Gelidium amansii Extract, a Potent α-glucosidase and α-amylase Inhibitor, Alleviates Postprandial Hyperglycemia in Diabetic Mice

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Gelidium amansii shows antioxidant and anti-obesity effects; however, the effect on postprandial blood glucose levels is not known. The objective of the present study was to investigate the inhibitory effect of Gelidium amansii extract (GAE) on carbohydrate-digesting enzymes and its ability to alleviate postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice. Gelidium amansii was extracted with 80% ethanol and concentrated for use in this study. The α-glucosidase and α-amylase inhibition assays were performed using the colorimetric method. ICR normal and STZ-induced diabetic mice were orally administered GAE (500 mg/kg body weight) or acarbose (100 mg/kg body weight) alone or soluble starch (2 g/kg body weight). Blood samples were taken from the tail vein at 0, 30, 60 and 120 min. Our results indicated that GAE markedly inhibited α-glucosidase and α-amylase activities with IC₅₀ values of 0.099±0.009 mg/ml and 0.178±0.038 mg/ml, respectively, and was a more effective inhibitor than acarbose, the positive control. Further, the postprandial blood glucose levels of STZ-induced diabetic mice in the GAE-administered group were significantly lower than those of control group mice (p<0.05). Moreover, the area under the curves (AUC) significantly decreased with GAE administration in STZ-induced diabetic mice (p<0.05). These results indicate that GAE may be effective in decreasing postprandial blood glucose levels by inhibiting carbohydrate-digesting enzymes such as α-amylase and α-glucosidase. Therefore, GAE could be used as a potential functional food for alleviating postprandial hyperglycemia.

Key words : Gelidium amansii, postprandial hyperglycemia, α-glucosidase, α-amylase

Introduction

Diabetes, an endocrine metabolic disorder, is a global health problem. It is characterized by chronic hyperglycemia caused by a relative or absolute lack of insulin secretion or insulin action [22, 23]. Hyperglycemia is classified as fasting hyperglycemia and postprandial hyperglycemia. The postprandial blood glucose level is usually less than 140 mg/dl in normal people, and diabetes is suspected when the glucose level is higher than 200 mg/dl [19]. The postprandial phase is characterized by a rapid increase in blood glucose levels, which may be associated with diabetic complications such as cardiovascular disease and thus lead to metabolic dysfunction [27]. Therefore, controlling postprandial hyperglycemia is the most important strategy for treating diabetes and preventing diabetic complications [2].

The therapeutic strategies used for reducing postprandial hyperglycemia generally suppress glucose absorption by inhibiting the activity of carbohydrate-digestive enzymes such as α-glucosidase and α-amylase [21]. Hence, synthetic α-glucosidase and α-amylase inhibitors, such as acarbose, miglitol, and voglibose, have been used to control hyperglycemia [3, 18, 20]. However, chronic use of such synthetic inhibitors can cause side effects such as abdominal cramps and diarrhea [3, 6, 18, 30]. Therefore, several studies have been conducted to identify natural inhibitors of α-glucosidase and α-amylase that do not have side effects.

Sea algae contain an abundance of phytochemicals and are potential sources of functional foods. The fucoxanthis, phlorotannins, and phenolic compounds isolated from sea algae have antioxidant and antimutagenic activities [31, 33, 35], and sulfated polysaccharides have anticoagulant, anti-hypertensive, antiviral, and anticancer activities [37]. Gelidium amansii is a red alga belonging to the order Gelidiales. Red algae are rich in bioactive compounds such as polyphenols, carotenoids, polysaccharides and phycocyanins [29]. Previous reports indicate that phenolic compounds in G. amansii...
extract (GAE) have antioxidant, immunomodulatory, anti-tumor, cytotoxic effects and anti-obesity effects [9, 32, 34, 36]. However, the effect of GAE on postprandial hyperglycemia is yet to be investigated. Therefore, this study was designed to investigate whether GAE inhibits a-glucosidase and a-amylase activities and alleviates postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice.

Materials and Methods

Preparation of GAE

G. amansii samples collected from Jeju Island in Korea were washed thrice to remove sand and salt, dried at room temperature, and ground into a powder. The powder was extracted with 80% ethanol for 24 hr at 40°C. The GAE was then concentrated in a rotary vacuum evaporator (N-1300 VW, EYELA Co., Japan) at 40°C and freeze-dried into powder form for use in experiments. The yield of GAE was 5.6%, and the total polyphenol and flavonoid contents of the GAE were 0.26±0.08 and 1.55±0.16 mg/ml, respectively [16].

a-Glucosidase inhibitory assay

The effect of GAE on a-glucosidase activity was determined by the a-glucosidase inhibitory assay, as described by Watanabe et al [30]. An enzyme solution, yeast a-glucosidase, (100 U, Sigma-Aldrich, St. Louis, MO, USA) prepared by dissolving 0.01 g of bovine serum albumin, 0.01 g of NaN₃, and 0.008 g of enzyme in 50 ml of phosphate-buffered saline (pH 7.0) was treated with p-nitrophenyl-α-D glucopyranoside (5 mM) (Sigma-Aldrich Co.). The sample (0.1 ml) was added to 0.5 ml of enzyme solution, and absorbance was measured at 405 nm. The mixture was then incubated at room temperature for 5 min, followed by the addition of 0.5 ml of substrate solution and measurement of absorbance after 5 min of reaction.

a-Amylase inhibitory assay

The a-amylase inhibitory assay was also performed as described by Watanabe et al. [30], using porcine pancreatic a-amylase (100 U, Sigma-Aldrich, St. Louis, MO, USA) and blocked p-nitrophenyl-α-D-maltopentoglycoside (Sigma-Aldrich Co.) as enzyme and substrate, respectively.

Measurement of cytotoxicity

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using 3T3-L1 cells purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). The 3T3-L1 cells were seeded in 96-well plates (1×10⁴ cells/well) and pre-incubated at 37°C for 24 hr in a humidified atmosphere containing 5% CO₂. The cells were then treated with various concentrations of GAE (0.1, 0.5, 1, and 2 mg/ml), cultured for 24 hr, and incubated with filtered MTT solution (Sigma-Aldrich Co.) added to each well at a final concentration of 0.5 mg MTT/ml at 37°C for 3 hr. The supernatant was carefully aspirated, and DMSO (200 μl) was added to each well. The plate was shaken to dissolve the formazan crystals, and absorbance of DMSO solutions was measured spectrophotometrically at 540 nm.

Experimental animals

Four-week-old male ICR mice (Central Laboratory Animal Inc., Seoul, Korea) were individually housed in a temperature-controlled room (25-30°C) with a relative humidity of 45-55%, under a 12 hr light/dark cycle. The animals had ad libitum access to pelleted food and tap water. Diabetes was induced by intraperitoneal injection of 60 mg/kg STZ (Sigma-Aldrich Co.) dissolved in citrate buffer (0.1 M, pH 4.5) after 2 weeks of adjustment period. After 7 days, tail bleeding was performed, and animals with a blood glucose concentration of 250 mg/dl or more were regarded as being diabetic. All animal handling and management procedures were in compliance with current international laws and policies (National Institutes of Health guidelines for the management and use of laboratory animals), and approved by the Animal Ethics Committee of Pusan National University (PNU-2016-1274).

Measurement of blood glucose level

Normal mice and STZ-induced diabetic mice were fasted overnight for 12 hr with ad libitum access to water and randomly divided into 3 groups (n=7). After overnight fasting, GAE (300 mg/kg body weight) or acarbose (100 mg/kg body weight) and soluble starch (2 g/kg body weight) were orally administered to mice [5]. Blood samples were taken from the tail vein, and blood glucose was measured using a glucose meter (Roche Diagnostics GmbH, Germany). The area under the curve (AUC) was calculated by the trapezoidal rule.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SAS version 9.1.
(SAS Institute, Cary, NC, USA). Student’s t-test was used for comparison between control and treatment groups. Differences between groups were assessed by one-way analysis of variance, followed by post-hoc Duncan’s multiple-range test. Values of $p<0.05$ were considered statistically significant.

**Results and Discussion**

**Inhibitory effect of GAE on $\alpha$-glucosidase and $\alpha$-amylase**

The $\alpha$-glucosidase inhibitory activity of GAE and acarbose, a commercial $\alpha$-glucosidase inhibitor, was compared, using p-nitrophenyl-$\alpha$-D-glucopyranoside as a substrate. The activity of $\alpha$-glucosidase was inhibited by GAE in a concentration-dependent manner by 23.11, 35.66, 50.03, and 54.72% at 0.02, 0.05, 0.10, and 0.20 mg/ml concentrations, respectively (Fig. 1). Acarbose, which is used as an oral hypoglycemic agent, inhibited the enzyme activity by 40.38% at 0.10 mg/ml. Thus, the $\alpha$-glucosidase inhibitory activity of GAE was significantly higher than that of acarbose at 0.10 mg/ml.

The $\alpha$-amylase inhibitory activity of GAE was determined similarly. GAE inhibited $\alpha$-amylase activity in a concentration-dependent manner by 16.43, 34.24, 42.46, and 52.05% at 0.02, 0.05, 0.10, and 0.20 mg/ml concentrations, respectively. Further, GAE inhibited $\alpha$-amylase activity more effectively than acarbose (38.11% at 0.10 mg/ml) did. The IC$_{50}$ values for $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities of GAE were 0.099±0.009 and 0.178±0.038 mg/ml, respectively (Table 1). These results show that GAE may be useful as a natural anti-hyperglycemic agent, owing to its potent $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities.

The treatment goal for people with diabetes is maintenance of normal blood glucose levels. Postprandial hyperglycemia in diabetes can be effectively controlled by inhibiting carbohydrate hydrolysis and delaying glucose absorption [1]. Representative carbohydrate-digesting enzymes include $\alpha$-amylase, which catalyzes the formation of disaccharides from polysaccharides via hydrolysis of $\alpha$-1,4-glycosidic linkages, and $\alpha$-glucosidase, which catalyzes the degradation of disaccharides into simple sugars for intestinal absorption [13, 24]. Suppressing $\alpha$-glucosidase and $\alpha$-amylase activities may therefore be an effective strategy for delaying carbohydrate digestion and lowering postprandial

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$-glucosidase (mg/ml)</th>
<th>$\alpha$-amylase (mg/ml)</th>
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<tr>
<td>GAE</td>
<td>0.099±0.009$^*$</td>
<td>0.178±0.038$^*$</td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.130±0.021</td>
<td>0.228±0.019</td>
</tr>
</tbody>
</table>

$^*$IC$_{50}$ value is the concentration of sample required for 50% inhibition of enzyme activity. Each value is expressed as the mean±SD of triplicate experiments.

**Table 1. IC$_{50}$ values of $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities of GAE**

Values with different letters are significantly different at $p<0.05$, as analyzed by Duncan’s multiple-range test.

Fig. 1. $\alpha$-Glucosidase inhibitory activity of *Gelidium amansii* extract (GAE). Each value is expressed as the mean±standard deviation (SD) of three experiments. $^*$Values with different letters are significantly different at $p<0.05$, as analyzed by Duncan’s multiple-range test. Acarbose (0.10 mg/ml) was used as a positive control.

Fig. 2. $\alpha$-Amylase inhibitory activity of *Gelidium amansii* extract (GAE). Each value is expressed as the mean±standard deviation (SD) of three experiments. $^*$Values with different letters are significantly different at $p<0.05$, as analyzed by Duncan’s multiple-range test. Acarbose (0.10 mg/ml) was used as a positive control.
blood glucose levels [10]. However, long-term use of commercial carbohydrate inhibitors such as acarbose leads to side effects such as pancreatitis, abdominal cramps, and diarrhea [3, 7, 11, 20]. Thus, researchers have long searched for an effective and non-toxic inhibitor of \( \alpha \)-glucosidase and \( \alpha \)-amylase.

Sea algae are an important source for drug discovery and have received much attention as sources of biologically active substances, including antioxidants, anti-hyperlipidemic agents, and hypoglycemic agents [22]. Additionally, algal flavonoids and polyphenols have been used to treat diabetes and dyslipidemia [23]. In the present study, we investigated the \( \alpha \)-glucosidase and \( \alpha \)-amylase inhibitory activities of GAE in an attempt to evaluate its efficacy as a natural inhibitor of postprandial hyperglycemia. GAE showed significantly higher inhibitory activity against both \( \alpha \)-glucosidase and \( \alpha \)-amylase than acarbose, the commercial inhibitor, with no cytotoxicity (Fig. 3).

The GAE used in the present study contained 1,810 \( \mu \)g/ml of polyphenols and 1,550 \( \mu \)g/ml of flavonoids [16]. Polyphenols have been reported to alleviate the main symptoms of type 2 diabetes, such as postprandial hyperglycemia, by inhibiting \( \alpha \)-amylase and \( \alpha \)-glucosidase-catalyzed disaccharide hydrolysis in the small intestine [11]. In addition, the inhibitory activities of carbohydrate digesting enzymes increased with an increase in the phenol content, and the highest inhibitory activity was observed at the maximum content of phenol compounds [7]. The GAE contained catechins, phlorotannins (eckol, dieckol and phloroglucinol) and proanthocyanidins [14, 25]. These polyphenolic compounds are known to form complexes with various proteins [28], and the hydroxyl groups in the polyphenolic compounds have a crucial role in inhibiting the activities of the enzymes [28, 17]. Thus, the high inhibitory effects of GAE on \( \alpha \)-glucosidase and \( \alpha \)-amylase activities may be attributable to the high content of polyphenols and the hydroxyl group in the polyphenol compounds.

**Effect of GAE on blood glucose levels**

The effect of GAE on postprandial blood glucose levels was measured both in STZ-induced diabetic mice (Fig. 4) and in normal mice (Fig. 5). The postprandial blood glucose levels were significantly lower in GAE-administered mice than in diabetic control mice. The increase in blood glucose level at 30, 60, and 120 min after starch administration (2 g/kg) was 398.23, 432.77, and 374.30 mg/dl, respectively, in diabetic control mice. However, the increase in blood glucose level at 30, 60 and 120 min after the administration of starch (2 g/kg) and extract (300 mg/kg) was 379.61, 390.23, and 343.86 mg/dl, respectively, which indicated that the hypoglycemic effect was more pronounced in GAE group mice than in diabetic control group mice. The maximum postprandial blood glucose level also significantly decreased when GAE was orally administered with starch in normal mice (Fig. 5). This confirms that GAE can alleviate starch-in-

**Fig. 3.** Cytotoxic effect of *Gelidium amansii* extract (GAE) in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0.1, 0.5, 1.0, and 2.0 mg/ml) of GAE for 20 hr, and cell viability was measured by MTT assay. Each value is expressed as the mean ± standard deviation (SD) of three experiments. NS: Not-significant.

**Fig. 4.** Effect of *Gelidium amansii* extract (GAE) on blood glucose levels in streptozotocin-induced diabetic mice. Distilled water (Control), GAE (300 mg/kg), or acarbose (100 mg/kg) was coadministered orally with starch (2 g/kg). Each value is expressed as the mean ± standard deviation (SD) of 7 mice. *“Values with different letters are significantly different each time (p<0.05), as analyzed by Duncan’s multiple-range test.*
In conclusion, the results of this study showed that GAE has significant inhibitory effects on α-glucosidase and α-amylase activities. In addition, GAE can inhibit postprandial increases in blood glucose levels. Therefore, we suggest that GAE may be used as a dietary supplement for diabetes.

References


This study investigated the anti-hyperglycemic effect of GAE in STZ-induced diabetic mice and normal mice after starch administration. GAE administration significantly inhibited the starch-induced increase in postprandial blood glucose levels in both diabetic and normal mice (Fig. 4 and 5), which showed that GAE could delay the absorption of carbohydrates and thus suppress the increase in postprandial blood glucose levels. In this study, GAE also decreased the AUC of the blood glucose response. This was consistent with previous studies, which have shown that medications that flatten the postprandial blood glucose peak reduce the AUC of the blood glucose response [15].

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal mice (mg.h/dl)</th>
<th>Diabetic mice (mg.h/dl)</th>
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<tbody>
<tr>
<td>Control</td>
<td>272.37±24.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>791.20±33.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAE</td>
<td>250.18±20.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>735.97±49.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acarbose</td>
<td>211.58±22.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>693.62±32.97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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<sup>a</sup>Distilled water (Control), GAE (300 mg/kg), or acarbose (100 mg/kg) was coadministered orally with starch (2 g/kg). Each value is expressed as the mean±standard deviation (SD) of 7 mice. <sup>b</sup><sup>c</sup>Values with different letters are significantly different each time (p<0.05), as analyzed by Duncan’s multiple-range test. GAE: Gelidium amansii extract.

Fig. 5. Effect of Gelidium amansii extract (GAE) on blood glucose levels in normal mice. Distilled water (Control), GAE (300 mg/kg), or acarbose (100 mg/kg) was coadministered orally with starch (2 g/kg). Each value is expressed as the mean±standard deviation (SD) of 7 mice. **Values with different letters are significantly different each time (p<0.05), as analyzed by Duncan’s multiple-range test. GAE: Gelidium amansii extract.**

It is important for diabetics to maintain normal blood glucose levels in both fasting and postprandial states. Postprandial hyperglycemia is an important risk factor in the development of type 2 diabetes, and its association with diabetic complications is well known. Postprandial hyperglycemia also induces vasoconstriction and stimulates the thrombus formation pathway, leading to increased free radical production, which increases the risk of microvascular and macrovascular disease that are a major cause of premature death in type 2 diabetic patients [4]. Thus, controlling postprandial blood glucose levels is the most important strategy for diabetes prevention and treatment.


초록: 당뇨 마우스에서 우뭇가사리(Gelidium amansii)의 식후 고혈당완화 효과

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(부산대학교 식품영양학과)

우뭇가사리(Gelidium amansii)는 홍조류로서 우뭇가사리과(Gelidiaceae)에 속한다. 현재까지 우뭇가사리의 항산화 및 항가단백 효과 등의 기능들이 연구되었으나 식후 혈당 수치에 미치는 영향에 관한 연구는 부족한 실정이다. 이에 본 연구에서는 Gelidium amansii extract (GAE)가 탄수화물 가수분해 효소(α-glucosidase, α-amylase)에 미치는 억제 효과 및 streptozotocin (STZ)으로 유도된 당뇨병 마우스의 식후고혈당에 미치는 완화효과를 조사하였다. 정상군과 STZ으로 유도된 당뇨병 마우스에 수용성 전분(2 g/kg body weight)을 경구투여한 후 GAE (300 mg/kg body weight) 또는 acarbose (100 mg/kg body weight)를 단독 또는 함께 투여하였다. 혈당은 꼬리채혈을 통해 0, 30, 60, 120분 간격으로 측정하였다. α-glucosidase와 α-amylase에 대한 GAE의 IC_{50} 값은 각각 0.099±0.009 mg/ml와 0.178±0.038 mg/ml의 결과값을 나타내어, 양성대조군인 acarbose보다 더 효과적이었다. STZ으로 유발된 당뇨병 마우스의 식후 혈당 수치는 대조군에 비해 GAE 투여 시 유의적으로 더 낮았다(p<0.05). 또한 GAE 투여는 당뇨병 마우스에서 포도당 반응에 대한 곡선면적 감소와 관련이 있었다(p<0.05). 이러한 결과는 GAE가 α-glucosidase, α-amylase와 같은 탄수화물 가수분해 효소를 억제함으로써 식후 고혈당을 완화시키는 유용한 천연 기능성 식품이 될 것으로 사료된다.