

Gynura procumbens Extract Alleviates Postprandial Hyperglycemia in Diabetic Mice

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ABSTRACT: This study was designed to investigate the inhibitory effect of *Gynura procumbens* extract against carbohydrate digesting enzymes and its ability to ameliorate postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice. *G. procumbens* extract showed prominent α -glucosidase and α -amylase inhibitory effects. The half-maximal inhibitory concentration (IC₅₀) of *G. procumbens* extract against α -glucosidase and α -amylase was 0.092±0.018 and 0.084±0.027 mg/mL, respectively, suggesting that the α -amylase inhibition activity of the *G. procumbens* extract was more effective than that of the positive control, acarbose (IC₅₀=0.164 mg/mL). The increase in postprandial blood glucose levels was more significantly alleviated in the *G. procumbens* extract group than in the control group of STZ-induced diabetic mice. Moreover, the area under the curve significantly decreased with *G. procumbens* extract administration in STZ-induced diabetic mice. These results suggest that *G. procumbens* extract may help alleviate postprandial hyperglycemia by inhibiting carbohydrate digesting enzymes.

Keywords: *Gynura procumbens* extract, α -glucosidase, α -amylase, postprandial hyperglycemia

INTRODUCTION

Diabetes mellitus is a common endocrine system disorder that involves metabolic dysfunction (1,2). In diabetes, a rapid increase in blood glucose levels appears in the postprandial phase. Postprandial hyperglycemia is strongly related to the development of type 2 diabetes and diabetic complications, such as macrovascular and microvascular diseases (3). It has been reported that regulating postprandial hyperglycemia is more important than high fasting blood glucose levels (4). Postprandial hyperglycemia is also an independent risk factor for vascular diseases. Therefore, controlling postprandial hyperglycemia is essential for treating diabetic patients as well as the prevention of diabetic complications.

One of the most effective ways to lower postprandial hyperglycemia is by limiting the activities of carbohydrate-hydrolyzing enzymes in order to inhibit the entry of glucose into the intestinal endothelial cells (5). In humans, dietary starches are digested by the actions of pancreatic α -amylase and intestinal α -glucosidase to yield individual monosaccharides (6). Inhibitors of α -glucosidase are effective at suppressing postprandial hyperglycemia by limiting glucose absorption (7). Widely used as oral hy-

poglycemic agents, α -glucosidase inhibitors such as acarbose and voglibose directly reduce postprandial hyperglycemia and prevent vascular complications (8,9). However, long-term use of these drugs can lead to unwanted side effects, including vomiting, weight gain, and diarrhea (10,11). Thus, studies have focused on developing natural, plant-based inhibitors of α -glucosidase and α -amylase without side effects.

Gynura procumbens (Lour.) Merr. (*G. procumbens*) is a member of the Asteraceae family. It has received attention as a folk remedy for treating diabetes. It has been reported that *G. procumbens* extract (GPE) showed anti-hypertensive (12), antioxidant (13), and anti-inflammatory effects (14). It has also been shown that a combination of *Azadirachta indica* and *G. procumbens* extracts (ratio of 50 mg/kg *A. indica* : 112.5 mg/kg *G. procumbens*) has anti-hyperglycemic effects in alloxan-induced diabetic rats (15). However, there are no studies thus far showing the effects of GPE on alleviating postprandial hyperglycemia through the inhibition of carbohydrate digestive enzymes in a diabetic animal model. Diabetes is induced by streptozotocin (STZ), a glucosamine-nitrosourea compound derived from *Streptomyces achromogenes* that is used clinically as a chemotherapeutic agent in the treatment of pan-

Received 14 April 2016; Accepted 16 August 2016; Published online 30 September 2016

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creatic β cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia. Therefore, the purpose of this study was to examine the effects of GPE on blood glucose levels in normal and STZ-induced diabetic mice. In addition, we investigated the inhibitory effects of GPE on α -glucosidase and α -amylase, as well as the effects of GPE on postprandial hyperglycemia.

MATERIALS AND METHODS

Materials and preparation of GPE

G. procumbens was collected from Uiwang, Gyeonggi, Korea. The plant was washed with distilled water, freeze dried, and ground into a powder. The *G. procumbens* powder was then extracted 3 times with water for 12 h at room temperature. The GPE was concentrated in a rotary vacuum evaporator, freeze-dried to a powder, and then stored in a deep freezer (-80°C). All other chemicals and reagents, including α -glucosidase and α -amylase, were of analytical grade and were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals and reagents were used without any further purification.

The total flavonoid content of GPE was 10.33 ± 0.88 mg catechin equivalents (CE)/g dry weight (DW). GPE contained 464.53 ± 1.81 $\mu\text{g/g}$ DW of kaempferol, 251.10 ± 3.67 $\mu\text{g/g}$ DW of myricetin, and 135.87 ± 0.40 $\mu\text{g/g}$ DW of quercetin (16).

Inhibition of α -glucosidase activity by GPE *in vitro*

The α -glucosidase inhibition assay was conducted using a chromogenic method, as described by Watanabe et al. (17), with a readily available yeast enzyme. Briefly, yeast α -glucosidase (0.7 U; Sigma-Aldrich Co.) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3 and used as an enzyme solution. Five mM *p*-nitrophenyl- α -D-glucopyranoside (*p*NGP) in the same buffer (pH 7.0) was used as the substrate solution. Next, 50 μL of the enzyme solution and 10 μL of sample dissolved in dimethylsulfoxide (5 mg/mL) were mixed in each well of a microtiter plate, and the titer was measured by determining the absorbance at 405 nm at time 0 using a microplate reader. After incubation for 5 min, the substrate solution (50 μL) was added and incubated for another 5 min at room temperature. The increase in absorbance from time 0 was measured. The inhibitory activity was expressed as 100 minus the absorbance difference (%) of the test compounds relative to the absorbance change of the control (the test solution was replaced by the carrier solvent).

Inhibition of α -amylase activity by GPE *in vitro*

The inhibitory activity of α -amylase was assayed in the

same way as described for α -glucosidase inhibition, except that porcine pancreatic amylase (100 U) and *p*-nitrophenyl- α -D-maltopentoglycoside were used as the enzyme and substrate, respectively.

Measurement of cytotoxicity

Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 3T3-L1 mouse embryo fibroblasts were seeded at the concentration of 1×10^4 cells/well in 96 well plates and pre-incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h. After that, the cells were treated with various concentrations (0.1, 0.5, 1, and 2 mg/mL) of GPE, and further incubated for 24 h. After completion of the treatment, the cells were incubated for 3 h at 37°C with filtered MTT (Sigma-Aldrich Co.) solution, which was added to each well to reach a final concentration of 0.5 mg MTT/mL. The supernatants were carefully aspirated, 200 μL of dimethyl sulfoxide was added to each well, and the plates were agitated to dissolve the crystal product. The absorbance was measured at 540 nm with a microplate reader.

Experimental animals

Male ICR mice (aged 4 weeks; purchased from Joong Ang Lab Animal Co., Seoul, Korea) were used. All animals were housed individually in a room with controlled light (12-h on/12-h off) and temperature, and pelleted food and water were available *ad libitum*. After an adjustment period of 2 weeks, diabetes was induced by intraperitoneal (i.p.) injection of 60 mg/kg STZ freshly dissolved in a citrate buffer (0.1 M, pH 4.5). After 7 days, tail bleeds were performed and animals with a blood glucose concentration above 250 mg/dL were considered diabetic. All procedures for handling and care of animals were approved by the animal ethics committee at Pusan National University (PNU2014-0619).

Measurement of blood glucose levels

Normal mice and STZ-induced diabetic mice were fasted overnight and randomly divided into 3 groups ($n=7$ mice each). Fasted animals were deprived of food for at least 12 h, but allowed free access to water. After overnight fasting, the mice were orally administered soluble starch [2 g/kg body weight (bw)] alone, with GPE (300 mg/kg bw), or with acarbose (100 mg/kg bw). Blood samples were taken from the tail vein at 0, 30, 60, and 120 min (18). Blood glucose was measured using a glucometer (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). The areas under the curve (AUC) were calculated using the trapezoidal rule.

Data and statistical analysis

The data are presented as the mean \pm standard deviation

(SD). Statistical analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The Student's t-test was used for comparisons between the control and sample groups. Differences were evaluated by one-way analysis of variance (ANOVA), followed by post-hoc Duncan's multiple range tests ($P < 0.05$).

RESULTS AND DISCUSSION

Inhibitory effect of GPE on α -glucosidase and α -amylase *in vitro*

The inhibitory effects of GPE against α -glucosidase were examined using *p*NGP as the substrates and were compared to that of the commercial α -glucosidase inhibitor acarbose (Fig. 1). The activity of α -glucosidase was inhibited by GPE in a concentration-dependent manner, with 36.88 ± 7.35 , 52.58 ± 5.28 , 63.78 ± 3.08 , and $75.81 \pm 3.31\%$ inhibition at concentrations of 0.05, 0.1, 0.25, and 0.50 mg/mL, respectively. Acarbose, an α -glucosidase inhibitor used as an oral hypoglycemic agent, inhibited the enzyme activity by 68.22% at a concentration of 0.10 mg/mL. The inhibitory effects of GPE on α -amylase activity were determined in a similar manner (Fig. 2). The inhibitory effects of GPE against α -amylase increased in a concentration-dependent manner (40.21 ± 6.55 , 54.33 ± 8.05 , 67.75 ± 4.42 , and $78.53 \pm 3.15\%$ activity inhibition at concentrations of 0.05, 0.1, 0.25, and 0.50 mg/mL, respectively). Moreover, the inhibitory effects of GPE were more effective than that of acarbose even at the low concentration (0.10 mg/mL). The IC_{50} values of GPE against α -glucosidase and α -amylase were 0.092 ± 0.018 and 0.084 ± 0.027 mg/mL, respectively (Table 1). These results indicate that GPE may prove useful as a natural anti-hyperglycemic material by inhibiting α -glucosidase and α -amy-

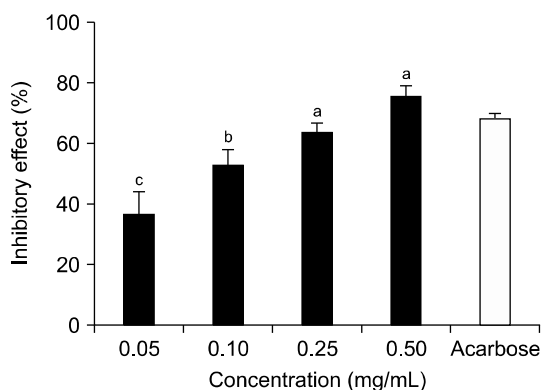


Fig. 1. Inhibitory effects of *Gynura procumbens* extract on α -glucosidase. Inhibitory effect was determined using *p*-nitrophenyl- α -D-glucopyranoside as the substrate. Acarbose was used as a positive control. Each value is expressed as the mean \pm SD of triplicate experiments. Values with different letters (a-c) are significantly different at $P < 0.05$ as analyzed by Duncan's multiple range test. The final concentration of acarbose was 0.1 mg/mL.

lase.

One effective method for controlling postprandial hyperglycemia is to inhibit carbohydrate hydrolyzing enzymes (intestinal α -glucosidase and pancreatic α -amylase) in order to slow down glucose absorption (18). Alpha-amylase begins the process by catalyzing the hydrolysis of polysaccharide α -1,4-glycosidic linkages, forming disaccharides; α -glucosidase further catalyzes the breakdown of disaccharides into simple sugars, which are available for absorption by intestinal cells (19,20). The inhibition of α -glucosidase and α -amylase activity might be an effective way to delay carbohydrate digestion and lower postprandial hyperglycemia. Therefore, identification of effective carbohydrate-hydrolyzing inhibitors from natural products to alleviate postprandial hyperglycemia without unwanted secondary effects is necessary (21).

This study was designed to investigate the inhibitory activities of GPE against α -glucosidase and α -amylase in an attempt to identify its efficacy as a natural product to alleviate postprandial hyperglycemia. GPE showed noticeable inhibition of both α -glucosidase and α -amylase activities, and it did not exert any cytotoxicity (Fig. 3).

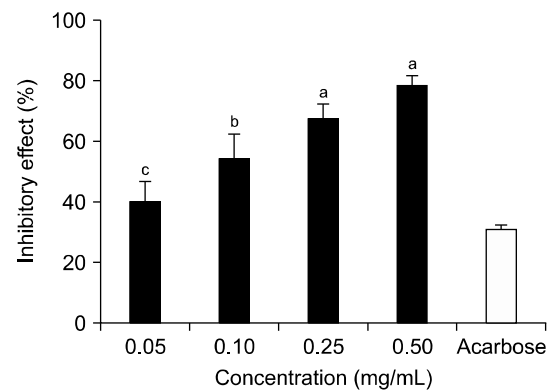


Fig. 2. Inhibitory effects of *Gynura procumbens* extract on α -amylase. Inhibitory effect was determined using *p*-nitrophenyl- α -maltopentoglycoside as the substrate. Acarbose was used as a positive control. Each value is expressed as the mean \pm SD of triplicate experiments. Values with different letters (a-c) are significantly different at $P < 0.05$ as analyzed by Duncan's multiple range test. The final concentration of acarbose was 0.1 mg/mL.

Table 1. IC_{50} values of inhibitory activity of GPE on α -glucosidase and α -amylase

Sample	IC_{50} (mg/mL) ¹⁾	
	α -Glucosidase	α -Amylase
GPE ²⁾	0.092 ± 0.018^{NS}	$0.084 \pm 0.027^*$
Acarbose	0.075 ± 0.006	0.164 ± 0.033

¹⁾The IC_{50} value is the concentration of sample required for 50% inhibition. Each value is expressed as the mean \pm SD in triplicate experiments.

²⁾GPE: *Gynura procumbens* extract.

NS: not significant.

* $P < 0.05$ compared to the control group.

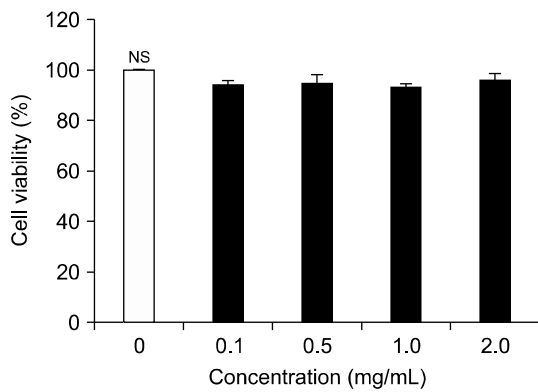


Fig. 3. Cytotoxic effect of *Gynura procumbens* extract (GPE) in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0.1, 0.5, 1.0, and 2.0 mg/mL) of GPE for 24 h, and cell viability was measured via the MTT assay. Each value is expressed as mean±SD of triplicate experiments. NS: not significant.

GPE contains various kinds of flavonoids, such as kaempferol, myricetin, and quercetin. Flavonoids have been reported to inhibit α -glucosidase and α -amylase activities (22). These flavonoids may inhibit the α -glucosidase and α -amylase activities by influencing their hydrophobic and hydrophilic properties at a molecular level (23). Tadera et al. (7) reported that hydroxyl substitution on the phenyl ring structure of myricetin might be effective in inhibiting the enzyme's activity. Additionally, kaempferol-3-O-glycoside has remarkable α -glucosidase inhibition ability (24). Therefore, it is possible that the flavonoids contained in GPE play a role in the inhibitory effects on α -glucosidase and α -amylase.

Effects of GPE on blood glucose levels *in vivo*

The effects of GPE on blood glucose levels following a meal were investigated in normal and STZ-induced dia-

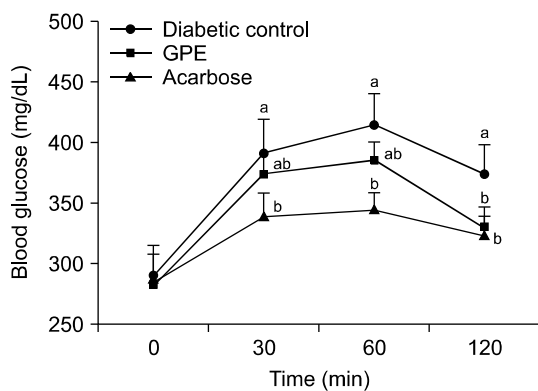


Fig. 4. Blood glucose levels after administration of *Gynura procumbens* extract (GPE) in STZ-induced diabetic mice. GPE (300 mg/kg), acarbose (100 mg/kg), and distilled water as a control were co-administered orally with starch (2 g/kg). Each value is expressed as the mean±SD of seven mice (n=21). Values with different letters (a,b) are significantly different at $P<0.05$ as analyzed by Duncan's multiple range test.

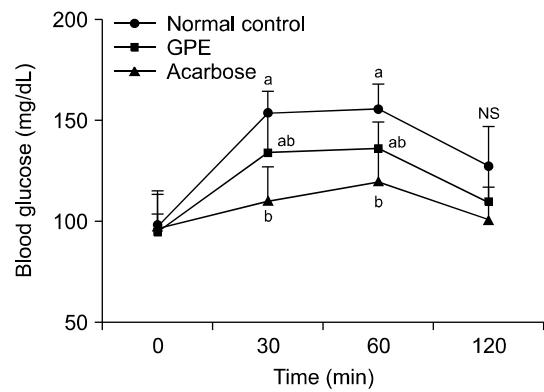


Fig. 5. Blood glucose levels after administration of *Gynura procumbens* extract (GPE) in normal mice. GPE (300 mg/kg), acarbose (100 mg/kg), and distilled water as a control were co-administered orally with starch (2 g/kg). Each value is expressed as the mean±SD of seven mice (n=21). Values with different letters (a,b) are significantly different at $P<0.05$ as analyzed by Duncan's multiple range test. NS: not significant.

betic mice. Postprandial blood glucose levels in mice administered GPE were significantly lower than those of the control diabetic mice (Fig. 4). Blood glucose levels in the control diabetic mice increased to 391.00 ± 28.35 at 30 min and 414.40 ± 26.19 mg/dL at 60 min after a meal, and then decreased to 374.00 ± 24.47 mg/dL at 120 min. However, when GPE was added to the food, the increase in postprandial blood glucose levels was significantly alleviated (374.01 ± 16.61 , 385.50 ± 14.57 , and 330.67 ± 16.10 mg/dL at 30, 60, and 120 min, respectively; $P<0.05$). Postprandial blood glucose levels also significantly decreased when normal mice were orally administered starch combined with GPE (Fig. 5; $P<0.05$). Collectively, the increase in postprandial blood glucose levels was suppressed significantly in both STZ-induced diabetic and normal mice treated with GPE. The AUC for the glucose response in diabetic mice administered GPE (723.13 ± 28.67 mg·h/dL) was significantly lower ($P<0.05$) than that in diabetic control mice (765.85 ± 52.20 mg·h/dL; Table 2). The AUCs in normal mice were consistent with

Table 2. AUC of postprandial glucose responses of normal and STZ-induced diabetic mice

Group ¹⁾	AUC (mg·h/dL)	
	Normal mice	Diabetic mice
Control	281.96 ± 31.65^{NS}	765.85 ± 52.20^a
GPE	247.24 ± 29.53	723.13 ± 28.67^{ab}
Acarbose	219.93 ± 33.02	661.67 ± 32.93^b

¹⁾Control (distilled water), *Gynura procumbens* extract (GPE) (300 mg/kg), and acarbose (100 mg/kg) were co-administered orally with starch (2 g/kg). Each value is expressed as the mean±SD of 7 mice (n=42). Values with different letters (a,b) are significantly different at $P<0.05$ as analyzed by Duncan's multiple range test. NS: not significant.

that in diabetic mice, demonstrating the anti-postprandial hyperglycemic effects of GPE.

Maintaining near normal blood glucose levels in both fasting and postprandial states is important for diabetic patients. It is well known that postprandial hyperglycemia is a major factor in the development of type 2 diabetes, and it is associated with diabetic complications (25, 26). Prolonged postprandial hyperglycemia may lead to the generation of oxidative stress, which can trigger inflammation and endothelial dysfunction, as well as diabetic complications, such as microvascular and macrovascular disease. Thus, alleviating postprandial hyperglycemia is crucial for the prevention and treatment of type 2 diabetes (27,28).

As shown in Fig. 3 and Fig. 4, postprandial hyperglycemia was significantly alleviated following the consumption of GPE-supplemented starch in both STZ-induced diabetic mice and normal mice. This may be due to the inhibition of carbohydrate hydrolyzing enzyme (pancreatic α -amylase and intestinal α -glucosidase) activities by GPE, thereby delaying the absorption of dietary carbohydrates in the small intestine epithelial cells. Chemicals that flatten the peak of postprandial hyperglycemia reduce the area under the blood glucose response curve (29). In this study, GPE reduced the peak of postprandial hyperglycemia and the AUC. These results also suggest that GPE may help alleviate postprandial hyperglycemia.

Alleviating postprandial hyperglycemia is important for the treatment of diabetic symptoms. Various synthetic compounds have been used in diabetes therapy. However, these products have usually been associated with unwanted side effects (30). Hence, many studies have tried to find natural resources that can alleviate postprandial hyperglycemia. The current study suggests that GPE might be effective for alleviating postprandial hyperglycemia and preventing diabetic complications. In conclusion, the results of this study showed that GPE has significant inhibitory effects on α -glucosidase and α -amylase activities. Furthermore, GPE likely alleviates postprandial hyperglycemia. Thus, GPE may be used as a functional food to alleviate postprandial hyperglycemia.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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