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Hypoglycemic Effect of *Sargassum ringgoldianum* Extract in STZ-induced Diabetic Mice

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Abstract

This study was designed to investigate whether *Sargassum ringgoldianum* extract may inhibit α-glucosidase and α-amylase activities, and alleviate postprandial hyperglycemia in streptozotocin-induced diabetic mice. The IC_{50} values of *Sargassum ringgoldianum* extract against α-glucosidase and α-amylase were 0.12 mg/mL and 0.18 mg/mL, respectively, which evidenced higher activities than those of acarbose. The blood glucose levels of the *Sargassum ringgoldianum* extract administered group were significantly lower compared to the control group in the streptozotocin-induced diabetic mice. Moreover, the area under the two-hour blood glucose response curve was significantly reduced and the absorption of dietary carbohydrates was delayed after administration of *Sargassum ringgoldianum* extract in the diabetic mice. Therefore, these results indicated that *Sargassum ringgoldianum* extract may help decrease the postprandial blood glucose level via inhibiting α-glucosidase.

Key words: *Sargassum ringgoldianum* extract, α-glucosidase, hypoglycemic effect, diabetic mice

INTRODUCTION

The prevalence of diabetes is increasing worldwide, and many diagnosed with this disease will die or become disabled due to complications (1,2). Postprandial blood glucose levels may elevate while fasting plasma glucose levels remain normal, which some have called "postprandial diabetes" (3). This state not only initiates the development of early microvascular and macrovascular complications, but it also can contribute to a more rapid progression to symptomatic diabetes by causing glucose toxicity in muscles and pancreatic beta cells. The control of postprandial hyperglycemia, therefore, offers the potential for early intervention and prevention of diabetes complications (4).

α-Glucosidase and α-amylase play a significant role in the digestive process of dietary complex carbohydrates; inhibition of both enzymes can retard the digestion of carbohydrates, delay glucose absorption, and reduce plasma glucose levels, resulting in a decrease in postprandial hyperglycemia (5). Therefore, reducing postprandial hyperglycemia levels has been considered one of the most effective therapeutic approaches, with fewer disadvantages than earlier diabetic treatments (6-8). Continuous use of synthetic agents, such as gliclazide, metformin and voglibose, should be limited because they may cause flatulence, abdominal cramps, vomiting, diarrhea, and other side effects (9). With respect to suppression of glucose production from carbohydrates and glucose absorption from the intestine, increasing efforts are being made to find and investigate potential inhibitors of α-glucosidase and α-amylase in natural products showing no side effects (6-8).

Marine macroalgae, or seaweeds, are one of nature's most biologically active resources and possess a wealth of bioactive compounds. Seaweed extracts have demonstrated various biological activities, such as antioxidant potential (10,11), anti-inflammatory properties (12), and anti-coagulant (13) and apoptotic activities (14). *Sargassum ringgoldianum*, belonging to the *Sargassaceae* family, is regarded as an edible brown alga and grows on the coast of Jeju Island, Korea. *S. ringgoldianum* extract (SRE) is rich in minerals, water-soluble polysaccharides and phenolic compounds (15). The SRE contained the highest amount of phenolic compounds among seaweeds screened for antioxidative activities. The SRE also had the strongest scavenging activity against the superoxide anion radical and hydroxyl radical among seaweeds (16). The biological benefits of SRE, including antioxidant (15-18), anti-tumor (18), anti-coagulant (19), anti-hyperlipidemic, anti-hypertensive and anti-arteriosclerosis activities (20,21), have been shown in several studies. However, the hypoglycemic effect of SRE has yet to be elucidated. Therefore, this study was designed to investigate whether or not SRE inhibits α-glucosidase and α-amylase activities, and alleviates postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice.

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MATERIALS AND METHODS

Materials and preparation of *S. ringgoldianum* extract

S. ringgoldianum algae was collected from the coast of Jeju Island, South Korea. The sample was first washed 3 times with tap water to remove salt, epiphytes, and sand attached to the surface, and then carefully rinsed with fresh water. Thereafter, the sample was lyophilized using a vacuum freeze dryer (Samwon Freezing Engineering Co., Busan, Korea) and homogenized with a grinder (Shinhan Science & Technology Co., Kyunggi, Korea). The *S. ringgoldianum* powder was extracted 3 times with 80% methanol, filtered through Whatman No. 1 filter paper, and evaporated under a vacuum using a rotary evaporator (BUCHI Co., Flawil, Switzerland). The extract was thoroughly dried for removal of solvents and stored in a deep freezer (-80°C) (22). 2.8 g of extract per 13.0 g of powdered *S. ringgoldianum* was obtained.

***In vitro* inhibition assay for α-glucosidase activity**

The α-glucosidase inhibitory assay was conducted using the chromogenic method developed by Watanabe et al. (23). Briefly, yeast α-glucosidase (0.7 U, Sigma-Aldrich, 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. A 5 mM p-nitrophenyl-α-D-glucopyranoside (pNGP) in the same buffer (pH 7.0) was used as the substrate solution. 50 μL of enzyme solution and 10 μL of sample dissolved in dimethyl sulfoxide at a 5 mg/mL concentration were mixed in a well and absorbance was measured at 405 nm using a microplate reader. After incubation for 5 min, substrate solution (50 μL) was added and incubated for another 5 min at room temperature. The increase in absorbance from zero time was measured. Inhibitory activity was expressed as 100 minus relative absorbance difference (%) of test compounds to absorbance change of the control where test solution was replaced by carrier solvent. The measurements were performed in triplicate and the IC_{50} value, i.e., the concentration of the extracts that results in 50% inhibition of maximal activity, was determined.

***In vitro* inhibition assay for α-amylase activity**

α-Amylase inhibitory activity was assayed in the same way (23) as described previously for α-glucosidase inhibitory assay, except that porcine pancreatic amylase (100 U, Sigma-Aldrich) and blocked p-nitrophenyl-α-D-maltopentoglycoside (Sigma-Aldrich) were used as enzyme and substrate, respectively.

Experimental animals

Four-week old male ICR mice (Orient Inc., Seoul, Korea) were kept under a 12 hr light/ 12 hr dark cycle with controlled room temperature. The animals were maintained with a 5L79 diet (Labdiet, Richmond, IN, USA), while tap water was available *ad libitum*. After an adjustment period of 2 weeks, diabetes was induced by intraperitoneal injection of STZ (60 mg/kg) freshly dissolved in a citrate buffer (0.1 M, pH 4.5) for the fasted (18 hr) animals. After 7 days, tail bleeds were performed and animals with a blood glucose concentration above 250 mg/dL were considered diabetic. The mice were administered orally soluble starch (2 g/kg BW) alone (control) or with SRE (300 mg/kg BW) or acarbose (50 mg/kg BW) dissolved in 0.2 mL of water.

Measurement of blood glucose level

Both normal mice and STZ-induced diabetic mice fasted overnight were randomly divided into 3 groups of 7 mice. Fasted animals were deprived of food for at least 12 hr but allowed free access to water. After overnight fasting, the mice were orally administered either soluble starch (2 g/kg BW) alone (control) or starch with SRE (300 mg/kg BW). 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). Areas under the curve (AUC) were calculated using the trapezoidal rule (24).

Statistical analysis

The data are represented as the mean±standard deviation (SD) of triplicate experiments. The statistical analysis was performed using SAS software. The Student's *t*-test was used for comparisons between control and sample groups. The values were evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests, and p-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

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

The inhibitory effect of SRE against α-glucosidase was determined using p-nitrophenyl-α-glucopyranoside (pNPG) as the substrate. SRE inhibited α-glucosidase activity in a dose-dependent manner by 42.88, 47.63, 52.85, and 61.79% at 0.05, 0.10, 0.15, and 0.20 mg/mL concentrations, respectively (Fig. 1). Acarbose, the α-glucosidase inhibitor used as an oral hypoglycemic agent, at 0.20 mg/mL, inhibited the enzyme activity by 48.04%. Thus, 0.20 mg/mL of SRE inhibited the α-glucosidase activity significantly higher than the same concentration of acarbose. The dose-depedent α-amylase inhibitory effect of SRE was illustrated using p-nitrophenyl-α-maltopenotoglycoside (pNPM) as a substrate (Fig. 2). The α-

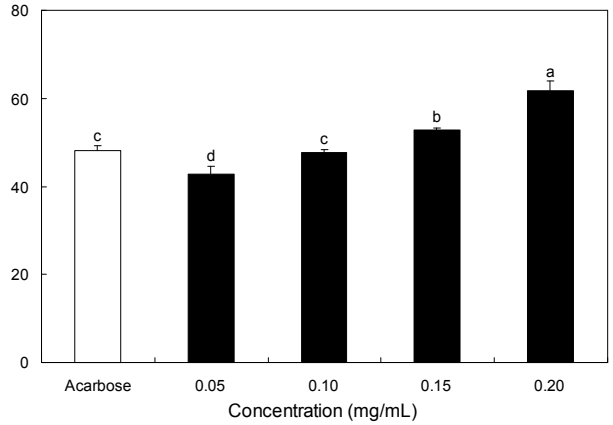

Fig. 1. Inhibitory activity of *S. ringgoldianum* extract on α-glucosidase. Each value is expressed as mean±SD in triplicate experiments. [a-d]Values with different alphabets on bars are significantly different at $p<0.05$ as analyzed by Duncan's multiple range test. The concentration of acarbose used as a positive control was 0.20 mg/mL.

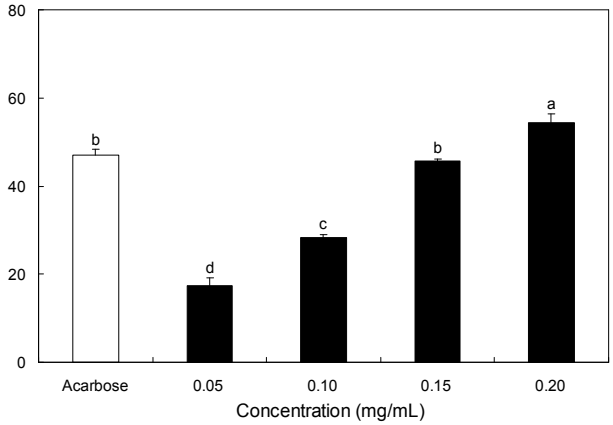

Fig. 2. Inhibitory activity of *S. ringgoldianum* extract on α-amylase. Each value is expressed as mean±SD in triplicate experiments. [a-d]Values with different alphabets on bars are significantly different at $p<0.05$ as analyzed by Duncan's multiple range test. The concentration of acarbose used as a positive control was 0.20 mg/mL.

amylase inhibitory activity of SRE at the concentration of 0.20 mg/mL was significantly higher than that of acarbose at the same concentration. IC_{50} values of SRE against α-glucosidase and α-amylase were 0.12 and 0.18 mg/mL, respectively (Table 1).

Postprandial hyperglycemia is a prominent and early defect in the development of diabetes. The treatment goal for patients with diabetes is to maintain near-normal levels of glycemic control, both in the fasting and postprandial states (4). While breaks down starch, glycogen and oligosaccharides by catalyzing the hydrolysis of α-1,4-glucosidic linkages, further breaks down the disaccharides into simpler sugars, readily available for intestinal absorption (8). Inhibitors of both α-amylase and α-glucosidase delay and prolong overall carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (25). According to our data, SRE had significantly higher inhibitory activities than acarbose on α-glucosidase and α-amylase, suggesting SRE has proven to be useful in terms of diabetic control.

Table 1. IC_{50} value of *S. ringgoldianum* extract (SRE) on α-glucosidase and α-amylase

Sample	IC_{50} (mg/dL)[1)]	
	α-Glucosidase	α-Amylase
SRE	0.12±0.02*	0.18±0.01*
Acarbose	0.26±0.04	0.23±0.02

[1)]IC_{50} value is the concentration of sample required for 50% inhibition. Each value is expressed as mean±SD (n=3). Significantly different from control at *$p<0.05$.

SRE contained a mixture of phlorotannins, high molecular weight polyphenols found to be polymerized bifuhalol (16). Polyphenolic compounds, such as tannins from terrestrial plants and phlorotannins from marine algae, are known to be associated with a variety of proteins to form complexes (26). The hydroxyl groups in phlorotannin derivatives may play an important role in promoting inhibitory activities. For example, *o*-quinones derived from catechols are covalently bound to amino acids and thiol groups; phlorotannins seem to be bound to active or binding sites of these enzymes, resulting in inhibition of their enzyme activities (27). Thus, SRE could be an effective inhibitor of intestinal α-glucosidase and α-amylase activities, which may prove to be synergistic in their potential therapeutic effects against the postprandial plasma glucose rise.

Effect of SRE on blood glucose levels *in vivo*

The effect of SRE on blood glucose levels after a meal was investigated in streptozotocin-induced diabetic and normal mice. Postprandial blood glucose levels of SRE-administered diabetic mice were significantly lower than control group mice (Fig. 3). The blood glucose levels of diabetic mice in the control group were 370.0, 404.5 and 403.5 mg/dL at 30, 60 and 120 min, respectively. However, the increase in postprandial blood glucose level was significantly reduced ($p<0.05$) in diabetic mice administered SRE (320.7, 337.3 and 307.0 mg/dL at similar respective time points). The postprandial blood glucose level was also significantly decreased when normal mice were orally administered starch with SRE (Fig. 4). In addition, the blood glucose levels of the SRE and acarbose administered groups were similar in both the streptozotocin-induced diabetic and the normal mice.

The area under the two-hour blood glucose response curve (AUC) of the SRE administered group (636.3±64.3 mg·hr/dL) was significantly lower ($p<0.05$) than the control group (762.5±48.0 mg·hr/dL) in diabetic

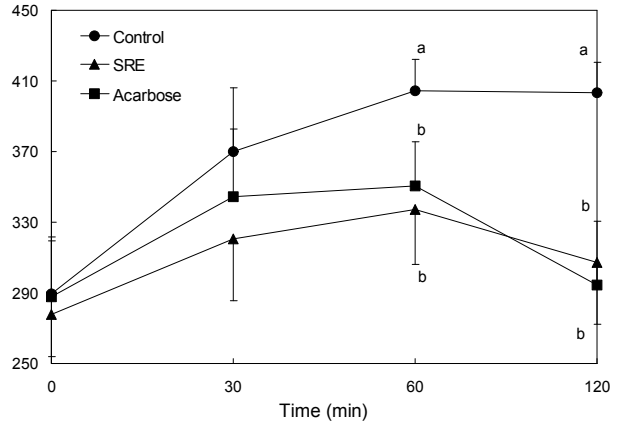

Fig. 3. Blood glucose level after administration of *S. ringgoldianum* extract (SRE) in STZ-induced diabetic mice. Control (distilled water), SRE (300 mg/kg), and acarbose (50 mg/kg) were co-administered orally with starch (2 g/kg). Each value is expressed as mean±SD of 7 mice (n=21). [a,b]Values with different alphabets are significantly different at $p<0.05$ as analyzed by Duncan's multiple range test.

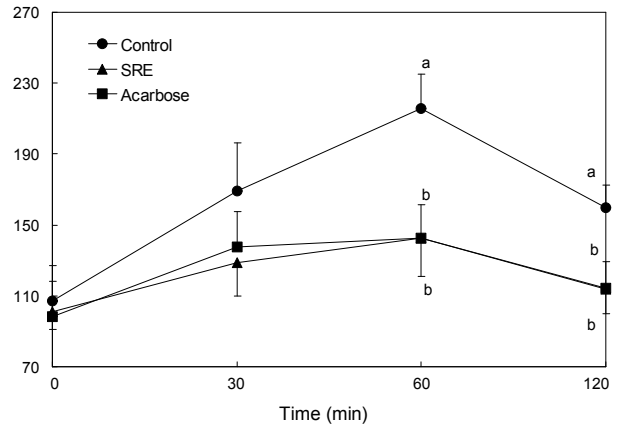

Fig. 4. Blood glucose level after administration of *S. ringgoldianum* extract (SRE) in normal mice. Control (distilled water), SRE (300 mg/kg), and acarbose (50 mg/kg) were co-administered orally with starch (2 g/kg). Each value is expressed as mean±SD of 7 mice (n=21). [a,b]Values with different alphabets are significantly different at $p<0.05$ as analyzed by Duncan's multiple range test.

mice (Table 2). The AUC in normal mice corroborated with the hypoglycemic effect of SRE.

Controlling not only fasting, but also postprandial hyperglycemia, are important in maintaing blood glucose levels, the major target of diabetic therapy (28). According to a previous study, postprandial hyperglycemia might be more strongly correlated with cardiovascular morbidity and mortality than fasting hyperglycemia (29). Early identification of postprandial hyperglycemia and its effective control, therefore, offer the potential for early intervention and prevention of diabetes complications (4). Thus, we determined the anti-hyperglycemic effect by SRE in streptozotocin-induced diabetic and normal mice after consumption of starch. The increase in postprandial blood glucose level was significantly suppressed by SRE administration in both streptozotocin-induced diabetic and normal mice groups. This result indicates that SRE may delay the absorption of dietary carbohydrates consumed, thereby suppressing an increase in postprandial blood glucose levels. Acarbose which flattens postprandial blood glucose peak reduces the AUC of the blood glucose response curve (30). In this study, SRE decreased both blood glucose level at the peak time point and the AUC.

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

Group[1)]	AUC (mg·hr/dL)	
	Normal mice	Diabetic mice
Control	353.1±39.5[a]	762.5±48.0[a]
SRE	253.5±34.9[b]	636.3±64.3[b]
Acarbose	257.6±36.3[b]	654.5±63.8[ab]

[1)]Control (distilled water), *S. ringgoldianum* extract (SRE, 300 mg/kg), and acarbose (50 mg/kg) were co-administered orally with starch (2 g/kg). Each value is expressed as mean±SD of 7 mice (n=42). [a,b]Values with different alphabets in a column are significantly different at $p<0.05$ as analyzed by Duncan's multiple range test.

Type 2 diabetes is a chronic disease characterized by simultaneous insulin deficiency and insulin resistance events, with the resultant hyperglycemia leading to microvascular and macrovascular complications. A large number of intervention trials demonstrated that improving glycemic control achieves considerable reduction of these complications (31-33). α-Glucosidase and α-amylase play a significant role during dietary complex carbohydrate digestion. The main benefits attributable to α-glucosidase inhibitors are reductions both in postprandial glycemia levels and in the total range of postprandial glucose levels (5). Many synthetic compounds such as gliclazide, metformin and voglibose have been used in the treatment of diabetes; however, many have been associated with marked toxic or undesirable side effects (9). Therefore, marine algae have become good candidates for the source of natural anti-diabetic materials (26). In an earlier report, phloroglucinol derivatives isolated from *Ecklonia cava* have the potential to prevent diabetes mellitus because of their high α-glucosidase and α-amylsase inhibitory activities (27). Brown algae extract may have a beneficial effect on controlling postprandial glucose levels in diabetic obese rats (34). Our current finding showed that SRE could potentially be developed as a novel natural nutraceutical to improve postprandial hyperglycemia and prevent diabetic complications because of its strong α-glucosidase inhibitory activity.

In conclusion, SRE inhibited α-glucosidase and α-

amylase activities followed by a diminished rise in blood glucose, resulting in a reduction in postprandial hyperglycemia. Furthermore, SRE may delay the absorption of dietary carbohydrates in the intestine, leading to suppression of an increased blood glucose level after a meal. Thus, we suggest that SRE could be a potential candidate in developing medicinal preparations and nutraceutical for diabetes and related symptoms.

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