulin resistance (DeFronzo, 1987). In addition to genetic factors, it is well known that lifestyle-related factors, such as overeating, obesity, lack of exercise and stress, are associated with the onset of type II diabetes mellitus. Until now many works concerning the hypoglycemic activities and improving diabetic complication were conducted on the crude extract of *A. senticosus* (Hong, Cha, & Rhee, 2009; Park, Lee, Kang, & Chung, 2006; Watanabea, Kamata, Sato, & Takahashi, 2010). However, there is not any report about the polysaccharide

* Corresponding author. Tel.: +86 319 2279988; fax: +86 319 2279988.
E-mail addresses: gkshan@tom.com (K. Guo), tu.fmmu@gmail.com, yanyangtu@yahoo.com.cn (Y. Tu).
[1] These authors contributed equally to this paper.

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from *A. senticosus* (ASP) to treat or improve type II diabetes mellitus symptom. Therefore, the purpose of this research is to test the efficacy of ASP on the FBG, TC, TG, HDL, LDL and insulin levels, as well as the body weight in alloxan-induced diabetic mice. We also identified the physicochemical properties of ASP, based on the chemical and instrumental analysis.

2. Experimental

2.1. Materials and chemicals

Acanthopanax senticosus were purchased from local drug market in Xian city of China. DEAE-cellulose and Sephadex G-100 were purchased from Amersham (Sweden). Alloxan, T-series dextran, standard sugars, D-glucuronic acid, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glucose Analyzer and strips were purchased from Roche Diagnostic Co. (USA). Reagents for total TC, TG, HDL and LDL were obtained from Beijing Chengxinde Biochemistry Reagent Company (Peking, China). Reagents for serum insulin were purchased from Adlitteram Diagnostic Laboratories Co. (USA). All of other reagents were analytical grade from Peking Chemical Co. (Peking, China).

2.2. Isolation and purification of polysaccharide fractions

The dried *A. senticosus* was extracted 3× with 95% EtOH at 75 °C for 5 h under reflux to remove lipid, and the supernatant was removed. The residue was then extracted 4× with 10 vol of distilled water at room temperature for 3 h. After centrifugation (1700 × *g* for 15 min, at 20 °C), the supernatant was concentrated 10-fold, and the crude polysaccharide fraction was precipitated with 95% EtOH (1:5, v/v). The precipitate, collected by centrifugation, was suspended in distilled water, followed by removal of the protein by the Sevag method (Sun & Liu, 2009) and exhaustively dialyzed against water for 2 days, then the dialyzed solution was collected and freeze dried to obtain the crude polysaccharide (CASP).

The sample (100 mg) was dissolved in distilled water, centrifuged, and then the supernatant was injected to a column (2.6 cm × 40 cm) of DEAE-cellulose equilibrated with 0.9% sodium chloride. After loading with sample, the column was eluted first with different concentrations of NaCl aqueous solution (0 and 1 M) stepwise at 1 ml/min. The eluted solution was separated into two fractions (CASP and CASPA), and then CASP was further purified by gel-permeation chromatography on a Sephadex G-100 column (2.6 cm × 100 cm), loading the above purified fraction for each run. The column was eluted with 0.15 M Nacl with a flow rate of 1 ml/min. Total sugar content of each tube was monitored at 490 nm by Dubois's method. Fractions (test tube nos. 36–39) were collected, and freeze dried, yielding only one fraction of ASP (41 mg). There are total 10 preparations for ASP as above description.

2.3. Molecular weight determination and monosaccharide composition analysis

Molecular weights of the different polysaccharide fractions were recorded on a Waters 515 instrument equipped with the GPC software (Millennium^{32}), a Waters 2410 RI detector and a TSK-GEL G3000 PWXL gel-filtration chromatographic column (7.8 mm × 300 mm), maintained at a temperature of 40 °C, eluted with 0.1 mol/L Na_2SO_4 solution at a flow rate of 0.6 ml/min. The columns were calibrated with T-series Dextran (T-2000, T-110, T-40, T-20 and T-10). A 20-μl aliquot was injected for each run.

Polysaccharide was also analyzed for monosaccharide by gas chromatography (GC). After hydrolysis with 2 M trifluoroacetic acid (TFA) and conversion of hydrolysate into alditol-acetates as previously described method (Johnes & Albersheim, 1972; Oades, 1967), the resulting alditol-acetates were analyzed by GC. GC was performed on a Vavian 3400 instrument (Hewlett-Packard Component, USA) equipped with DM-2330 capillary column (30 m × 0.32 mm × 0.2 μm) and flame-ionization detector (FID). The column temperature was kept at 120 °C for 2 min, and increased to 250 °C (maintained for 3 min) at a rate of 8 °C/min. The injector and detector heater temperature were 250 and 300 °C, respectively. The rate of N_2 carrier gas was 1.2 ml/min.

2.4. Measurement of carbohydrate, protein contents and uronic acid

Total carbohydrate contents of the polysaccharide fractions were determined by phenol–sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as the standard. Proteins in the polysaccharides were quantified according to the Bradford's method (Bradford, 1976) using bovine serum albumin (BSA) as the standard. In addition, total uronic acid contents were measured by *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as the standard.

2.5. Experimental animal

Male mice of original Kun-ming strain (18–22 g each) were used for the study. All the procedures were carried out in strict accordance with the "Principles of Laboratory Animal Care". All mice were housed under standard conditions at 22 ± 1 °C, with humidity of 50 ± 10%, and a constant 12-h light and dark cycle. Rodent laboratory chow pellets and tap water were supplied ad libitum.

2.6. Preparation of alloxan-induced diabetic mice

Diabetes was induced in mice that had been fasted for 12 h by injection of alloxan (60 mg/kg body weight) freshly dissolved in 0.9% NaCl injectable solution via the tail vein. Diabetes was confirmed by the measurement of blood glucose from the tail vein at 72 h after feeding alloxan. The mice with a blood glucose level above 11 mmol/L, as well as with polydipsia, polyuria and polyphagia were selected for the experiment (Zhang, Huang, Hou, & Wang, 2006).

2.7. Experimental design and oral administration

Sixty Male mice were randomly divided into six groups and each group consisted 10 rats as follows:

- Group 1: Normal control group (NC)
- Group 2: Diabetic control group (DC)
- Group 3: Diabetic plus metformin (100 mg/kg) group (DM)
- Group 4: Diabetic plus ASP (100 mg/kg) group (DAL)
- Group 5: Diabetic plus ASP (200 mg/kg) group (DAM)
- Group 6: Diabetic plus ASP (400 mg/kg) group (DAH)

The rats were orally administered once daily for four weeks.

Group 1 containing 10 normal rice served as normal control group, which was orally administered 10 ml/kg of body weight/day (BW/D) of 0.9% NaCl solution (Vehicle); Group 2 containing 10 type 2 diabetic mice was treated in the same condition as Group 1; Group 3 was orally administered the standard oral hypoglycemic agent metformin (2 mg/kg BW/D) in the same vehicle; Group 4–6 received oral ASP administration by a cannula in the dose of 100 mg/kg, 200 mg/kg and 400 mg/kg BW/D, respectively. All the groups (groups 1–6) were still fed with common pellet diets during the study.

2.8. Determination the body weight and the level of blood glucose, serum lipids and insulin in mice

Blood was collected from tip of the tail vein and FBG level was measured for once every week by using a glucose analyzer. Meanwhile the body weight of experimental mice was recorded on the balance every week until they were sacrificed. On 28th day of experiment, the mice were sacrificed by decapitation in order to minimize the handling stress, and blood was collected from dorsal aorta and serum was separated by centrifugation for 5 min and was kept at −20 °C for the biochemical assay of total TC, TG, HDL and LDL. Their levels in serum were measured spectrophotometrically by the prescribed method after four weeks (Allain, Poon, Chan, Richmond, & Fu, 1974; Buccolo & David, 1973). The fasting serum insulin level was determined by using a radioimmunoassay kit.

2.9. Acute toxicity studies

Acute oral toxicity assay was evaluated on ASP in male Kunming mice (weight of 20–24 g). We divided mice to three groups, each group containing five mice. The mice were oral administration of ASP at doses of 500, 1000, 2000 mg/kg. All external morphological, behavioral, neurologic, autonomic changes, toxic effects were recorded continuously for two days.

2.10. Statistical analysis

All the data were expressed as means ± standard deviations (SD) of three replicates. Statistical calculations by SPSS version 10.0 software (SPSS Inc, Chicago, USA) were carried out. Data were analyzed 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. Values of $P < 0.05$ were considered to be significantly different.

3. Investigations and results

3.1. Purification of polysaccharides and its physicochemical properties

In this study, the purified polysaccharide of ASP was obtained by DEAE-cellulose anion-exchange and Sephadex G-100 gel filtration chromatography, as seen from Fig. 1.

ASP had a negative response to the Bradford's method and no absorption was detected by the UV spectrum at 280 and 260 nm indicating the absence of protein and nucleic acid. The ASP showed a single and symmetrically sharp peak, indicating its homogeneity on HPSEC (data not shown). According to the retention time, its molecular weight was estimated to be 59 kDa. The total carbohydrate content of ASP was 95.5% determined by phenol–sulfuric acid method. No uronic acid was measured by *m*-hydroxydiphenyl method. GC analysis showed ASP was composed of three kinds of monosaccharides, namely glucose, galactose and arabinose in a molar ratio of 2.1:1.9:1.

3.2. The effect of ASP on the body weight in mice

The data in Fig. 2 indicated that there was no significant difference ($P > 0.05$) of body weight among all groups at the beginning of the experiment. After mice were treated with alloxan, the body weight of mice in DC group exhibited greater loss than that in NC group during the experiment ($P < 0.01$). The body weight of all three groups administrated with ASP in the dose of 100, 200 and 400 mg/kg were significantly increased as compared to the DC group from the second week after alloxan injection ($P < 0.05$ or $P < 0.01$). Moreover administration of ASP at the dose of 200 mg/kg

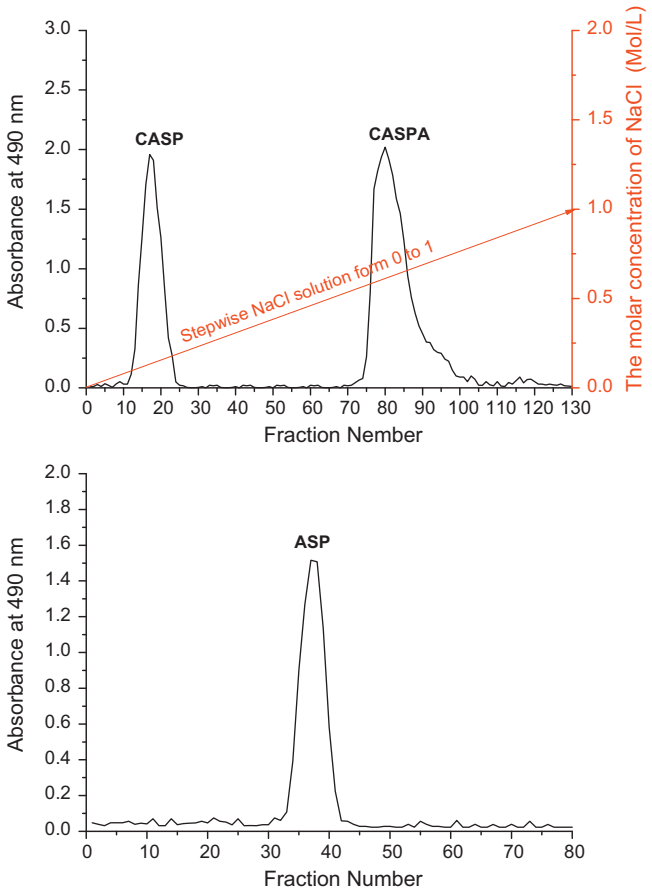

Fig. 1. The profiles of ASP on the DEAE-cellulose anion-exchange and Sephadex G-100 gel filtration chromatography.

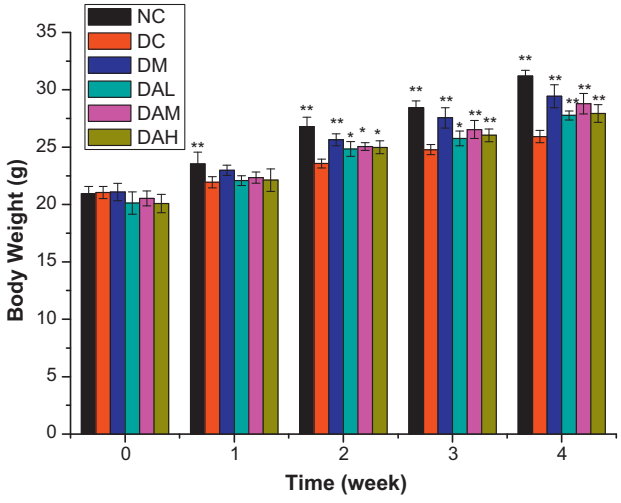

Fig. 2. The effect of ASP on the body weight in mice.

significantly improved the body weight loss than the other doses, which was comparable to that of the DM group.

3.3. The effect of ASP on blood glucose level

The FBG level of different groups was presented in Table 1. The FBG in DC group, treated with alloxan, exhibited a high level as compared to the NC group ($P < 0.01$). When the diabetic mice was orally administrated with a clinical drug for diabetes mellitus, the

Table 1
The effect of ASP on FBG in mice.

Groups	Blood glucose levels (mmol/l)				
	Weeks after oral administration (week)				
	0	1	2	3	4
NC	4.12 ± 0.12**	4.05 ± 0.06**	4.11 ± 0.13**	3.99 ± 0.18**	4.05 ± 0.15**
DC	14.67 ± 0.17	14.64 ± 0.20	14.72 ± 0.22	14.71 ± 0.21	14.69 ± 0.24
DM	14.53 ± 0.13	8.19 ± 1.24**	5.74 ± 0.83**	5.06 ± 1.04**	4.97 ± 0.61**
DAL	14.63 ± 0.21	13.78 ± 1.37*	9.43 ± 1.02**	8.04 ± 1.37**	7.18 ± 1.17**
DAM	14.71 ± 0.27	9.43 ± 1.53**	8.43 ± 1.29**	6.67 ± 1.01**	5.76 ± 0.83**
DAH	14.65 ± 0.14	11.84 ± 1.64**	8.68 ± 1.33**	7.89 ± 1.00**	6.49 ± 1.11**

All values represent the mean ± standard deviation ($n = 3$). Significant differences with DC group were designated as *$P < 0.05$ and **$P < 0.01$.

Table 2
The effects of ASP on blood lipids and insulin levels in mice.

Groups	TC (mmol/l)	TG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	HDL/TC (%)	Insulin (mIU/l)
NC	3.11 ± 0.21**	1.32 ± 0.12**	1.95 ± 0.09**	0.34 ± 0.03**	62.70 ± 4.34**	19.43 ± 0.14**
DC	9.23 ± 0.57	2.16 ± 0.21	3.16 ± 0.34	1.04 ± 0.11	34.24 ± 4.31	31.54 ± 2.73
DM	7.52 ± 0.73*	1.83 ± 0.19	3.08 ± 0.42	0.78 ± 0.09**	40.96 ± 3.26**	30.02 ± 3.63
DAL	6.42 ± 0.92**	1.87 ± 0.13	2.94 ± 0.45	0.75 ± 0.10	45.79 ± 3.57**	24.32 ± 2.92**
DAM	5.83 ± 0.67**	1.46 ± 0.21**	3.02 ± 0.36	0.58 ± 0.13	51.80 ± 3.41**	21.42 ± 3.11**
DAH	6.05 ± 0.51**	1.66 ± 0.12*	2.67 ± 0.31*	0.58 ± 0.12	44.13 ± 4.18**	23.12 ± 2.82**

All values represent the mean ± standard deviation ($n = 3$). Significant differences with DC group were designated as *$P < 0.05$ and **$P < 0.01$.

DM group reverse this bad situation as evidenced by the lower FBG level in alloxan-induced diabetic mice form the beginning to the end of this experiment. At the same time, we can see that there was a significant decrease in the groups treated with ASP in three doses of 100, 200 and 400 mg/ml after 7 days, as compared with the DC group (*$P < 0.05$ and **$P < 0.01$). Among these three groups, DAM groups showed a greater hypoglycemic effect than that observed for the mice treated at the dose of 100 and 200 mg/ml, and there were also no differences observed between the DM group and DAM group. Moreover with time continuing, the hypoglycemic effect in groups of DM, DAL, DAM and DAH all became stronger than ever. Thus, from the above findings we can draw a conclusion that ASP at a suitable dose can lower the blood glucose levels in alloxan-induced diabetic mice.

3.4. The effect of ASP on blood lipids and insulin levels

The effect of ASP on blood lipids and insulin levels were evaluated on 28th day, as seen in Table 2. There is an obvious difference ($P < 0.01$) on the value of TC, TG, HDL, LDL, HDL/TC and insulin between the NC and DC group. After oral administration of metformin to mice, the blood lipids level was down-regulated more positively as compared to that in the DC group. On the other hand, the blood insulin level in DM group did not changed any more. In addition, the ratio of HDL/TC was significantly different ($P < 0.01$) from that in DC group, with a higher scale. The above results conformed to the mechanism by which metformin take effect against the type II diabetes mellitus. In all ASP-administrated groups, we can found that ASP could raise the ratio of HDL/TC and attenuate the TC, TG, LDL and insulin levels, especially at the dose of 200 mg/ml. Based on the results, the effect of ASP on the blood lipids in diabetic mice was comparable to the metformin at the dose of 200 mg/ml. Moreover apart from decreasing blood lipids, ASP also acted on the insulin level, which is different from the metformin.

3.5. Acute toxicity studies

In the present study, even at high dose of 2000 mg/kg ASP did not exhibit any sign of toxicity. The behavior of the treated mice appeared normal, and no toxic effect and death were detected during this investigation.

4. Discussion

Insulin resistance and impairing insulin secretion act as the main causes for the type II diabetes, leading to the defective glucose oxidation and hyperglycemia. Diabetes mellitus is also strictly related to other metabolic abnormalities, in which the most important one is lipid abnormalities, characterized mainly by high TC, TG, LDL levels and low content of HDL. Accumulating evidence suggests that hyperglycemia and hyperlipemia play an important role in the process of diabetic complication. At present, the main medicines treating Type II diabetes are biguanide and sulfonylurea drugs. However, they both have side effects more or less to destroy the balance of human health (Liu et al., 2009). At the same time, diabetes mellitus is one of the most challenging diseases, which puts a large burden on society and the public health. Therefore most researchers have to pay attention to finding out one anti-diabetic drug which has a good efficacy but little toxic side-effects. Considering low side-effects and low cost, polysaccharide from natural resources open new avenues for the treatment of various diseases including diabetes. It has been well established that non-starch polysaccharides are likely to be associated with less risk of diabetes. Many of these polysaccharides have been reported to have effects on hyperglycemia (Li, Fu, Rui, Hu, & Cai, 2005; Li et al., 2006; Lo, Tsai, Wasser, Yang, & Huang, 2006; Luo, Cai, Yan, Sun, & Corke, 2004).

In the present study, we successfully purified one homogenous polysaccharide from the root of *A. senticosus*, and preliminarily elucidated its physicochemical characteristics. ASP oral administration to alloxan-induced diabetic mice could improve their body weight and reverse the hyperglycemia and hyperlipemia status, as well as lowering the insulin level. Acute toxicity assay had already proved that the ASP did not show any toxic reactions even at the high dose in male mice. Taken together, the results indicated that ASP may be useful as a good natural material to be developed as a promising agent to treat diabetes. However further chemical and pharmacological investigation should be carried out to clarify the detailed mechanism by which ASP take effect against diabetes mellitus.

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