Inhibitory effects of extract from the *Schizandra chinensis* on rat small intestinal α-amylase activity and postprandial blood glucose

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Abstract

Postprandial hyperglycemia plays an important role in the development of type 2 diabetes and complications associated with the disease such micro-and macro-vascular disease. The present study investigated the effect and action mechanism of a ethanolic extract from the *Schizandra chinensis*(*SC*-E) on hyperglycemia in vivo and in vitro. In vitro, *SE*-E demonstrated a potent inhibitory effects on α-amylase activity (IC_{50} : 4 μg/ml). Its inhibition on α-amylase was determined to be competitive type. Oral administration of *SE*-E markedly lowered plasma glucose levels in non-fasted streptozotocin induced diabetic rats (45 mg/kg BW). In addition when it was orally administrated to rats with starch (2g/kg BW), *SC*-E (50 and 100 mg/kg BW) significantly suppressed the increase of blood glucose levels after starch loading . These results suggest that some edible plants merit further evaluation for clinical usefulness as anti-diabetic drugs.

Introduction

Postprandial hyperglycemia plays an important role in the development of type2 diabetes mellitus and complications associated with the disease such micro- and macro-vascular disease. However, therapeutic approaches to the treatment of postprandial hyperglycemia have been ineffective except for insulin therapy, creating a challenge for researchers involved in the development of new agents to control postprandial hyperglycemia. One strategy used to stabilize postprandial blood glucose has been the utilization of specific enzyme in-

hibitors which act against gut digestive enzyme by interrupting rapid nutrient absorption[1].

α-Amylase is an enzyme catalyzing the digestion of carbohydrates, and the uptake of carbohydrates in the intestines can be slowed down by the powerful synthetic inhibitors of the enzymes. However, the powerful inhibitory action caused side effects like flatulence, diarrhea and abdominal cramping, all of which are associated with incomplete[2]. The purpose of this study is to investigate and evaluate α-amylase inhibitory effects from *Schizandra chinensis*(*SC*) and to provide scientific evidence of *SC* as a natural anti-diabetic or functional food.

Material & Method

1. Extract of α-amylase inhibitor

The dried of plant sample was extract with 10 times weight of ethanol 80% (1:10 w/v) for 7 days at 4℃. The obtained extract was concentrated and dried in vacuum falling evaporator after filtration. The dried extract was dissolved in distilled water and stored in -70℃, until further use.

2. Streptozotocin(STZ) induced diabetic rats

Six-weeks-old Sprague-Dawely male rats, approximately 180 g were purchased from Daehan Biolink Co., Ltd. and were made diabetic by injection of freshly prepared solution of streptozotocin(STZ, Sigma, USA) dissolved in 0.01 M citrate buffer (pH 4.5) at the dose of 45 mg/kg body weigh after an overnight fast. After 48 h, the rats having blood glucose levels above 250 mg/dl were used in this study[3].

3. Preparation of α-amlyase

The brush-border mucosal layer of small intestine from a diabetic rats overnight fasted was obtained by careful scraping, and diluted with cold saline. Next to breakdown on a sonicator for 15 sec, it was centrifuged at 1,000×g, 4℃ for 30 min and the supernatant was used as crude enzyme solution in vitro[4].

4. Inhibition of α-amylase activity

The α-amylase activity was measured by the method of Bernfeld(1955)[5]. To 0.1 ml of sample, 0.25 ml 1% starch solution in 40 mM phosphate buffer(pH 6.9), 0.05 ml crude enzyme were added and the mixture was incubated at 37℃, 5 min. Reaction was stooped by addition of 0.4 ml of dinitrosalicylic acid reagent(DNS). This solution was boiled for 10 min, then cooled and diluted with 0.4 ml of distilled water. The absorbance of reaction products was measured at 540 nm. One unit of α-amylase inhibition was defined as the amount of inhibition agent required to inhibit 1% of α-amylase activity under reaction conditions. Appropriate blanks were prepared using crude enzyme boiled for 3 min.

$$Inhibition(\%) = (1 - \frac{[A(inhibition) - A(control)]}{[A(enzyme) - A(blank)]}) \times 100$$

5. Kinetics of enzyme inhibition

In order to examine the inhibition mode by *SC*-E, α-amylase activity was measured with increasing concentration of 1% starch(0.5-10 mM) in the absence or presence of *SC*-E at different concentrations (5, 10, 100, and 1000 ㎍/ml). Optimal doses of *SC*-E were determined based on the results from inhibitory activity assay as described above. Inhibition type for *SC*-E was determined by Lineweaver-Burk plot analysis of the resulted from enzyme assays containing various concentrations of 1% and *SC*-E.

6. Measurement of *SE*-E on blood glucose levels after starch loading

Animals were allocated into five groups of six rats and starch (2 g/kg BW) was administered simultaneously with *SC*-E (50 and 100 mg/kg BW) after deprivation of food for 16 h. The rats of normal and control groups were given only starch in saline solution. Blood samples were taken from the tail vein before and 0(preintubation), 30, 60, 90, 120, and 180 min after the starch administration, and assessed for the glucose levels using glucometer and test-strips(Roche Ltd., Accu-chek active, Germany).

Result & Discussion

The treatment goal for patient with type 2 diabetes mellitus is generally agreed to be to maintain near-normal levels of glycemic control, both in the fasting and postprandial states. Postprandial hyperglycemia is the earliest metabolic abnormality to occur in type 2 diabetes mellitus[6]. Postprandial blood glucose levels may be elevated in the presence of normal levels of fasting plasma glucose(FPG), constituting an early stage in type 2 diabetes, which some have called "postprandial diabetes"[7]. This state not only initiates the development of early microvascular and macrovascular complications, but also can contributes to a more rapid progression to symptomatic diabetes by causing glucose toxicity in muscle and pancreatic beta cells[1].

α-Amylase is an enzyme catalyzing the digestion of carbohydrates, and the uptake of carbohydrates in the intestines can be slowed down by the powerful synthetic inhibitors of the enzymes. α-Amylase inhibitors block the actions of α -amylase enzymes in the small intestine, which is rate-limitng in the conversion of oligosaccharides and disaccharides to monosaccharidesm necessary for gastrointestinal absorption[2]. In the present study, acarbose, used as a positive control, effectively inhibited α-amlyase activity (IC_{50}: 0.145 mg/ml). *SC*-E showed a more potent effect on α-amylase activity (IC_{50}: 0.044 mg/ml) (Table 1).

The inhibition mode of *SC*-E against α-amylase activity was analyzed by Lineweaver-Burk plots using the data derived from enzyme containing various concentration of 1% starch (0.5-10 mM) at each different concentration of *SC*-E. Double-reciprocal plots of enzyme kinetics demonstrated competitive inhibition of α-amylase activity by *SC*-E (Fig. 1). The K_m value of 1% starch for α-amylase was 0.296 mM and the K_i value of *SC*-E was 0.77 mg/ml.

In present study examined whether *SC*-E could exert the inhibitory effect on the increase of the blood glucose level after starch loading in the rats fasted for 16 h. Blood glucose concentration increased from 162 to 565 mg/dl 30 min after administration of starch (2 g/kg BW), and decreased thereafter. Acarbose (20 mg/kg BW), as a reference drug, was also shown to suppress the rise of blood glucose level after starch loading (Fig. 2).

In the present study, oral administration of *SC*-E extract lowered plasma glucose

levels in SD rats. Furthermore, this treatment inhibited the increase of plasma glucose levels after starch loading in rats. The results suggest that *SC*-E extracts may be have therapeutical potential in postprandial hyperglycemia

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Table 1. α-Amylase inhibitory activities of *SC*-E.

	Yield (㎎/㎖)	Inhibition(%) Concentration(㎍/㎖)[1] 10	50	100	IC_{50}[2] (㎎/㎖)
SE-E	71	51.75	50	57.84	0.044
Acarbose[3]	-	43.86	44.35	47.58	0.145

[1]The final concentration in the reaction mixture.

[2]The concentration which caused 50% inhibition of rat intestinal α-amylase activity were calculated from regression equation.

[3]Acarbose : reference drug, Glucoby®-Bayer

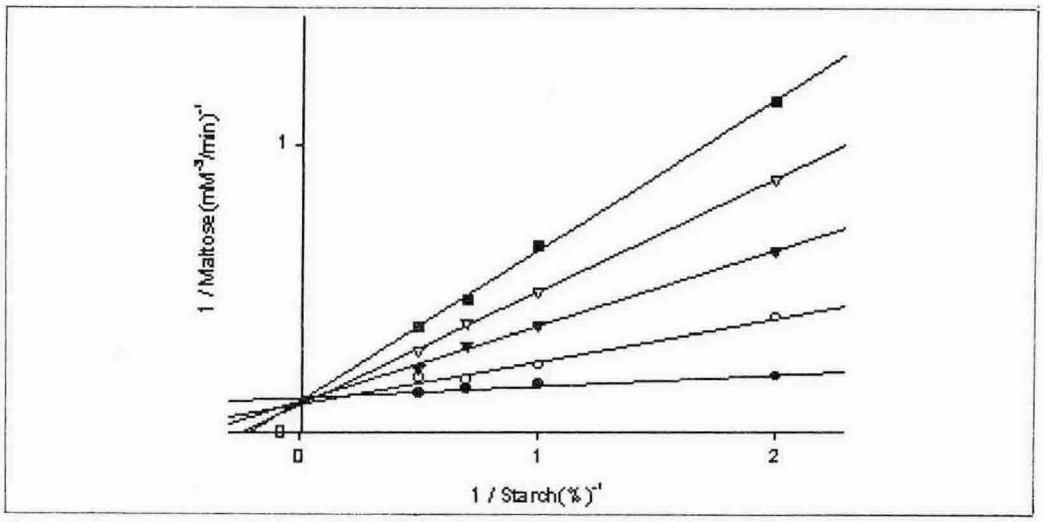

Fig. 1. Lineweaver-Burk plots of the inhibition of rat intestinal α-amylase by *SC*-E. (symbols : -■-, 1000 ㎍/㎖ of *SE*-E; -▽-, 100 ㎍/㎖ of *SE*-E; -▼-, 10 ㎍/㎖ of *SE*-E; -○-, 5 ㎍/㎖ of *SE*-E; -●-, control(without inhibitor))

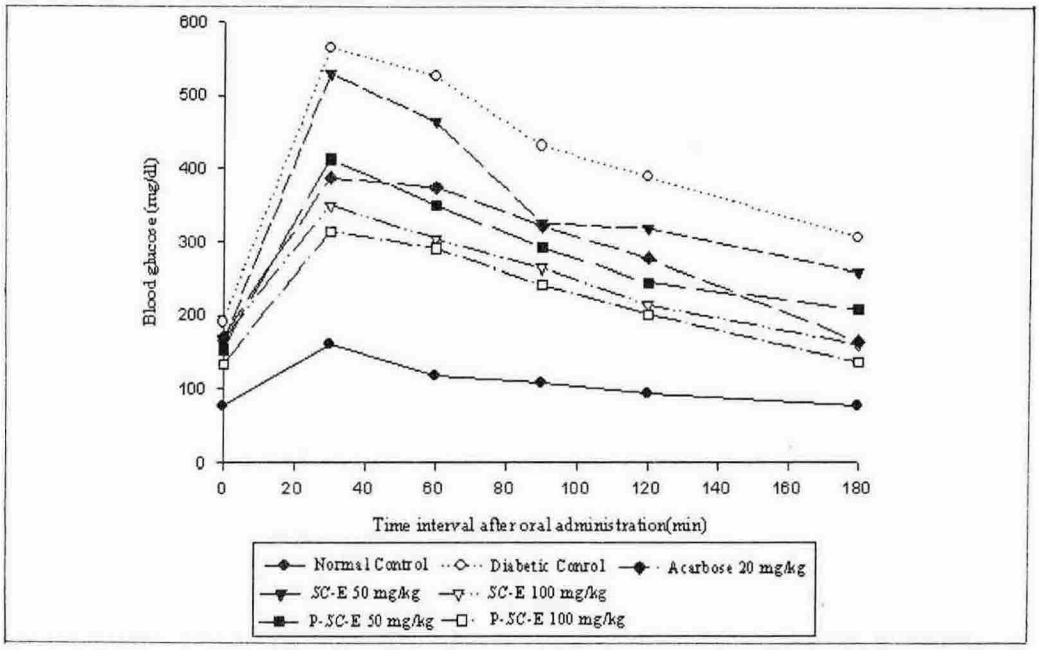

Fig. 2. Inhibitory effect of *SC*-E and acarbose on blood glucose elevation in diabetic rat loaded starch.

P-*SE*-E : On days 7, after pre-treatment of *SC*-E, diabetes was induced by STZ. Acarbose:reference drug, Glucoby®-Bayer