—Regular Article—

Comparative Study for α-Glucosidase Inhibitory Effects of Total Iridoid Glycosides in the Crude Products and the Wine-processed Products from *Cornus officinalis*

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To compare the difference of α-glucosidase inhibitory effect of total iridoid glycosides from crude products and processed products (Paozhi in Chinese) of *Cornus officinalis* Sieb. et Zucc. by α-glucosidase and glucose tolerance test in mice. Alcohol 40% was employed to extract the total iridoid glycosides from the crude products and the processed products then the total iridoid glycosides were enriched through macroporous resins to obtain SZY-1 and SZY-2 from the crude products and the processed products, respectively. The inhibitiory activity of these extracts was investigated on α-glucosidase inhibition *in vitro* and glucose tolerance test *in vivo*. The results demonstrated that both SZY-1 and SZY-2 were successfully enriched by macroporous resins. The contents of the total iridoid glycoside in SZY-1 and SZY-2 were 75.3% and 42.8%, respectively, and exerted inhibitory effects at various concentrations on α-glucosidase. The maximal inhibition ratio (86%) of SZY-1 was markedly higher than that of SZY-2. Meanwhile, SZY-1 was more potent than SZY-2 in decreasing blood sugar in diabetic mice induced by streptozotocin. Their effects were dependent on the dosage. In conclusion, the iridoid total glycoside from crude products and processed products of *C. officinalis* had α-glucosidase inhibitory effects *in vitro* and *in vivo*, and the effects of the crude products ware superior to those of processed products. It was also suggested that the hypoglycemic effects of *C. officinalis* may be attributable to its total iridoid glycoside.

Key words——*Cornus officinalis*; iridoid glycoside; hypoglycemic effect; α-glucosidase

INTRODUCTION

Cornus officinalis Sieb. et Zucc., which is mainly distributed in Sichuan, Hubei, and Henan provinces of China, has been used as an important traditional Chinese medicine for a long history in China, Korea, and Japan.[1] It is widely used for the treatment of such symptoms as dizziness and tinnitus, waist or knee soreness, asynodia and spermatorrhea, uracratia, frequency of micturition, uterine bleeding, and leukorrhea and abiotrophy.[2] *C. officinalis* consists of many ingredients such as iridoids, tannic acid, polysaccharides, saponin, amino acid, and minerals.[3,4] It has been reported that *C. officinalis* exhibits immune-regulating function, anti-inflammatory and anti-shock effects, and protective effect on experimental diabetic nephropathy.[5,6] Traditional Chinese medicine processing methods (Paozhi in Chinese) that play an essential role in detoxification can decompose the toxic ingredients into less or non-toxic derivatives and can improve the effects of Chinese medicine.[7–11] The processing method is multiform in traditional Chinese medicine; generally, *C. officinalis* is processed by wine.

It was also reported that the total iridoid glycoside of *C. officinalis* possesses many therapeutic effects such as hypoglycemic effect by diminution of glucose absorption and postprandial hyperglycemia, as well as decreasing cholesterol and triacylglycerides levels.[12–15] By these data, it is assumed that the antidiabetic effect of total iridoid glycoside from *C. officinalis* is still controversial and that the mechanism has to be further investigated.

In this paper, the α-glucosidase inhibitory effect of the crude extract and processed products from *C. officinalis* were compared. Macroporous absorbent resin was used to enrich the ingredients of the total iridoid glycoside in the crude and processed products. The inhibitory effects of α-glucosidase and their influence on glucose tolerance of diabetic mouse induced by streptozotocin were also investigated.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts The fresh products of *C. officinalis* Sieb. et Zucc were collected from Anxian County, Sichuan Province, China. A voucher specimen has been deposited in the Herbarium of Hunan Normal University. Crude

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products were obtained by ripping of nuclear of *C. officinalis* and drying under the sun. The processed products were prepared by the following method. The nuclear of *C. officinalis* was ripped from fresh specimens by appropriate cooking, and the pulp was stuffed and wetted in rice wine for 1 h and put into the appropriate container then sealed tightly and heated with water for 4 h. Finally, the pulp was dried at 60℃ for 8 h to obtain the processed products of *C. officinalis*.

The crude products of *C. officinalis* were extracted with 40% alcohol using cold maceration. The extraction solvent was replenished every 24 h for 7 days. The extraction from previous days was collected and filtered through a porous plug of absorbable cotton. Then, the filtrate was concentrated at 60℃ by rotary evaporator (Buchi Labortechnik, Flawil, Switzerland). The concentrated filtrate was uploaded into a, macroporous absorbent resin column. The column was first eluted with water then with 70% alcohol. The 70% alcohol solution was collected, concentrated, and dried to obtain SZY-1 with pale yellow powder. The yield of the extract was 2.1%. The processed products were treated by the same method as the crude products. Thus SZY-2 was obtained with a yield 5.2%.

The total iridoid glycosides were determined by colorimetry method;[16] the contents of the total iridoid glycosides in the extracts of SZY-1 and SZY-2 were 75.3% and 42.8%, respectively.

Chemicals α-Glucosidase (Cat. No. G 5003) and *p*-nitrophenyl-α-*D*-glucopyranoside (Cat No: N 1377) were purchased from Sigma Aldrich Chemical Co, USA. Macroporous absorbent resins of HPD100 were purchased from Hebei Cangzhou Baoeng Chemical Co., Ltd. Acarbose (Glucobay, 50 mg/tablet) was obtained from Bayer Medical Co. Leverkusen, Germany. All other chemicals and solvents used were in analytical grade.

Animals Kunming mice weighing 20–22 g of either sex obtained from Animal House, Pharmacy Discipline, Hunan Normal University, Hunan province were used. The animals were provided with a rodent standard diet with free access to water *ad libitum* and housed in rooms under conventional conditions of controlled temperature. The temperature was kept at 25±1℃ with a 12-h light/dark cycle. All experimental protocols were in accordance with the Principles of Laboratory Animal Care and Use in Research established by the Ministry of Health of P. R. China and approved by the local Animal Ethics Committees of the Faculty of Medicine, Hunan Normal University.

Test for Inhibition Activity of α-Glucosidase The test for α-glucosidase inhibitory activity *in vitro* was established by chromogenic method with reference to Matsui *et al.*[17] with slight modifications. Yeast α-glucosidase at a concentration of 0.1 U/ml was dissolved in 100 mM phosphate buffer (pH 7.0) containing 0.2% bovine serum albumin and 0.02% sodium azide, which was used as enzyme solution; *p*-nitrophenyl-α-*D*-glucopyranoside (5 mM) was used as substrate solution. SZY-1 and SZY-2 were weighed and serial dilutions of both 3.2 and 100 mg/ml were made up with equal volumes of distilled water. Ten microliters of SZY-1 and SZY-2 dilutions were incubated for 5 min with 50 μl enzyme solution. After incubation at 37℃, 50 μl of substrate solution was added and the mixture solution was further incubated at room temperature for 5 min. The presubstrate and postsubstrate solution addition absorbance was measured at 405 nm on a microplate reader (Multiskan MS model 352, Labsystemms Inc., Finland). The α-glucosidase inhibitory activity was calculated as follows: $(1-A_S/A_0)\times 100$, where A_0 is the absorbance of control and A_S that of samples containing extracts. All experiments were performed in duplicate. Acarbose was dissolved in distilled water; dilution of 10 mg/ml was made and performed as positive control.

Assay of Oral Carbohydrate Tolerance Tests The oral carbohydrate tolerance test was established according to the detailed method of Tiwari *et al.*[18] The studies were carried out by oral administration of starch in normal and diabetic groups of mice. Experimental diabetes was induced in mice by intravenous (i.v.) administration of freshly prepared streptozotocin (Sigma Chemical Co., USA) solution (50 mg/kg, citrate buffer 0.01 M, pH 4.5). Blood glucose levels were constantly monitored by glucose oxidase method with a Spectrophotometer S500 (Secomam, France). Animals with glycemia value both 10 and 15 mmol/l were selected for the experimentation. Acarbose at a dose of 10 mg/kg was used as positive control. Mice were divided into eight groups consisting of ten mice in each group; the control group, positive group, treatment groups D1-D3 (SZY-1) and treatment groups D4-D6 (SZY-2). The mice of each group were restricted from food over-

night but had free access to water. For diabetic controls (DC), mice were administered deionized water 4 ml/kg. The mice of treatment group 1 were administered orally with 200 mg/kg body mass of SZY-1 (D1), treatment group 2 was administered orally with 100 mg/kg body mass of SZY-1 (D2), treatment group 3 was administered orally 50 mg/kg body mass of SZY-1 (D3), treatment group 4 was administered orally with 200 mg/kg of SZY-2 (D4), treatment group 5 was administered orally with 100 mg/kg of SZY-2 (D5), and treatment group 6 was administered orally 50 mg/kg of SZY-2 (D6). After 10 min, all mice were administered orally with starch 3 g/kg, then the eye orbit was snipped for blood glucose assaying at 0 min (before starch administration) and 30, 60, 90, and 120 min after starch administration. Blood glucose concentrations were recorded by glucose meter. The maximum blood glucose concentration found during blood glucose determination was taken as the peak blood glucose (PBG).

Statistical Analysis Data are expressed as mean ±S.E.M. Statistical difference in PBG between control and treatment groups was determined by Statistical Package for Social Sciences (SPSS11.5). One-way analysis of variance (ANOVA) was performed by Tukey's test for *post hoc* analysis. $p<0.05$ was considered as significant.

RESULTS

Inhibitory Effect of α-Glucosidase Both SZY-1 extract and SZY-2 extract h exhibited α-glucosidase inhibitory effects. The percent inhibition at 3.2～100 mg/ml concentrations of both SZY-1 extract and SZY-2 extract showed significant dose-dependent relations. Thus the highest concentration of 100 mg/ml observed a maximum inhibition of nearly 86%. Percent inhibition exerted according to the lowest concentration of 3.2 mg/ml to the highest concentration of 100 mg/ml varied at 5.7–86%. However, SZY-2 showed less inhibitory potential with percent inhibitions ranging at 4.8–49.7%. The inhibitory activity of SZY-1 and SZY-2 against yeast α-glucosidase is shown in Fig. 1. It can be observed from Fig. 1 that the inhibitory activities of SZY-1 were superior to those of SZY-2, and the inhibitory effects of high doses (100 mg/ml) were equivalent to that of acarbose.

Oral Starch Tolerance Test In normal mice oral starch tolerance tests revealed that both SZY-1 and SZY-2 significantly decreased PBG compared with control mice (Tables 1, 2). D1, D2, D3, and D4 also reduced blood glucose levels significantly at 30 min ($p<0.05$). Moreover, blood glucose levels in D1 and D2 decreased highly significantly ($p<0.01$) compared with control group. D3 showed significantly ($p<0.05$) reduced blood glucose levels at 30 and 60 min. D4, D5, and D6 had no significant variance in decreasing blood glucose levels at 30, 60, 90, and 120 min. These data were dependent on the dose of SZY-1 and SZY-2, and the effect of D1 was similar to that of acarbose.

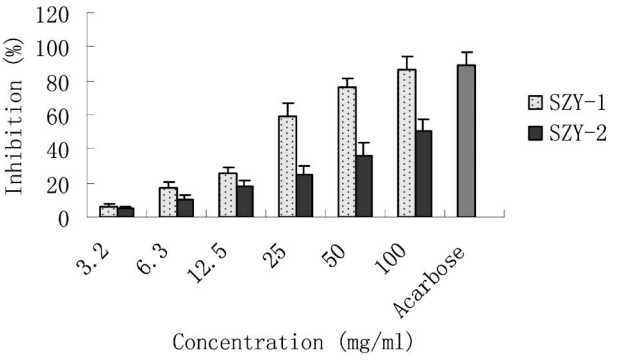

Fig. 1. Inhibition Activity of SZY-1 and SZY-2 against Yeast α-Glucosidase

Inhibition activity of the extract for the crude products (SZY-1), processed products (SZY-2) of *C. officinalis* and acarbose (10 mg/ml) against α-glucosidase. Experiments were run as detailed in materials and methods, result represented as mean±S.D. of percent enzyme activity.

DISCUSSION

α-Glucosidases are a series of enzymes located on the intestinal brush-border.[18] Most carbohydrates in starchy food are hydrolyzed to glucose and fructose by α-glucosidase and absorbed into the blood, thereby increasing blood glucose. These processes usually occur in the upper portion of the small intestine and greatly increase BG concentration, especially in diabetic patients.[19] However, α-glucosidase inhibitor can prolong the processes on the entire intestine, extend the time of carbohydrate absorption, and flatten blood glucose concentrations over time.[20] It was reported that acarbose, a first-line α-glucosidase inhibitor drug for the treatment of type 2 diabetes, is not controlled through diet alone.[21,22] In our study, acarbose prevented abnormally high rise in postprandial blood glucose concentrations. Both SZY-1 and SZY-2 reduced the increases of blood glucose concentrations and decreased blood glucose level after starch loading in normal and diabetic mice. However, in normal

Table 1. The Blood Glucose Response during Oral Starch Tolence Test in Normal Mice Treated with SZY-1, SZY-2 and Acarbose

Groups	Number	Dosage (mg/kg)	Peak blood glucose (mmol/l)				
			0 h	0.5 h	1 h	1.5 h	2 h
Normal contrast group	10	—	6.49±1.52	6.23±3.96	6.02±3.77	5.92±3.51	5.78±3.13
Starch load group	10	—	6.44±1.49	15.26±3.82	14.07±3.74	11.64±3.26	9.98±3.07
Acarbose group	10	10	6.58±1.32	9.07±1.97**	10.05±2.33**	9.37±2.44	8.45±2.36
SZY-1 (D1)	10	200	6.73±1.62	11.94±2.18*	10.77±2.06*	8.38±1.37*	7.59±1.13*
SZY-1 (D2)	10	100	6.74±1.63	12.06±2.78*	10.57±1.74*	8.09±1.23*	7.75±1.48*
SZY-1 (D3)	10	50	6.53±0.89	12.64±2.48	11.27±1.43*	8.67±1.54*	8.48±0.93*
SZY-2 (D4)	10	200	6.63±1.31	12.36±1.72*	11.78±3.05	10.06±1.87	9.32±1.51
SZY-2 (D5)	10	100	6.24±1.23	14.19±2.59	12.38±2.15	11.62±1.92	10.18±1.46
SZY-2 (D6)	10	50	6.56±1.87	14.33±3.25	13.02±2.73	11.98±1.97	10.75±1.49

Comparison with the contrast group, $^{*}p<0.05$, $^{**}p<0.01$.

Table 2. The Blood Glucose Response during Oral Starch Tolence Test in Diabetic Mice Treated with SZY-1, SZY-2 and Acarbose

Groups	Number	Dosage (mg/kg)	Peak blood glucose (mmol/l)				
			0 h	0.5 h	1 h	1.5 h	2 h
Normal contrast group	10	—	25.44±8.65	24.65±7.62	21.72±8.47	20.68±8.54	17.52±6.33
Starch load group	10	—	25.44±9.32	44.43±9.10	41.72±7.55	39.95±9.32	36.74±8.42
Acarbose group	10	10	26.82±4.76	28.54±3.67**	30.77±5.23**	30.86±4.59*	31.38±5.32
SZY-1 (D1)	10	200	27.57±4.54	30.88±4.45**	31.92±4.84**	31.65±4.63*	31.07±4.78
SZY-1 (D2)	10	100	27.50±4.38	32.41±5.72*	34.24±6.18*	34.26±5.38	32.84±4.02
SZY-1 (D3)	10	50	25.94±8.74	34.49±5.07*	35.62±6.39*	35.27±7.42	33.89±6.64
SZY-2 (D4)	10	200	25.53±4.32	38.91±3.95	37.97±5.72	37.61±5.44	36.37±6.22
SZY-2 (D5)	10	100	25.10±4.51	39.42±4.83	38.02±5.59	37.88±5.75	37.02±7.35
SZY-2 (D6)	10	50	25.78±5.42	38.15±3.27	37.36±7.24	36.94±6.18	36.56±6.14

Comparison with the contrast group, $^{*}p<0.05$, $^{**}p<0.01$.

mice, both SZY-1 and SZY-2 did not affect glucose absorption in the small intestine. It was demonstrated that both SZY-1 and SZY-2 delayed the rapid digestion of starch and reduced the postprandial zenith of blood glucose concentration. These effects are similar to those for the α-glucosidase inhibitor, acarbose. At the same time, the glucose tolerance test suggested that SZY-1 inhibits elevation of blood glucose in normal and diabetic mice. Thus it is effective for controlling the rise of postprandial blood sugar. However, different doses of SZY-2 had no significant effect in decreasing blood glucose. Therefore wine-processed products of *C. officinalis* are not beneficial for the treatment of diabetes.

Macroporous resin, a kind of high polymer adsorbent, is a rapid, highly effective, convenient, sensitive, and selective tool that has been widely used for the separation and purification of Chinese medicinal herbs.[23] In this study, the application of macroporous resin separated and enriched the iridoid glycoside ingredients. It was found that the content of the iridoid glycoside of the wine-processed products was lower than that of the crude products. Therefore we supposed that the iridoid glycoside may be the one of the main active ingredients for reducing postprandial blood sugar, In a previous study it was reported that many iridoid glycosides were separated such as loganin, morronisides, sweroside, cornuside II, cornuside I, 7α-*O*-ethylmorronside, and 7-β-*O*-ethylmorronside.[24]

Our results also suggest that the mechanism for traditional processing technology of traditional Chinese medicine is not clear. These traditional methods may result in the loss of some effective ingredients. Therefore our results demonstrate that the crude product of *C. officinalis* is more appropriate for the treatment of diabetes. Further study will highlight the mechanism for traditional processing technology of *C. officinalis*.

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