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The Inhibitory Activity of *Polygonum Multiflorum* Thunberg and its Effect on Postprandial Hyperglycemia in Streptozotocin-induced Diabetic Rats*

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To keep blood glucose levels as close to normal as possible is the major goal of diabetes mellitus treatment. α-Glucosidase is the enzyme that digests dietary carbohydrate and inhibition of this enzyme may suppress postprandial hyperglycemia. The methanol extract of *Polygonum multiflorum* Thunberg was tested for inhibitory activity against α-glucosidase *in vitro* and *in vivo*. *Polygonum multiflorum* Thunberg extract inhibited yeast α-glucosidase activity in a concentration-dependent manner. *Polygonum multiflorum* Thunberg showed an IC_{50} value of 0.48 mg/mL. The ability of *Polygonum multiflorum* Thunberg extract to lower postprandial glucose was studied in streptozotocin-induced diabetic rats. A starch solution (1 g/kg) with and without the methanol extract of *Polygonum multiflorum* Thunberg extract (500 mg/kg) was administered to diabetic rats by gastric intubation after an overnight fast. A single oral dose of *Polygonum multiflorum* Thunberg extract significantly inhibited increases in blood glucose levels at 60 and 90 min ($P<0.05$) and significantly decreased incremental response areas under the glycemic response curve ($P<0.05$). These results suggest that *Polygonum multiflorum* Thunberg may have an antihyperglycemic effect by inhibiting α-glucosidase activity in the animal model of diabetes mellitus.

Key words: *Polygonum multiflorum* Thunberg, α-glucosidase, Antihyperglycemic effect, Diabetes mellitus

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INTRODUCTION

Diabetes is the fourth leading cause of death among Koreans[1)] and the prevalence of diabetes mellitus among Koreans is increasing due to an aging population, increased urbanization and more sedentary lifestyles.[2)] Cardiovascular disease (CVD), a major complication of diabetes, is the leading cause of premature death among patients with diabetes.[3)] It is well known that achieving near-normal glycemic control in patients with diabetes mellitus is associated with sustained, decreased rates of diabetes-related cardiovascular complications.[4,5)] Avignon *et al.*[6)] have reported that postprandial glucose levels are a better marker of glycemic control than are fasting blood glucose levels in patients with type 2 diabetes. Microvascular and macrovascular complications are associated with postprandial hyperglycemia but not with fasting blood glucose levels.[7-10)]

α-Glucosidase is an enzyme that catalyzes the final step in the carbohydrate digestive process and, hence, α-glucosidase inhibitors could delay the use of dietary carbohydrates and minimize increases in postprandial glucose levels. α-Glucosidase inhibitors such as acarbose,[11)] voglibose[12)] and miglitol[13)] are used as oral hypoglycemic agents. However, chronic use of these agents could result in side effects such as flatulence, abdominal cramping, vomiting and diarrhea so that their use may be limited.[14)] Therefore, numerous studies have been carried out to isolate α-glucosidase inhibitors from natural products without side effects.[15-17)]

Polygonum multiflorum Thunberg is a medicinal plant distributed in Korea, China, Japan and Vietnam.[18)] Traditionally, it has been used to treat elevated serum cholesterol and coronary heart diseases. It was also reported that *Polygonum multiflorum* Thunberg has a strong antioxidant effect.[19)] However, the hypoglycemic

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effect of *Polygonum multiflorum* Thunberg has not been studied. Thus, in this study we measured the α-glucosidase inhibitory activity of *Polygonum multiflorum* Thunberg *in vitro* and *in vivo* to evaluate its possible use as an antihyperglycemic agent.

MATERIALS AND METHODS

1. Reagents

Yeast α-glucosidase, p-nitropheny-α-D-glucopyranoside and streptozotocin (STZ) were purchased from Sigma Chemical Co (St. Louis, MO, USA) and a glucose assay kit was purchased from Yeongdong Co (Seoul, South Korea).

2. Preparation of the Methanol Extract

Polygonum multiflorum Thunberg was obtained from a local market in Busan, Korea. *Polygonum multiflorum* Thunberg was powdered and extracted with ten volumes of methanol for 12 hrs three times at room temperature. The solvent was removed by rotary evaporation at 40 ℃. The extract was dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/mL to be used as a test sample.

3. Measurement of Yeast α-glucosidase Inhibitory Activity *in vitro*

Yeast α-glucosidase inhibitory activity was determined using the chromogenic method developed by Watanabe *et al.*[16] Yeast α-glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin, 0.2 g/L NaN_3 and 5 mM p-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as an enzyme and a substrate solution. 50 μL of the enzyme solution and 10 μL of the test sample at various concentrations were mixed and absorbance at 405 nm was measured using a microplate reader (model 550, BioRad, Hercules, CA, USA). After incubation for 5 min, 50 μL of the substrate solution was added and incubated for an additional 5 min. The increase in absorbance from zero time was measured and inhibitory activity was calculated as a percent of blank control. The final concentration of the extract and acarbose used was 0.5 mg/mL. The inhibitory activity of the extract against α-glucosidase at concentrations of 0.25, 0.10 and 0.05 mg/mL was also measured. 50% inhibitory concentration (IC_{50}) was defined as the concentration that inhibited 50% of enzyme activity under the assay conditions.

4. Animals

Male Sprague-Dawley rats weighing between 270 and 300 g were purchased from Bio Genomics, Inc. (Seoul, South Korea). The rats were housed individually in stainless steel wire-bottomed cages and located in a room temperature (23~27 ℃), humidity (50~60%), and lighting cycle (0600~1800 hr light and 1800~0600 hr dark) were controlled. The animals were fed a commercial chow diet (Samyang Co., Seoul, Korea) *ad libitum* for 7 days after arrival. The animals were rendered diabetic by intraperitoneal injection of STZ (60 mg/kg) in citrate buffer, pH 4.5. Blood samples were taken from the tail tip after 7 days and blood glucose concentration was measured using a glucometer (Glucotrend Roche Diagnostics, United Kingdom). Animals showing fasting blood glucose levels higher than 200 mg/dL were considered diabetic and used for further study. All the animals continued to be fed a commercial chow diet.

5. Measurement of Postprandial Blood Glucose

The effect of *Polygonum multiflorum* Thunberg extract on postprandial glucose was measured in STZ-induced diabetic rats (n = 16). The rats were randomly divided into two groups. After being starved overnight, fasting blood samples were collected from the tail tip. The rats were offered starch (1 g/kg) alone or starch with methanol extract of *Polygonum multiflorum* Thunberg (500 mg/kg) by gastric intubation. Blood samples were collected from the tail tip at 30, 60, 90, 120, 180 and 240 min. Feed was withheld during the test. Plasma glucose was measured using a commercial glucose oxidase kit (Yeongdong Co., Seoul, South Korea). Plasma glucose level was expressed as increments from baseline. Incremental areas under the response curve (AUC) were calculated using the trapezoidal rule with fasting levels as the baseline.

6. Statistical Analysis

Increment plasma glucose level and AUC of the glucose response curve are expressed as mean±standard error of the mean (S.E.M.). Differences between incremental plasma glucose levels and AUC of the control and *Polygonum multiflorum* Thunberg group were assessed using Student's t-test. Significance was defined as $P<0.05$.

RESULTS

1. Inhibition of α-glucosidase Activity *in vitro*

The inhibitory activity of the methanol extract of *Polygonum multiflorum* Thunberg against yeast α-glucosidase is shown in Table 1. The methanol extract of *Polygonum multiflorum*

Table 1. Inhibitory activities of the methanol extract of *Polygonum multiflorum* Thunber on yeast α-glucosidase

Sample	α-glucosidase inhibitory activities(%)
Polygonum multiflorum Thunberg	51.8
Acarbose	28.4

The methanol extract of *Polygonum multiflorum* Thunber and acarbose were dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/mL, respectively.

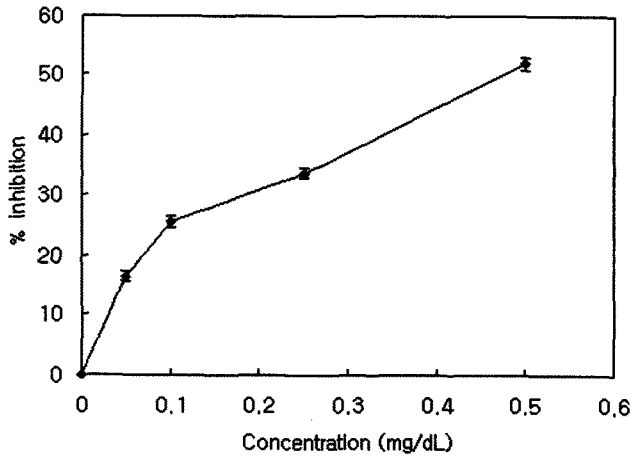

Fig. 1 Dose-dependent inhibition of yeast α-glucosidase activity of *Polygonum multiflorum* Thunber

The methanol extract of *Polygonum multiflorum* Thunber was dissolved in dimethylsulfoxide(DMSO) at a concentration of 0.5, 0.25, 0.1, 0.05 mg/mL, respectively.

Thunberg inhibited yeast α-glucosidase activity by 51.8% at a concentration of 0.5 mg/mL *in vitro*. Acarbose, an α-glucosidase inhibitor, which is used for an oral hypoglycemic agent inhibited enzyme activity by 28.4%. Inhibitory activity of *Polygonum multiflorum* Thunberg extract against yeast α-glucosidase was 33.4%, 25.5% and 16.5% at concentrations of 0.25, 0.10 and 0.05 mg/mL, respectively (Fig. 1). *Polygonum multiflorum* Thunberg extract showed an IC_{50} value of 0.48 mg/mL.

2. Inhibition of α-glucosidase Activity *in vivo*

The plasma glucose response to a single oral dose of starch (1 g/kg) alone or starch with *Polygonum multiflorum* Thunberg extract (500 mg/kg) is shown in Fig. 2. Fasting plasma glucose levels of the control group and *Polygonum multiflorum* Thunberg extract group were 258.2±10.1 mg/dL and 263.4±11.9 mg/dL, respectively. The incremental plasma glucose levels of the rats that consumed starch were 66.0±10.8, 111.6±10.2, 95.1±9.2, 66.8±4.5, 24.7±5.0 and 1.4±4.9 mg/dL at 30, 60, 90, 120, 180 and 240 min, respectively. The incremental plasma glucose levels of the rats that consumed *Polygonum multiflorum* Thunberg extract with starch were 55.9±6.5, 82.6±6.9, 64.3±7.9, 54.6±6.8,

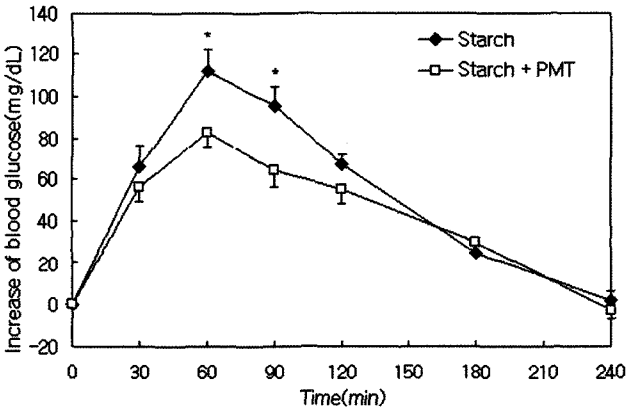

Fig. 2 Postprandial glucose response to *Polygonum multiflorum* Thunber extract in streptozotocin (STZ)-induced diabetic rats

Control group (◆): Starch (1 g/kg) was administered orally to a rat after an overnight-fast.

PMT group (□): Starch (1 g/kg) with the methanol extract of *Polygonum multiflorum* Thunber (500 mg/kg) was administered orally to a rat after an overnight-fast.

The values were expressed as the mean±SEM.

*Significantly different at P<0.05

Table 2. Area under the curve (AUC) of postprandial glucose responses of streptozotocin-induced diabetic rats

	AUC (mg · min/dL)
Control group[1)]	12,718±535
PMT group[2)]	10,146±765*

1) Starch (1 g/kg) was administered orally to a rat after an overnight-fast.

2) Starch (1 g/kg) with the methanol extract of *Polygonum multiflorum* Thunber (500 mg/kg) was administered orally to a rat after an overnight-fast.

The values were expressed as the mean±SEM.

*Significantly different at P<0.05

30.1±5.9, -3.1±4.1 mg/dL at 30, 60, 90, 120, 180 and 240 min, respectively. Consumption of *Polygonum multiflorum* Thunberg extract significantly decreased incremental plasma glucose levels at 60 and 90 min (P<0.05).

The area under the curve (AUC) for glucose response of the *Polygonum multiflorum* Thunberg group (10,146± 765 mg · min/dL) was significantly lower than that of the control group (12,718±535 mg · min/dL, P<0.05, Table 2).

DISCUSSION

α-Glucosidase is a key enzyme in carbohydrate digestion in the small intestine.[20)] Therefore, α-glucosidase inhibitors could delay digestion to reduce postprandial glucose. At present, α-glucosidase inhibitors are the most common oral agents in improving postprandial hyperglycemia.[21)] Chronic consumption of acarbose, voglibose and miglitol was reported to improve fasting hyperglycmia and decrease glycated hemoglobin.[21)] It was also reported that acarbose was effective in preventing obesity.[22)] However, it is well

documented that synthetic α-glucosidase inhibitors have undesirable side effects, such as flatulence, diarrhea and abdominal cramping.[23] Therefore, many natural plants with fewer side effects have been used in the prevention and treatment of diabetes mellitus.[20,24-26] *Punica granatum* flower,[20] *Commelina communis* L.[24] and gall of *Rhus chinensis*[25] inhibit α-glucosidase activity. Nishioka *et al.*[26] demonstrated that the methanol extracts of *Scutellaria baicalensis, Rheum officinale,* and *Paeonia suffruticosa* showed potent inhibitory activity against α-glucosidase.

In this study, we investigated the inhibitory effect of *Polygonum multiflorum* Thunberg against α-glucosidase to elucidate the possible use of *Polygonum multiflorum* Thunberg as an antihyperglycemic agent. Inhibition activity of the methanol extract of *Polygonum multiflorum* Thunberg against yeast α-glucosidase was about 180% that of acarbose *in vitro* (Table 1). *Polygonum multiflorum* Thunberg extract showed inhibitory activity against yeast α-glucosidase in a dose-dependent manner (Fig. 1).

We determined the antihyperglycemic effect of *Polygonum multiflorum* Thunberg in STZ-induced diabetic rats after consumption of starch. Postprandial plasma glucose peaked at 60 min after consumption of starch in the control group. The *Polygonum multiflorum* Thunberg extract significantly suppressed incremental plasma glucose at 60 and 90 min (Fig. 2). These data demonstrate that *Polygonum multiflorum* Thunberg exerts α-glucosidase inhibitory activity *in vivo* to attenuate peak postprandial glucose level by delayed glucose absorption. Consumption of the *Polygonum multiflorum* Thunberg extract AUC for the glucose response curve compared with that of the control group is shown in Table 2.

The main benefit of α-glucosidase inhibitors, including acarbose, is to reduce postprandial glycemic levels, which are associated with micro- and macrovascular complications, to a greater extent than fasting glucose.[9] Inoue *et al.*[27] reported the medication which flattens peak postprandial blood glucose showed a reduction of AUC of the blood glucose response curve. In this study, *Polygonum multiflorum* Thunberg extract decreased incremental glucose at peak time point and AUC. Postprandial hyperglycemia is one of the earliest observable abnormalities in diabetes mellitus. Postprandial hyperglycemia is highly correlated with glycated hemoglobin levels and is a better predictor of glycated hemoglobin levels than fasting glucose.[28] Postprandial hyperglycemia contributes to complications associated with the disease. Bastyr *et al.*[29] demonstrated that diabetes therapy focused on lowering postprandial glucose versus fasting glucose could be a better treatment. Thus, chronic consumption of *Polygonum multiflorum* Thunberg could be helpful in improving postprandial hyperglycemia and preventing diabetic complications.

Further study to isolate and identify the active component responsible for the inhibition of α-glucosidase is strongly recommended. Also, it might be worthwhile to evaluate the effect of chronic consumption of *Polygonum multiflorum* Thunberg on fasting and postprandial hyperglycemia.

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