Prev. Nutr. Food Sci. 2017;22(3):166-171
https://doi.org/10.3746/pnf.2017.22.3.166
pISSN 2287-1098 · eISSN 2287-8602

Sargassum yezoense Extract Inhibits Carbohydrate Digestive Enzymes *In Vitro* and Alleviates Postprandial Hyperglycemia in Diabetic Mice.

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ABSTRACT: In this study, we investigated whether *Sargassum yezoense* extract (SYE) could inhibit α-glucosidase and α-amylase activities, and alleviate postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice. Freeze-dried *S. yezoense* was extracted with 80% ethanol and concentrated for use in this study. The hypoglycemic effect was determined by evaluating the inhibitory activities of SYE against α-glucosidase and α-amylase as well as its ability to decrease postprandial blood glucose levels. The half-maximal inhibitory concentrations of SYE against α-glucosidase and α-amylase were 0.078±0.004 and 0.212±0.064 mg/mL, respectively. SYE was a more effective inhibitor of α-glucosidase and α-amylase activities than the positive control, acarbose. The increase in postprandial blood glucose levels was significantly alleviated in the SYE group compared with that in the control group of STZ-induced diabetic mice. Furthermore, the area under the curves significantly decreased with SYE administration in STZ-induced diabetic mice. These results suggest that SYE is a potent inhibitor of α-glucosidase and α-amylase activities and alleviates postprandial hyperglycemia caused by dietary carbohydrates.

Keywords: *Sargassum yezoense*, α-glucosidase, α-amylase, postprandial hyperglycemia, diabetic mice

INTRODUCTION

Diabetes mellitus is a serious chronic disease that is increasing with obesity and aging in the worldwide population (1). Diabetes patients have high blood glucose levels because either they cannot produce enough insulin or the cells of their liver, muscle, and fat tissue do not respond to insulin normally (2). In diabetes, the postprandial phase is accompanied by rapid and large increases in blood glucose levels. Postprandial hyperglycemia negatively affects the development of type-2 diabetes and causes diabetic complications, including dyslipidemia and hypertension (3). In addition, postprandial hyperglycemia was reported as a direct risk factor for cardiovascular diseases, and most cardiovascular risk factors are affected by acute blood sugar increases (4). Therefore, controlling postprandial hyperglycemia is the most important factor for treating patients with diabetes to reduce the risks of diabetic complications (5).

One therapeutic approach to suppressing postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase in digestive organs (6). Synthetic α-glucosidase inhibitors, such as acarbose, miglitol, and voglibose, are available to treat postprandial hyperglycemia in patients with type-2 diabetes. However, chronic use of these agents can result in side effects, such as flatulence, abdominal cramping, vomiting, and diarrhea; therefore, their use may be limited (7-10). Therefore, many studies have been performed to identify more effective and safer inhibitors of α-glucosidase and α-amylase from natural sources.

Marine macroalgae, or seaweeds, are one of nature's most biologically active resources and contain a wealth of bioactive compounds. Seaweed extracts have demonstrated various biological activities, such as antioxidant (11), anti-inflammatory (12), anticoagulant (13), and apoptotic activities (14). *Sargassum yezoense* is a brown algae belonging to the *Sargassum* genus. It is one of the most abundant marine algae on the east coast of Korea and is popular in Korea and Japan as a food ingredient and marine herb (15). *S. yezoense* extract (SYE) showed various bioactivities including anti-inflammatory, antibacterial, and anti-atopy activities (16-19). Most notably,

Received 7 March 2017; Accepted 21 April 2017; Published online 30 September 2017
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SYE has strong antioxidant and peroxisome proliferator-activated receptor (PPAR)α and PPARγ stimulating effects in 3T3-L1 cells because it contains biologically active substances, such as sargaquinoic acid and sargahydroquinoic acid (20).

However, there are presently no studies reporting the ability of SYE to alleviate postprandial hyperglycemia through the inhibition of carbohydrate digestive enzymes in diabetic mice. Therefore, this study was conducted to determine whether SYE inhibits α-glucosidase and α-amylase activities *in vitro* and alleviates postprandial hyperglycemia in diabetic mice *in vivo*.

MATERIALS AND METHODS

Materials and preparation of SYE

The brown algae, *S. yezoense*, were collected from the east coast of Korea. After collection of *S. yezoense*, the samples were washed three times under running tap water to remove foreign substances and sand on the surface, carefully rinsed with fresh water, and then freeze-dried. The lyophilized samples were homogenized with a grinder prior to extraction. The sample was extracted three times with ten volumes of 80% ethanol for 12 h at room temperature. The filtrate was then evaporated at 40°C using a rotary evaporator (N-1300VW, EYELA, Tokyo, Japan). After the solvent had been completely removed from the SYE, it was stored in a deep freezer (−80°C).

Inhibition assay for α-glucosidase activity *in vitro*

The α-glucosidase inhibitory assay was based on the chromogenic method developed by Watanabe et al. (21), and it was performed using a readily available yeast enzyme. Briefly, yeast α-glucosidase (0.7 U, Sigma-Aldrich Co., St. Louis, MO, USA) 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 the enzyme solution. Five mM *p*-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as the substrate solution. Fifty μL enzyme solution and 10 μL SYE [5 mg/mL in dimethyl sulfoxide (DMSO)] were mixed in a well, and the absorbance at 405 nm was measured as time zero using a microplate reader. After incubation for 5 min, the substrate solution (50 μL) was added, and the incubation continued for another 5 min at room temperature. The increase in absorbance from the zero time point was then measured. The inhibitory activities of varying concentrations of SYE were expressed as 100 minus the absorbance difference (%) of the test compounds relative to the absorbance change of the negative control (i.e., DMSO used as the test solution). The measurements were performed in triplicate, and the IC_{50} value (i.e., the concentration of SYE that result in 50% inhibition of maximal activity) was determined.

Inhibition assay for α-amylase activity *in vitro*

The α-amylase inhibitory activity was assayed in the same manner as described previously for α-glucosidase (21), except that porcine pancreatic amylase (100 U, Sigma-Aldrich Co.) and blocked *p*-nitrophenyl-α-D-maltopentoglycoside (Sigma-Aldrich Co.) were used as the enzyme and substrate, respectively.

Measurement of cytotoxicity

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and 3T3-L1 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). 3T3-L1 cells were seeded at 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. Afterward, the cells were treated with various concentrations (0.1, 0.5, 1, and 2 mg/mL) of SYE, and incubated for 20 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 at a final concentration of 0.5 mg MTT/mL. The supernatants were carefully aspirated, 200 μL of DMSO was added to each well, and the plates were agitated to dissolve the crystal product. The absorbance of DMSO solutions was measured spectrophotometrically at 540 nm.

Experimental animals

Four-week old male mice (ICR, Central Laboratory Animal Inc., Seoul, Korea) were housed individually in a temperature-controlled room (25~30°C) with a relative humidity of 45~55% and 12 h on/12 h off light/dark cycles. The animals were provided pelleted food and tap water *ad libitum*. After an adjustment period of 2 weeks, diabetes was induced by an intraperitoneal injection of streptozotocin [STZ; 60 mg/kg body weight (b.w)] that was freshly dissolved in citrate buffer (0.1 M, pH 4.5) (22). After seven days, tail bleeds were performed, and animals with a blood glucose concentration above 250 mg/dL were considered to be diabetic. The procedures for the handling and care of mice adhered to the guidelines that comply with current international laws and policies (NIH guide for the Care and Use of Laboratory Animals), and all procedures were approved by the animal ethics committee at Pusan National University (PNU-2016-1273).

Measurement of blood glucose levels

Both normal mice and STZ-induced diabetic mice were fasted overnight and randomly divided into three groups of 7 mice. Before testing blood glucose levels, animals were kept in a fasting state for at least 12 h, but had free access to water. The mice were orally administered as

follows: control, mice received starch orally (2 g/kg b.w); SYE, mice received starch with SYE orally (300 mg/kg b.w); acarbose, mice received starch with acarbose orally (100 mg/kg b.w). Blood samples were taken from the tail vein at 0, 30, 60, and 120 min. Blood glucose was measured using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). The areas under the curve (AUC) were calculated using the trapezoidal rule (23).

Data statistical analysis

The data are presented as mean±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 treatment 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 SYE on α-glucosidase and α-amylase *in vitro*

The inhibitory effects of SYE against α-glucosidase activity were determined using *p*-nitrophenyl-α-D-glucopyranoside as the substrate, and were compared to the effects of a commercial α-glucosidase inhibitor, acarbose. SYE inhibited α-glucosidase activity in a dose-dependent manner by 39.76±2.38, 57.83±1.43, 75.90±1.51, and 78.92±2.11% at concentrations of 0.05, 0.10, 0.25, and 0.50 mg/dL, respectively (Fig. 1). Acarbose, an α-glucosidase inhibitor used as an oral hypoglycemic agent, inhibited the enzyme activity by 58.03±3.22% at a concentration of 0.25 mg/dL. The α-glucosidase inhibitory activity of SYE was significantly higher than that of acarbose at the same concentration (0.25 mg/dL).

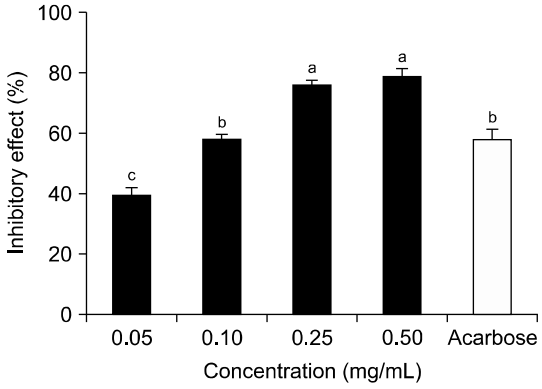

Fig. 1. Inhibitory activity of *Sargassum yezoense* extract (SYE) against α-glucosidase. Each value is expressed as mean±SD in triplicate experiments. Values with different letters (a-c) are significantly different at $P<0.05$ as analyzed by Duncan's multiple range test. The concentration of acarbose used as a positive control was 0.25 mg/mL.

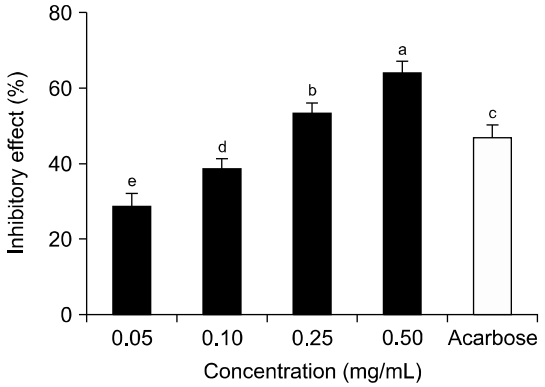

Fig. 2. Inhibitory activity of *Sargassum yezoense* extract (SYE) against α-amylase. Each value is expressed as mean±SD in triplicate experiments. Values with different letters (a-e) are significantly different at $P<0.05$ as analyzed by Duncan's multiple range test. The concentration of acarbose used as a positive control was 0.25 mg/mL.

As shown in Fig. 2, the inhibitory effects of SYE on α-amylase increased in a dose-dependent manner by 29.03 ±3.20, 38.71±2.59, 53.76±2.60, and 64.52±2.75% at concentrations of 0.05, 0.10, 0.25, and 0.50 mg/dL, respectively. SYE also inhibited α-amylase activity more effectively than acarbose. The IC_{50} values of SYE against α-glucosidase and α-amylase were 0.078±0.004 and 0.212 ±0.064 mg/mL, respectively. The IC_{50} values of SYE against α-glucosidase and α-amylase were significantly lower than those of acarbose, indicating that SYE has stronger inhibitory effects than the positive control (Table 1). These results indicate that SYE may prove useful as a natural, postprandial anti-hyperglycemic agent owing to its inhibitory effects against α-glucosidase and α-amylase.

The treatment aim for diabetic patients is to maintain normal levels of glycemic control, both in fasting and postprandial states. Postprandial hyperglycemia is the first sign that appears in diabetes mellitus-related metabolic abnormalities. α-Amylase is the key enzyme for catalytic hydrolysis of the α–1,4 glycosidic linkages of starch to form maltose, which is further hydrolyzed to glucose prior to absorption in the small intestine (24). α-Glucosidases are a family of membrane-bound enzymes in the

Table 1. IC_{50} values of the inhibitory effect of *Sargassum yezoense* extract (SYE) against α-glucosidase and α-amylase activities

Sample	IC_{50} (mg/mL)[1)]	
	α-Glucosidase	α-Amylase
SYE	0.078±0.004*	0.212±0.064*
Acarbose	0.189±0.013	0.262±0.037

Each value is expressed as mean±SD in triplicate experiments.
*Significantly different from acarbose at $P<0.05$.
[1)]IC_{50} value is the concentration of sample required for 50% inhibition.

intestine that are involved in the digestion and uptake of carbohydrates into the bloodstream. Monosaccharides such as glucose and fructose, are absorbed directly in the small intestine. Disaccharides such as sucrose, and polysaccharides such as starch, must be degraded before being absorbed. This digestive process is performed by the α-glucosidases (10).

Since the activity of α-glucosidase in the small intestine of diabetic patients is higher than that of normal people, the intake of carbohydrates in food significantly increases postprandial blood glucose levels (25). Inhibition of α-amylase and α-glucosidase attenuates the increase in postprandial blood glucose after ingestion of carbohydrates to manage postprandial blood glucose in type 2 diabetic patients (10). α-Glucosidase inhibitors are typically compounds such as acarbose, which inhibit the α-glucosidase in the small intestine, thereby reducing the degradation of polymeric sugar compounds and suppressing the rapidly increasing blood glucose levels that occur after ingestion of carbohydrate (26). However, acarbose, which is used as a commercial carbohydrate inhibitor, has side effects such as flatulence, abdominal cramping, and diarrhea when used long-term (7,8). Thus, scientists have long considered alternative effective and non-toxic inhibitors of α-glucosidase and α-amylase.

In this study, we investigated the inhibitory effect of the natural product, SYE, against α-glucosidase and α-amylase to elucidate its possible use as an anti-postprandial hyperglycemic agent. SYE afforded significantly higher inhibitory activities against both α-glucosidase and α-amylase than the commercial inhibitor, acarbose. It also did not show any cytotoxicity (Fig. 3). SYE contains various bioactive compounds, such as sargaquinoic acid and sargahydroquinoic acid, which are types of plastoquinones. According to another study, sargaquinoic acid and sargahydroquinoic acid have remarkable α-glucosidase inhibition ability (25). Plastoquinones contain hydroxyl groups, and Tadera et al. (27) reported that hydroxyl substitution on the phenyl ring structure could be effective in inhibiting the enzyme activity. Thus, the inhibitory effect of SYE on α-glucosidase and α-amylase appears to be due to the plastoquinones in SYE.

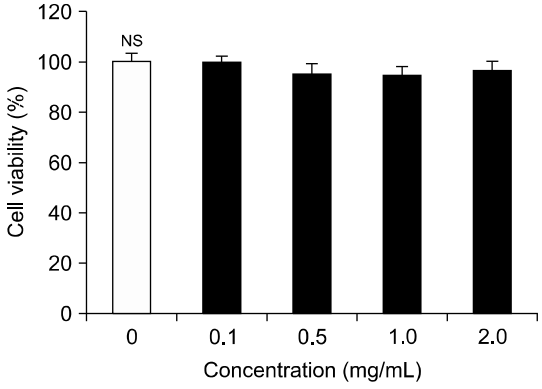

Fig. 3. Cytotoxic effect of *Sargassum yezoense* extract (SYE) in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0.1, 0.5, 1.0, and 2.0 mg/mL) of SYE for 20 h, and cell viability was measured via MTT assay. Each value is expressed as mean±SD in triplicate experiments. NS: non-significant.

Effects of SYE on blood glucose levels *in vivo*

The effects of SYE on blood glucose levels after a meal were investigated in normal and STZ-induced diabetic mice. The postprandial blood glucose levels of the SYE administered mice were lower than those of the diabetic control mice (Fig. 4). Blood glucose levels in the diabetic control mice increased to 378.7±27.9 mg/dL at 30 min and 412.0±16.5 mg/dL at 60 min after a meal, and then decreased to 381.0±18.6 mg/dL at 120 min. However, when SYE was added to starch, the increase in postprandial blood glucose levels was significantly suppressed (351.5±19.8, 384.5±23.7, and 335.5±20.4 mg/dL at 30, 60, and 120 min, respectively; $P<0.05$). The peak postprandial blood glucose levels also significantly decreased when the normal mice were orally administered starch with SYE (Fig. 5). Thus, this confirms that SYE can suppress the postprandial hyperglycemia that is caused by starch in normal mice. The AUC for the glucose response in diabetic mice administered SYE (706.7±43.9 mg·h/dL) was significantly lower ($P<0.05$) than that in diabetic control mice (764.1±45.1 mg·h/dL) (Table 2).

The ability to control postprandial hyperglycemia is important in achieving the tight glycemic control that is targeted in diabetes treatment (28). In addition, postprandial hyperglycemia increases the risk of cardiovascular disease, increases free radical production, induces vasoconstriction, and plays a negative role in type 2 dia-

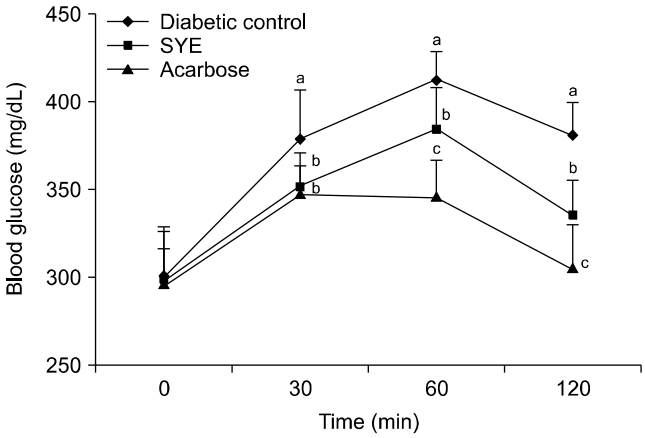

Fig. 4. Blood glucose levels after the administration of *Sargassum yezoense* extract (SYE) in streptozotocin-induced diabetic mice. Each value is expressed as mean±SD of seven mice. Values with different letters (a-c) are significantly different at each time ($P<0.05$) as analyzed by Duncan's multiple range test. Control, mice received starch orally (2 g/kg b.w); SYE, mice received starch with *Sargassum yezoense* extract orally (300 mg/kg b.w); Acarbose, mice received starch with acarbose orally (100 mg/kg b.w).

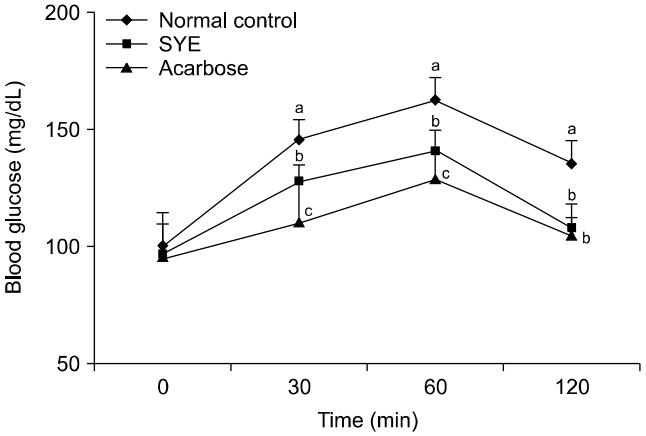

Fig. 5. Blood glucose levels after the administration of *Sargassum yezoense* extract (SYE) in normal mice. Each value is expressed as mean±SD of seven mice. Values with different letters (a-c) are significantly different at each time ($P<0.05$) as analyzed by Duncan's multiple range test. Control, mice received starch orally (2 g/kg b.w); SYE, mice received starch with *Sargassum yezoense* extract orally (300 mg/kg b.w); Acarbose, mice received starch with acarbose orally (100 mg/kg b.w).

betes; therefore, controlling postprandial hyperglycemia plays an important role in diabetic patients (29).

Thus, we determined the anti-postprandial hyperglycemic effect of SYE in diabetic and normal mice after consumption of starch. The increase in postprandial blood glucose levels was suppressed significantly in both diabetic and normal mice when treated with SYE. These results show that SYE may delay the absorption of dietary carbohydrates, resulting in suppression of the increase in postprandial blood glucose levels. Inoue et al. (30) reported that medications flatten the peak of postprandial blood glucose and decrease the AUC of the blood glucose response curve. In this study, SYE was shown to reduce both blood glucose levels at the peak time point and the AUC in diabetic mice. The AUCs in normal mice were also lowered by SYE, paralleling that observed in diabetic mice. As shown in Fig. 4 and Fig. 5, postprandial hyperglycemia was significantly alleviated after ingestion of starch supplemented with SYE in both diabetic and normal mice. This may be due to inhibition of the activity of carbohydrate degrading enzymes (e.g., pancreatic α-amylase and intestinal α-glucosidase) by SYE, thereby delaying the absorption of dietary carbohydrates in the epithelial cells of the small intestine.

Table 2. Areas under the curve (AUC) of the postprandial glucose responses of normal and streptozotocin-induced diabetic mice

Group[1)]	AUC (mg·h/dL)	
	Normal mice	Diabetic mice
Control	287.5±20.4[a]	764.1±45.1[a]
SYE	247.6±13.9[b]	706.7±43.9[b]
Acarbose	228.4±23.7[c]	660.6±39.4[c]

Each value is expressed as the mean±SD of seven mice. Different letters (a-c) in a column are significantly different at $P<0.05$ using Duncan's multiple range tests.
[1)]Control, mice received starch orally (2 g/kg b.w); SYE, mice received starch with *Sargassum yezoense* extract orally (300 mg/kg b.w); Acarbose, mice received starch with acarbose orally (100 mg/kg b.w).

Recently, marine algae have been recognized as a good resource for anti-diabetic materials derived from nature (31). Results from our investigation suggest that SYE from brown algae is helpful in preventing postprandial hyperglycemia and diabetic complications, as assessed by the anti-hyperglycemic effects of SYE in both diabetic and normal mice. This study demonstrates that SYE may prove useful as an effective, natural anti-diabetic substance.

In conclusion, SYE inhibited α-glucosidase and α-amylase activities, suppressing the formation of glucose from starch, and resulting in a reduction in postprandial hyperglycemia. Furthermore, SYE may delay the absorption of dietary carbohydrates in the intestine, resulting in suppression of increased blood glucose levels after a meal. Thus, SYE may be used as a functional food to alleviate postprandial hyperglycemia.

ACKNOWLEDGEMENTS

This work was supported by a 2-Year Research Grant of Pusan National University.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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