

Gelidium elegans Regulates Blood Glucose Homeostasis in ICR Mice

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Abstract *Gelidium elegans* has been reported to improve metabolic function, but it has not been studied in non-obese mice with glucose tolerance. To evaluate the effect of *Gelidium elegans* (50 or 200 mg/kg doses) on glucose homeostasis, an oral glucose tolerance test (OGTT), oral maltose tolerance test (OMTT), and insulin tolerance test (ITT) were performed. The non-obese group of mice that was administered 200 mg/kg *Gelidium elegans* had significantly lowered blood glucose levels. By revealing that *Gelidium elegans* may improve glucose homeostasis, this study expands our understanding of the anti-diabetic effect of *Gelidium elegans* and its biological importance.

Keywords: *Gelidium elegans*, *Gelidium amansii*, Hyperglycemia, Diabetes, Glucose absorption

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1. Introduction

Hyperglycemia is characterized by an excessive amount of blood glucose and is often observed in obese and diabetic patients [1,2]. Hyperglycemia is known to cause numerous hematogenous-associated complications, such as cardiovascular disease (CVD), oxidative stress, and ketoacidosis [3,4,5]. To maintain the homeostasis of hyperglycemic conditions outside of the cells, glucose moves into the cell via sodium-dependent glucose co-transporter (SGLT) and facilitative glucose transporters (GLUTs) [6]. SGLT works against an electrochemical gradient in which glucose is transported from the intestines or nephrons by the Na⁺-K⁺ ATPase pump [7,8]. GLUTs act as glucose carriers and are mediated by an energy-independent glucose transport process that is common to almost all cell [9,10]. GLUT4 is the most abundant type in muscle and adipose tissues and is essential for glucose-stimulated insulin secretion to control blood glucose homeostasis [11,12]. Stimulation of GLUT4 mediates glucose translocation to the plasma membrane; hence, it mediates glucose uptake in muscles.

For many decades, several pharmaceutical compounds have been developed to prevent the development of hyperglycemia and its associated diseases, including obesity and diabetes. Pioglitazone, metformin, and rosiglitazone are well known Food and Drug Administration (FDA)-approved anti-hyperglycemic pharmaceutical compounds [13]. These anti-hyperglycemic compounds decrease excessive amounts of blood glucose through the peripheral cell recognition of insulin as well as activation of the GLUT4 signaling pathway [14,15]. Although anti-hyperglycemic

compounds have a beneficial effect on the regulation of blood glucose homeostasis, pioglitazone, metformin, and rosiglitazone have been shown to have a number of adverse effects, such as liver toxicity, lactic acidosis, and diarrhea [16]. Increasing the impact of novel therapy strategies and/or developing effective anti-hyperglycemic compounds without adverse effects is therefore necessary.

Recently, several studies reported that the phytochemicals and polyphenols extracted from fruits, vegetables, and edible seaweeds stimulate glucose uptake via modulation of the GLUT4 signaling pathway and insulin sensitivity both in vitro and in human clinical trials [17,18,19]. In particular, previous studies revealed that seaweed-derived phytochemicals and dietary seaweed attenuate blood glucose levels in vivo [20,21], indicating that seaweed might be a suitable resource for ameliorating blood glucose levels without adverse effects.

Gelidium elegans, previously known as *Gelidium amansii*, is an edible seaweed native to the Asian Pacific Region [22,23]. Along with other group, we have reported the bioactivity of *Gelidium elegans*, including its anti-oxidative stress, anti-lipogenesis, and anti-obesity properties [23,24,25]. In addition, Kang *et al.* suggested the potential effect of *Gelidium elegans* on blood glucose regulation in vivo [25]. Although these reports potentially indicate that *Gelidium elegans* regulates the level of hematogenous-circulating glucose and diabetes, they have not yet been studied. In the present study, we therefore investigated the potential effect of *Gelidium elegans* on anti-diabetes activity in vivo. To determine the anti-diabetic effect of *Gelidium elegans*, we performed three independent assays, including an oral glucose tolerance test (OGTT), oral maltose tolerance test (OMTT), and insulin tolerance test (ITT).

2. Materials and Methods

2.1. Reagents

Gelidium elegans extract was provided by NEWTREE Inc. (Seongnam, Kyonggi, South Korea). The composition of the *Gelidium elegans* extract is described in Table 1. Glucose, insulin, and methylcellulose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Metformin was purchased from Cayman Chemical (Ann Arbor, MI, USA).

Table 1. The composition of *Gelidium elegans* extract [26]

Component	<i>Gelidium elegans</i>
Moisture	5.1 %
Crude protein	16.7 %
Crude ash	24.1 %
Carbohydrate	47.6 %
Total polyphenols	8.79 mg per 1g

2.2. Animals and Treatment

All mice were housed in a specific pathogen-free facility at CHA University, Seongnam, Republic of Korea. The project was approved by the Institutional Animal Care and Use Committee of CHA University (IACUC Approval Number 150075). Five-week-old male ICR mice were purchased from Orient Bio Co. (Kapyong, Republic of Korea).

Mice were provided a NIH-07 rodent chow diet (Zeigler Brothers, Gardners, PA, USA). Animals were acclimated to temperature (20-24 °C) and humidity (44.5-51.8 %) with a 12-h light/dark cycle for 1 week prior to use. After the 1-week adaptation period, mice were randomly divided into twelve group. Each group consisted of 6 mice. *Gelidium elegans* and/or metformin were provided through oral administration.

2.3. Oral Glucose Tolerance Test

After 5 weeks, mice were randomly divided into four group (n=6 per group). An OGTT was performed following a 12-h fast. There were four group of mice. The first group was orally administered 1.5 g/kg glucose. Two group were administered 1.5 g/kg glucose and 50 or 200 mg/kg *Gelidium elegans*. The fourth group (positive control) was orally administered 1.5 g/kg glucose and 140 mg/kg metformin. Tail-vein blood samples were collected at 0 (before the glucose challenge), 30, 60, 90, 120, and 150 min.

2.4. Oral Maltose Tolerance Test

After 5 weeks, mice were randomly divided into four group (n=6 per group). An OMTT was performed following a 12 h fast. There were four group of mice. The first group was orally administered 3 g/kg maltose. Two group were administered 3 g/kg maltose and 50 or 200 mg/kg *Gelidium elegans*. The fourth group (positive control) was orally administered 3 g/kg maltose and 140 mg/kg metformin. Tail-vein blood samples were

collected at 0 (before the glucose challenge), 30, 60, 90, and 120 min.

2.5. Insulin Tolerance Test

After 5 weeks, mice were randomly divided into four group (n=6 per group). An ITT was performed following a 12 h fast. There were four group of mice. The first group was intraperitoneally challenged with 0.05 U/kg insulin. Two group were intraperitoneally challenged with 0.05 U/kg insulin, and three min afterwards, they were orally administered 50 or 200 mg/kg *Gelidium elegans*. As a negative control (fourth group), we used mice that did not receive insulin. Tail-vein blood samples were collected at 0 (before the glucose challenge), 30, 60, 90, 120, and 150 min.

2.6. Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences version 12.0 (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used for comparisons among the group. Significant differences between the mean values were assessed using Duncan's test. *p* values less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Oral Glucose Tolerance Test

Hyperglycemia is a metabolic disorder characterized by an excessive amount of circulating glucose in the blood plasma [27,28]. OGTT reflects the degree of glucose tolerance and can be used to diagnose hyperglycemia and diabetes [29,30]. Therefore, we performed OGTT to evaluate the effects of *Gelidium elegans* on hyperglycemia.

All mice were fasted for 12 h. The control group was administered 1.5 g/kg glucose. Each of the two independent group (n=6) received oral administrations of 50 or 200 mg/kg *Gelidium elegans* in addition to 1.5 g/kg glucose. The positive control group was orally administered 1.5 g/kg glucose and 140 mg/kg metformin.

To evaluate the effect of *Gelidium elegans* on glucose tolerance, blood samples were collected from the tail veins. Blood glucose levels were measured over 2 h. As shown in Figure 1A, all group had glucose levels within the normal range of 106.0 ± 14.0 mg/dL at 0 min.

Insulin release in response to a glucose load occurs within the first 15-30 min and is responsible for limiting the initial rise in glucose levels upon meal ingestion [31]. The glucose-only control group reached a glucose level of 176.0 ± 20.2 mg/dL at 30 min. On the other hand, the group that received 50 or 200 mg/kg *Gelidium elegans* had a significantly suppressed rise in blood glucose, with glucose levels of 149.0 ± 18.5 mg/dL and 136.0 ± 36.3 mg/dL at 30 min, respectively. In comparison with the control group, the group that was administered 200 mg/kg *Gelidium elegans* significantly decreased their blood glucose levels by approximately 10.4 % at 30 min.

At 60 min, the glucose level of the control group reached 172.2 ± 31.4 mg/dL. On the other hand, the group

that received 50 or 200 mg/kg *Gelidium elegans* had a significantly suppressed the rise in blood glucose, with glucose levels of 154.7 ± 13.7 mg/dL and 141.8 ± 23.4 mg/dL, respectively, when compared to the control group at 60 min.

The group that were administered 50 or 200 mg/kg of *Gelidium elegans* exhibited glucose levels of 143.7 ± 19.6 mg/dL and 135.8 ± 28.9 mg/dL at 90 min, respectively. In comparison with the control group, the group that received 200 mg/kg *Gelidium elegans* had blood glucose levels that were significantly decreased by approximately 12.9 % at 90 min.

At 120 min, the group that were administered 50 or 200 mg/kg *Gelidium elegans* had significant attenuation of blood glucose (glucose levels of 126.2 ± 28.9 mg/dL and 132.3 ± 27.7 mg/dL, respectively) when compared to the control group. The group that received 140 mg/kg metformin displayed significant attenuation at 30, 60, 90, 120, and 150 min when compared to the control group. By the 150-min time period, the glucose levels of all group had declined to within the normal fasting range of 114.3 ± 30.2 mg/dL. When compared with the control group, the group that received 200 mg/kg *Gelidium elegans* showed glucose levels that were significantly decreased from their fasting level at 30, 60, 90, and 120 min.

Area under the curve (AUC) is a useful measure for identifying the average concentration over a time interval [32]. We therefore calculated the AUC by the trapezoidal rule to evaluate the OGTT of *Gelidium elegans*. As shown Figure 1B, AUC_{glucose} levels also correlated with Figure 1A.

In the current study, we found that *Gelidium elegans* can impact glucose homeostasis in ICR mice. In particular, the group that was administered 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels after glucose loading. Several studies have reported that phytochemical compounds, such as those found in flavonoid-rich foods, have the potential to regulate glucose homeostasis [33].

To maintain the level of blood glucose within a normal range, there are two major strategies involving the regulation of insulin resistance in peripheral tissues and the regulation of insulin production in pancreatic tissue. When the proper concentration of insulin is insufficient to stimulate the glucose uptake from blood stream into peripheral tissues causes insulin resistance [34]. The critical initial steps in the development of insulin resistance include inactivation of insulin receptor, insulin receptor substrate 1 (IRS1), and IRS2 [35].

It have been shown that flavonoid-rich foods ameliorate insulin resistance in diabetes model [36,37,38,39]. In particular, blue berry has been used traditional medicine for diabetic patients due to its biological activity that promote to decrease insulin resistance in peripheral tissues [40]. In addition, curcumin decreased the level of blood glucose through the suppression of glucose production in hepatic tissue or the attenuation of insulin resistance peripheral tissues [41].

Oral administrations of 50 or 200 mg/kg *Gelidium elegans* with glucose had significant attenuation of blood glucose when compared to control group. These results indicate that *Gelidium elegans* may be expected to increase the peripheral and hepatic insulin sensitivity through activating IRS expression.

Flavonoid-rich foods can also reduce glucose uptake by modifying the activity of other carbohydrate-digestive

enzymes, such as α -glucosidase [42,43]. Therefore, we performed an OMTT to indirectly investigate whether *Gelidium elegans* inhibits α -glucosidase.

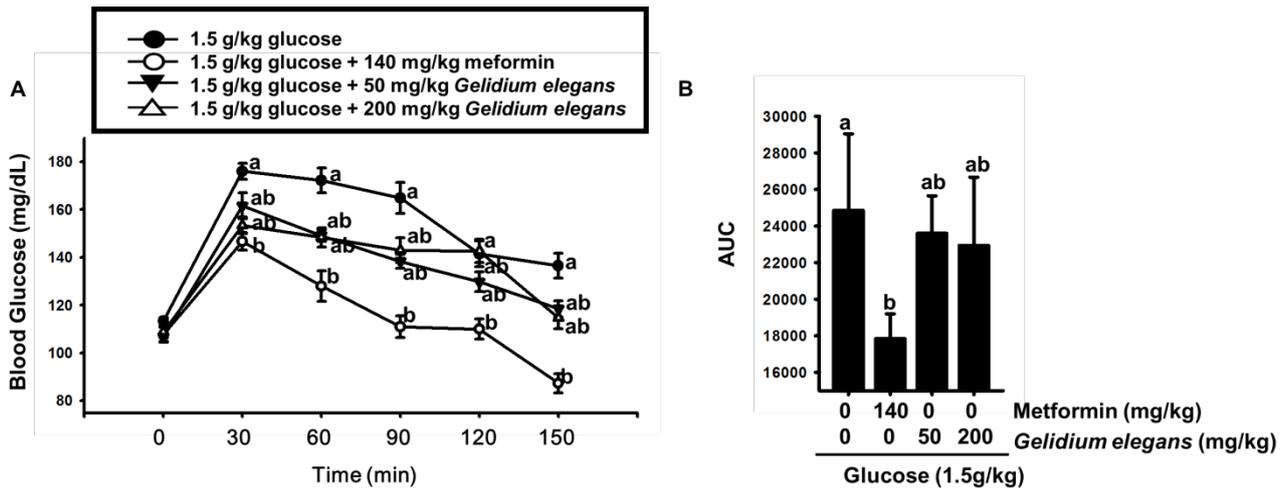
3.2. Oral Maltose Tolerance Test

Maltose is added to a wide variety of foods, including candies, cereal bars, bagels, pies, sweet potatoes, and honey [44]. Maltose is a disaccharide composed of two glucose molecules and is obtained from beverages [45]. After food intake, the enzymes maltase and α -glucosidase break down maltose into two glucose molecules, which results in an increased blood glucose concentration in the small intestine [46,47,48]. α -glucosidase inhibitor, such as miglitol, lower blood glucose levels after a meal by interfering with the conversion of disaccharides to monosaccharides in the small intestine [48,49,50]. Therefore, we performed OMTT to investigate whether *Gelidium elegans* inhibits the α -glucosidase activity.

Prior to performing the OMTT, all mice were fasted for 12 h [51,52]. Four group were tested: a control group that received 3 g/kg maltose, a reference drug group that received 3 g/kg maltose and 140 mg/kg metformin, and group that received 3 g/kg maltose and two different doses of *Gelidium elegans* (oral administrations of 50 or 200 mg/kg). Blood samples were collected from the tail veins at 0, 30, 60, 90, and 120 min after maltose loaded. All group showed the glucose levels within the normal range of 114.3 ± 12.1 mg/dL at 0 min. As shown Figure 2A, the group that received 50 or 200 mg/kg *Gelidium elegans* exhibited glucose levels of 134.0 ± 11.9 mg/dL and 122.4 ± 7.5 mg/dL at 60 min, respectively. In comparison to the control group, the group that received 3 g/kg maltose with 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 10.4 % at 60 min. The group that received 50 or 200 mg/kg *Gelidium elegans* exhibited glucose levels of 127.4 ± 10.6 mg/dL and 123.6 ± 14.7 mg/dL at 120 min, respectively. In comparison with the control group, the group that received 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 12.9 % at 120 min. The group that was administered 140 mg/kg metformin showed significant attenuation at 30, 60, 90, and 120 min compared to the control group. Therefore, we calculated the area above the axis minus the area below the axis (in their respective coordinates) to evaluate the OMTT of *Gelidium elegans*. As shown Figure 2B, AUC_{maltose} levels also correlated with Figure 2A.

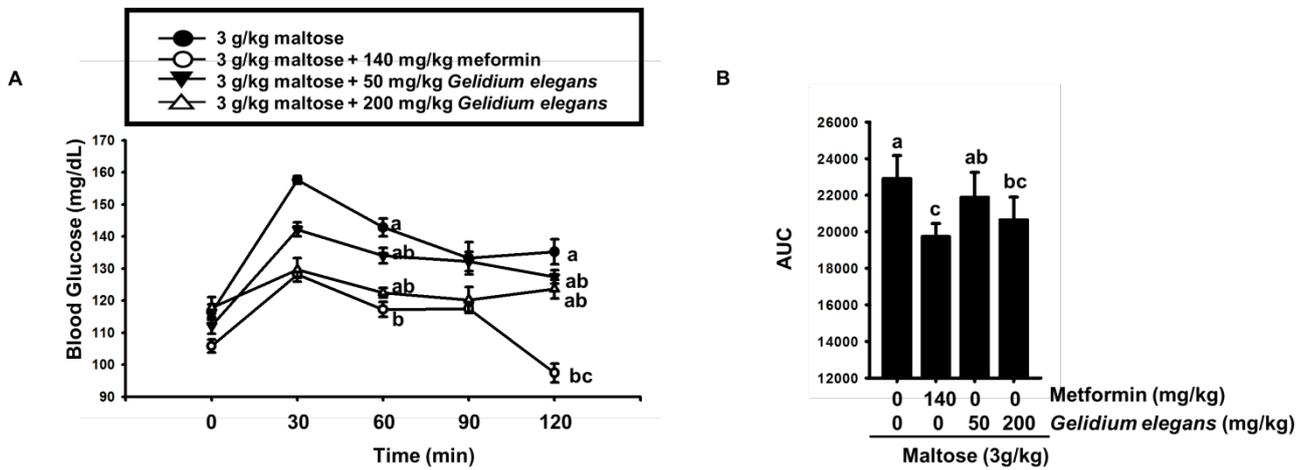
The group that were administered 50 or 200 mg/kg *Gelidium elegans* had significant attenuation of blood glucose in OMTT when compared to control group. Certainly, 50 mg/kg *Gelidium elegans* was sufficient to reduce the level of blood glucose. These results indicate that *Gelidium elegans* may be expected to inhibit the activity of α -glucosidase in the intestines.

Although we sought that *Gelidium elegans* might suppress the insulin resistance and α -glucosidase activity (Figure 1A, Figure 1B, Figure 2A, and Figure 2B) however it is still remain unclear whether *Gelidium elegans* stimulates the production or sensitivity of insulin in vivo. To examine whether *Gelidium elegans* affect the sensitivity or production of insulin in vivo, we therefore performed an ITT.



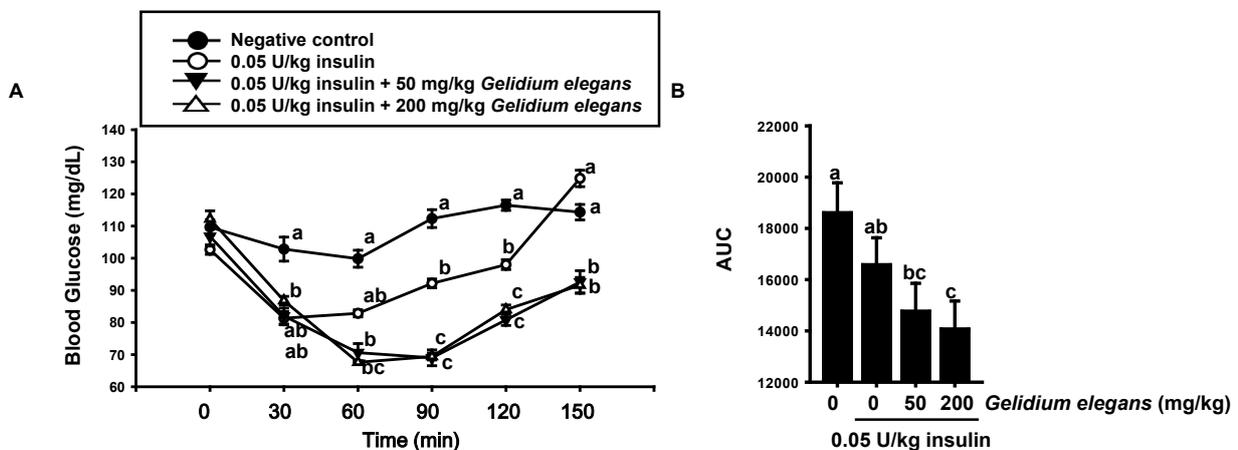
After a 12-h fast, four groups of male mice (5 weeks old) were orally administered 1.5 g/kg glucose. The first group received only glucose, the second and third group received glucose and 50 or 200 mg/kg *Gelidium elegans*, and the fourth group received glucose and 140 mg/kg metformin. Blood glucose levels were measured at the indicated times (0, 30, 60, 90, 120, and 150 min) (A). Area under the curve for glucose (AUC_{glucose}) was calculated using the trapezoidal rule (B). Data are mean \pm SD (n=6). The results were subjected to ANOVA and Duncan's tests ($p < .05$).

Figure 1. *Gelidium elegans* improves glucose tolerance in ICR mice



After a 12-h fast, four groups of male mice (5 weeks old) were orally administered 3 g/kg maltose. The first group only received maltose, the second and third group received maltose and 50 or 200 mg/kg *Gelidium elegans*, and the fourth group received maltose and 140 mg/kg metformin. Blood glucose levels were measured at the indicated times (0, 30, 60, 90, and 120 min) (A). Area under the curve for maltose (AUC_{maltose}) was calculated using the trapezoidal rule (B). Data are mean \pm SD (n=6). The results were subjected to ANOVA and Duncan's tests ($p < .05$).

Figure 2. Plasma glucose during oral maltose tolerance test in ICR mice with *Gelidium elegans* supplement



After a 12-h fast, three groups of male mice (5 weeks old) were intraperitoneally injected with 0.05 U/kg insulin. The first group received only insulin, and the second and third group received insulin and oral administrations of 50 or 200 mg/kg *Gelidium elegans*. A fourth group did not receive insulin. Blood glucose levels were measured at the indicated times (0, 30, 60, 90, 120, and 150 min) (A). Area under the curve for insulin (AUC_{insulin}) was calculated using the trapezoidal rule (B). Data are mean \pm SD (n=6). The results were subjected to ANOVA and Duncan's tests ($p < .05$).

Figure 3. Plasma glucose during insulin tolerance test in ICR mice with *Gelidium elegans* supplement

3.3. Insulin Tolerance Test

Hyperglycemia and diabetic patients with insulin resistance occur with critical metabolic syndromes, including visceral obesity, hypertension, dyslipidemia, and IGT [53,54]. Metformin, a representative insulin-sensitizing drug, improves insulin sensitivity [55,56]. An ITT is the most commonly used method for estimating insulin resistance by measuring blood glucose levels [57,58,59]. Therefore, we performed an ITT to investigate whether *Gelidium elegans* improves insulin resistance in blood glucose levels.

Prior to an ITT, mice were fasted for 12 h [51,52]. Four groups were tested: a negative control (non-insulin) group, a control group that received 0.05 U/kg insulin, and two groups that received 0.05 U/kg insulin along with dose-dependent 50 or 200 mg/kg *Gelidium elegans* by oral administration. Blood samples were collected from the tail veins at 0, 30, 60, 90, 120, and 150 min. Prior to performing the ITT, all mice were fasted for 12 h. All groups showed glucose levels within the normal range of 107.3 ± 13.1 mg/dL. As shown in Figure 3A, the groups that were administered 50 or 200 mg/kg *Gelidium elegans* exhibited glucose levels of 70.6 ± 16.9 mg/dL and 67.6 ± 3.2 mg/dL at 60 min, respectively. In comparison to the control group, the group that received 0.05 U/kg insulin with 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 18.4% at 60 min. The groups that received 50 or 200 mg/kg *Gelidium elegans* exhibited glucose levels of 69.0 ± 14.8 mg/dL and 69.4 ± 6.5 mg/dL at 90 min, respectively. In comparison with the control group, the group that was administered 0.05 U/kg insulin with 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 24.7% at 90 min. The groups that were administered 50 or 200 mg/kg of *Gelidium elegans* exhibited glucose levels of 80.8 ± 10.3 mg/dL and 84.0 ± 8.7 mg/dL at 120 min, respectively. In comparison to the control group, the group that received 0.05 U/kg insulin with 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 14.3% at 120 min. The groups that received 50 or 200 mg/kg *Gelidium elegans* exhibited glucose levels of 92.6 ± 21.1 mg/dL and 91.6 ± 14.5 mg/dL at 150 min, respectively. In comparison with the control group, the group that received 0.05 U/kg insulin with 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels of approximately 26.6% at 150 min. There was no significant difference in the negative control group's glucose attenuation at 30, 60, 90, and 120 min. This study has shown that insulin sensitivity can be attributed to *Gelidium elegans*. We therefore calculated the area above the axis minus the area below the axis (in their respective coordinates) to evaluate the ITT of *Gelidium elegans*. As shown in Figure 3B, AUC_{insulin} levels also correlated with Figure 3A.

Flavonoids may exert beneficial effects in diabetes by enhancing insulin secretion and suppressing hyperglycemia through regulation of glucose metabolism. Resveratrol attenuates hyperglycemia through the stimulation of IRS and GLUT4 proteins in diabetes model [60].

Administered insulin with 50 or 200 mg/kg *Gelidium elegans* had significant attenuation of blood glucose in

ITT when compared to control group. These results indicate that *Gelidium elegans* may partially reflect an increase in the production of insulin from pancreatic tissues or the stimulation of protein kinase B (Akt) and GLUT4 protein in peripheral tissues. In addition, this experiment might imply that *Gelidium elegans* has anti-hyperglycemic effects. Further experiments are necessary to clarify the actual mechanisms involved.

4. Conclusion

Taken together, these results from the OMTT, OGTT, and ITT showed that *Gelidium elegans* decreased blood glucose levels when compared to the control. More specifically, the group that was administered 200 mg/kg *Gelidium elegans* had a significant decrease in blood glucose levels. Insulin is one of the well-known hormones that can reduce blood glucose levels by promoting insulin release or improving insulin sensitivity. *Gelidium elegans* is widely distributed in Asia and is commonly used as an edible item, with a known lack of toxicity. *Gelidium elegans* therefore represents a potentially useful dietary addition for the treatment of diabetes.

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Gelidium elegans" which was funded by the Ministry of Oceans and Fisheries, Korea. The funders have no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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